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[54] STABLE INTRAVENOUSLY INJECTABLE PLASMA PROTEIN FREE FROM HYPOTENSIVE EFFECTS AND PROCESS FOR ITS PRODUCTION

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[58] **Field of Search** 424/177

[56] **References Cited**

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[57] **ABSTRACT**

The depressor effect observed when heat-treated plasma protein fractions are rapidly infused is eliminated by separating, e.g., with a surface active adsorbent or cation exchanger, ultrafiltration membrane or by gel molecular sieve, a low molecular weight depressor substance having a molecular weight of less than 10,000 from the plasma proteins.

27 Claims, No Drawings

STABLE INTRAVENOUSLY INJECTABLE PLASMA PROTEIN FREE FROM HYPOTENSIVE EFFECTS AND PROCESS FOR ITS PRODUCTION

BACKGROUND OF THE INVENTION

This invention relates to stable plasma proteins, substantially free of blood pressure depressant components, and to a method for producing such depressant-free plasma proteins.

Solutions of heat-treated human plasma protein fractions, e.g., those described in U.S. Pat. No. 2,958,628, have been used extensively for a number of years in clinics and hospitals in the treatment of hypoproteinemia, shock and other conditions requiring the use of a plasma expander. More recently, the value of such solutions has become enhanced by virtue of the increased risk of hepatitis associated with the infusion of whole blood or plasma which has not been heat-treated.

In most cases, solutions of plasma protein fractions are infused into the patient at a slow rate and heretofore no side effects of any significance have been experienced with such solutions. Recently, however, solutions of plasma protein fractions have been used in numerous heart-lung bypass procedures for open-heart surgery where the infusion rate is necessarily much greater, i.e., on the order of 100 ml. of 5 percent heat-treated plasma protein solution within about five minutes. The arterial pressure has been observed to drop markedly in a number of such patients whereas this undesirable effect could be controlled if the infusion rates were reduced. Thus, the use of such plasma solutions in situations requiring rapid infusion could be seriously detrimental to the patient when such a depressor effect occurs.

It is an object of this invention to provide a method of removing the depressor substance from heat-stable human plasma protein fractions. Another object is the provision of novel heat stable plasma protein, substantially free of blood pressure depressants. Other objects will be apparent to those skilled in the art to which this invention pertains.

SUMMARY OF THE INVENTION

The depressor substance which causes a decrease in arterial pressure during the rapid infusion of solutions of heat-treated plasma protein fractions, e.g., in heart-lung by-pass techniques, can be substantially removed by contacting a solution of a heat-treated plasma protein fraction with a fractionating substance, including surface active adsorbents, cation exchangers, ultrafiltration membranes and gel filtration particles.

DETAILED DISCUSSION

The starting materials for the process of this invention are stable human blood plasma protein fractions which contain a depressor, i.e., a substance which lowers blood pressure significantly when plasma containing it is infused rapidly. Stable plasma protein fractions are those which have been rendered heat stable by heating, up to 60° C., for up to 10 hours. Such stable plasma protein fraction typically consist predominantly of albumin plus small amounts of alpha and beta globulins.

A preferred class of starting material is a non-homogeneous plasma protein fraction, e.g., that obtained from Supernatant IV-1 by precipitation with ethanol (Cohn Method 6 process), which protein fraction has been reconstituted to a 5 percent solution containing NaCl and a stabilizer, e.g., acetyl tryptophan and/or sodium caprylate, and then heated to 60° C. for 10 hours to destroy hepatitis virus. This stable plasma protein fraction is described in Japanese Patent Publication No. 5297/60 which issued as Japanese Pat. No. 265704, the counterpart of U.S. Pat. No. 2,958,628. Another group of starting materials are solutions of plasma protein fractions which have been heated at about 60° C. for shorter periods of time, e.g., between about 2 to 10 hours, can also be used. For example, it has been observed that heating solutions of stable plasma protein fractions at 60° C. for 10 hours or longer to inactivate any hepatitis virus which may be present, sometimes causes a small amount of precipitate to form.

In a preferred aspect of this invention, a solution of a stable plasma protein fraction is heated at about 60° C. for a few hours, for example, about 1 to 4 hours. Any precipitate which forms is removed, e.g., by filtration or centrifugation, and the clear plasma solution is then treated with any one of the means of this invention for removing depressor substances. Subsequently, heating at 60° C. for 10 hours or longer may then be performed for the purpose of destroying any hepatitis virus in the plasma. Another starting material is the heat-treated stable plasma protein fraction obtained from human placenta.

The exact nature of the material employed for removing the depressor is not critical and can readily be determined according to methods well known in the art, given the knowledge that the depressor is a relatively low molecular weight, readily separable material. For example, using an ultrafiltration membrane which allows materials of a molecular weight below 10,000 to pass through, the depressor can be separated from any starting plasma protein solution. The operability of any fractionating material which is inert to plasma proteins for separating the depressor substance from the starting plasma solution can then be determined using this separated solution of the depressor to determine experimentally the optimum conditions using that fractionating material.

It will also be apparent that once the depressor substance has been characterized, its removal from any starting plasma protein solution by a selected fractionating material can be readily determined by routine experimentation.

Preferred fractionating materials for removing the depressor are surface active adsorbents, cation exchangers, preferably cationic ion exchange resins, ultrafiltration membranes and gel filtration molecular sieves, and accordingly, the preferred methods of removal of the depressor are surface active adsorbent chromatography, cation exchanger chromatography, ultrafiltration and gel molecular sieve filtration.

A preferred treatment of the protein solution for removal of the depressor substance or substances comprises treating the solution with a surface active adsorbent, for example, silica gel, hydrated alumina gel, magnesium hydroxide gel or barium sulfate, followed by separation of the adsorbent from the mixture to give a plasma protein solution substantially free of depressor substances. Although more concentrated or less concentrated solutions of plasma protein can be used, a concentration of about 5 percent is preferred. The

optimum amount of adsorbent used will vary, depending upon the particle size of the adsorbent, the relative concentration of the protein, and the desired level to which the depressor substance is to be reduced, i.e., either complete removal or substantially complete removal, i.e., to a point where a slight depressor effect by rapid infusion of the solution would not be harmful to the recipient. In general, silica gel having a particle size of about 10–40 microns is a preferred adsorbent although particle sizes greater or smaller than 10–40 microns are effective. Typical of the preferred silica gels are Aerosil 200 (Degussa Inc.) and Merck Silica Gel H.

The starting protein solution can be mixed with the adsorbent batchwise with gentle agitation or it can be passed through a suitable column of the adsorbent. Treatment time is not critical, and, in fact, in the batchwise procedure, mixing for several minutes or for several hours appears to have substantially the same effect in the successful removal of the depressor substances. The temperature at which the operation is performed also is not critical so long as it is maintained below that which is detrimental to the plasma proteins and generally is in the range of about 5° to about 60° C.

Following treatment of the protein solution with the adsorbent, the latter can be removed by conventional means, e.g., centrifugation and/or filtration. The solution of plasma protein can then be sterile-filtered into suitable containers and preferably then heated at about 60° C. for at least 10 hours to inactivate any hepatitis virus that may be present, if the solution has not previously been subjected to prolonged heating.

Although surface active adsorbents are the preferred agents for the removal of depressor substances from plasma proteins, other agents or conditions can be used to bring about the desired effect. Solutions of stable plasma protein fractions can be treated with cation exchangers, for example, carboxymethyl cellulose; carboxymethyl Sephadex (Pharmacia Fine Chemicals), which is a cross-linked dextran with terminal carboxymethyl groups; Amberlite CG-50, which is a sulfonated polystyrene cross-linked with divinylbenzene sold by Rohm and Haas and Co.; and Dowex 50-X2 which is a similar cation exchanger sold by Dow Chemical Co. The protein solutions may be allowed to pass through columns of the ion exchangers such as those previously equilibrated with 0.25 percent sodium chloride solution, at a rate of about 50–150 ml./hr./cm^2, and at a temperature of about 4° to 60° C. The eluates are then substantially free of depressor substances.

Another useful means for the removal of depressor substances from stable plasma protein fractions is by ultrafiltration. The starting protein solutions containing depressor substance can be subjected to ultrafiltration using a suitable membrane of a porosity which allows passage essentially only of low molecular weight species below about 10,000 (which includes the depressor substance) under conditions which are well known to those practicing ultrafiltration procedures.

One of a variety of suitable membranes is that described as UM10 Diaflo ultrafiltration membrane, which is a non-cellulosic polymer with ionic groups on the surface, available from Amicon Corporation. Other acceptable membranes are PM10 Diaflo membranes (also from Amicon), similar to UM10 membrane but nonionic; Pellicon type PSED (Millipore Corporation) and Nitrocellulose membrane S-12136 (Sartorius Division of Brinkman Instruments).

The ultrafiltration procedure is well described in H. J. Bixler, R. W. Hausslein, L. M. Nelsen, Separation and purification of biological materials by ultrafiltration, Nat. Meeting Am. Inst. of Chem. Eng., Cleveland, May, 1969; C. J. Van Oss, P. M. Bronson, Removal of IgM from serum by ultrafiltration, Anal. Biochem., 36, 464 (1970); D. Boutin, J. Brodeur, Ultrafiltration of human serum, evidence of low-molecular weight cholinesterase activity (in French), Rev. Can. Biol., 29 (2), 187 (June, 1970); Ultrafiltration for laboratory and clinical uses, Publication No. 403, 1970, put out by Amicon Corporation, Lexington, Mass.

Still another means for removal of depressor substances from stable plasma protein fractions according to the present invention is by gel filtration. Similar in some respects to ultrafiltration, such a system depends on the ability of lower molecular weight species, including the depressor substance, to pass through the interstices of bead-formed gel particles and become more or less entrapped thereby. The procedure for the use of molecular sieve gels in gel filtration is well known in the art and is exemplified in numerous references, including Whitaker, J.R., Determination of molecular weights of proteins by gel filtration on Sephadex, Anal. Chem., 35, 1950–1953 (1963); Andrews, P., Estimation of the molecular weights of proteins by Sephadex gel filtration, Biochem., J., 91, 222–233 (1964); Laurent, T. C., Killander, J., A theory of gel filtration and its experimental verification, J. Chromatog., 14, 317–330 (1964); Carnegie, P. R., Estimation of molecular size of peptide by gel filtration, Biochem. J., 95, 9 P (1965). Among those gels suitable for use in the gel filtration method but not limited thereby to these specific examples are Sephadex G- 25, Sephadex G-50, Sephadex G-75 and Sephadex G-100 which are a variety of cross-linked dextran available from Pharmacia Fine Chemicals. Also useful is Sepharose 6B, a bead-formed agarose gel available from Pharmacia.

The depressor substance which is removed from stable plasma protein fractions is a polypeptide having a molecular weight between 1,000 and 10,000. the depressor substance is believed to be generated primarily during the heating of the protein solution. However, we have found that this purified stable plasma protein fraction may thereafter be heated up to 60° C. for extended periods without generating additional depressor substance.

After contacting plasma protein fractions which contain this depressor substance with any one of the materials described herein for removing the depressor substance according to the present invention, viz., a surface active adsorbent, a cation exchanger, an ultrafiltration membrane or by gel filtration particles, the resulting purified, stable plasma protein fraction, which is substantially free of depressor substance can be rapidly infused intravenously into a patient without the danger of depressing the blood pressure, particularly in cases of heart-lung by-pass. As a result of the present invention, a purified stable plasma protein fraction is obtained, whose scope of clinical application is extended over that of previously obtainable plasma protein fractions.

The term "substantially free of depressor" as used herein means that the plasma protein fraction lacks true depressor activity, as distinguished from the volume effect which causes a nominal drop in blood pressure, when any liquid, including saline or isotonic solu-

tion, is injected. This effect can be distinguished by the dog isolated hind limb test, which detects only true depressor effect. The novel depressor-free plasma protein fraction products of this invention give a negative response in this test and in the smooth muscle rat uterus test for kinin and kinin-like substances.

It is believed that the depressor substance may be bradykinin or a kinin-like material, since it is well established that blood plasma contains kininogens capable of being converted to kinins when activated by a certain specific enzyme or enzymes. Treatment of plasma protein fractions with either surface active adsorbents or cation exchangers or by ultrafiltration or gel filtration results in the removal of any kinin or kinin-like substance, as evidenced by lack of significant depressor effect when the treated protein is subjected to testing on smooth muscle or perfused in the isolated hind limb of a dog or administered by systemic infusion in dogs. Contractions of the smooth muscle of a rat uterus is a highly sensitive test and quite specific for indicating the presence of kinin or kinin-like substances. The isolated hind limb test is a highly sensitive test for detecting depressor substances which cause dilation of the peripheral circulatory system thus producing a fall in blood pressure. Systemic infusion is an in vivo test which simulates effects on blood pressure following rapid infusion in a patient.

For a description of techniques for the fractionation of human plasma proteins with adsorbents, ion exchange resins, molecular sieve gel filtration and ultrafiltration, see H. E. Schutze and J. F. Hermans, "Molecular Biology of Human Proteins," Vol. I, pp. 285–303 (Elsevier. Pub., N.Y. 1966). For references to the removal of a kinin from blood with a cationic ion exchange resin, see M. E. Webster and J. P. Gilmore, Biochem. Pharm., Vol. 14, 1,161–**1,163** (1965); J. A. Bates, L. Gillespie and D. T. Mason, The Lancet, Vol. 70, 514–517 (1964); and J. V. Pierce and M. E. Webster, Biochem. and Biophys. Res. Comm., Vol. 5, No. 5, 353–357 (1961); ibid, with siliconized silica gel, H. Yoshida, K. Matsumoto, T. Nakajima and Z. Tamura, Chem. Pharm. Bull. 19(8) 1,691–1,695 (1971).

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Starting Materials - Plasma Protein Fraction, Human

A. From blood plasma, heat-treated at 60° C. for 10 hours.

Most of the experimental studies were conducted on non-homogeneous plasma protein fractions obtained by the process described in U.S. Pat. No. 2,958,628. Various lots containing 5% protein were used which by electrophoretic analysis contained at least 83 percent albumin and no more than 17 percent of alpha globulin and beta globulin. In addition, the 5 percent protein solutions were stabilized with sodium acetyltryptophanate (or N-acetyltryptophan) and sodium caprylate (0.004 M with respect to each) and also contained sodium chloride in amounts which rendered the solution slightly less than isotonic. The protein solutions had also been heated at about 60° C. for 10 hours to destroy any hepatitis virus.

B. From blood plasma, heat-treated at 60° C. for 2 hours.

The dried plasma protein powder reconstituted to a 5 percent protein solution in water and stabilized with 0.004 M quantities of sodium acetyltryptophanate and sodium caprylate, and also containing sodium chloride in an amount to make the solution slightly less than isotonic (as described in U.S. Pat. No. 2,958,628) was heated at 60° C. for about 1 to 4 hours, in this instance about 2 hours, rather than for 10 hours as in the case of Starting Material A. A small amount of flocculent precipitate generally formed. After cooling to about room temperature, the precipitate was removed by filtration and the clear solution was then ready for use.

C. From placenta.

Human placentas which were frozen immediately after each delivery were finely crushed. An extract from 20 kg. of this tissue with 40 liters of 1 percent sodium chloride solution yielded a precipitate of gamma globulin. The supernatant was mixed with butyric acid in amounts to make the solution 2–6 percent with respect to the acid. The pH was adjusted to 4.5 – 5.5, the solution was heated at 57°–60° C. for 1 to 2 hours, and the precipitate which formed was removed. Ammonium sulfate was added to the supernatant in amounts to provide a concentration of 35–40 percent. The precipitated protein was collected as paste and dialyzed against running water at 4° C for 24 hours. The resulting product was a non-homogeneous plasma protein fraction comparable to Starting Material A, above. Its solutions in water were adjusted to about pH 7 for ultrafiltration, gel filtration or cation exchanger treatment.

Methods for Testing Depressor Substances

A. Smooth Muscle Contractibility.

Smooth muscle contractibility was measured by the method of Magnus (Trautshold, K., Handbook of Experimental Pharmacology, Vol. XXV, p. 55, Springer-Verlag, New York, 1970). A 1.5 cm. strip of uterus muscle was isolated from a virgin Wistar rat weighing about 150 g. The strip was suspended in 8.6 ml. de Jalon solution saturated with air and containing 0.1 mg. percent of atropine sulfate. Kymograph recordings of the contractile forces were made before and for 90 seconds following the addition of 0.4 ml. of the test solution.

B. Blood Pressure Following Systemic Infusion of Test Solution

Male dogs of about 8 kg. body weight were anesthetized by intramuscular injection of urethane (2.2 g./kg.), and the left carotid artery was cannulated for recording arterial pressure with a polygraph transducer. The 5 percent test solution was administered through a cannula inserted in the right femoral vein at a dose of 125–214 mg. of protein/kg. and at a rate of 18–30 ml./minute. A depressor effect was expressed as a percentage decrease of the mean arterial pressure following infusion as compared to the pressure before infusion, i.e.,

$$\% \text{ Decrease} = \frac{\text{Mean arterial pressure before infusion} - \text{Mean arterial pressure after infusion}}{\text{Mean arterial pressure before infusion}} \times 100$$

C. Blood Pressure Following Infusion of Test Solution in the Isolated Hind Limb.

Dogs weighing 15 to 20 kg. were anesthetized with thiopentalchloralose (20 and 60 mg./kg., respectively). The right femoral vein was isolated for injection of maintenance anesthetics and the right femoral artery was cannulated for pressure recording on a polygraph. About 6 cm. of the left femoral artery was exposed and tied midway. A loop of rubber tubing was connected by way of polyethylene catheters at each end to the tied artery, one catheter being placed well into the portion of the artery leadidng to the heart and the other catheter being placed about 6–10 cm. in the direction leading toward the limb extremity. A Sigma motor pump was connected directly into the loop below the first named catheter and a pressure transducer was connected into the loop on the peripheral side of the pump. Heparin (10 mg./kg.) was injected I.V. and after 30 minutes, the pump was started and adjusted so that the pressure of the blood leaving the pump to flow into the limb essentially matched the arterial pressure recorded from the right femoral artery. Test solutions of 5 percent plasma protein at a total dose of 250 mg. of protein (5 ml.) were infused into the artery of the isolated hind limb at a rate of 1 ml./5 seconds. A Sigma motor pump which provides a substantially constant pulsatile flow was used so that base lines in the pressure recordings could be determined more accurately. A base line is that figure obtained by adding to the diastolic pressure one-third of the difference between the systolic and diastolic pressures. Decreases following the infusion of the test solutions were expressed as the actual difference in mm. Hg. between the base line before infusion and the base line after infusion.

This isolated hind limb procedure is more sensitive than the systemic infusion method (above) and will show direct effects of depressor substances on arterial pressure as a result of changes in peripheral resistance.

Removal of Depressor Substance

A. Surface Active Adsorbent.

Example 1

A 5 percent solution of heat-treated plasma protein fraction (Starting Material A) was passed through a column of Merck H silica gel previously equilibrated with 0.25 percent sodium chloride. The flow rate of the protein solution was 50–150 ml./hr./cm². About 95 percent of the protein was recovered in the effluent as determined by optical density at 280 nm. in a Hitachi Spectrophotometer. By electrophoretic analysis, the final product comprised 88.5 percent albumin, 7.5 percent alpha globulin and 4.0 percent beta globulin.

	Depressor Substance Method A	Method B
Starting Material A	Strong Contraction[1]	11.5%[2]
Product of Example 1	No Contraction	0.0%

[1] Contraction was slightly greater than that obtained by 10 ng. of Bradykinin.
[2] Dose of 187 mg. protein/kg. at a rate of 18 ml./min.

Silica gel effectively removed depressor substance from non-homogeneous plasma protein.

Example 2

100 ml. of heat-treated plasma protein solution (Starting Material A) was stirred gently at about 25° C. with 2.0 g. silica gel (Aerosil 200) for 5 hours, then centrifuged and the clear supernatant solution was tested for depressor substance.

	Depressor Substance - Method C			
	Before Infusion	After Infusion	No. Determinations	Avg. Decrease
Starting Material A	161	78	7	83
Product of Example 2	148	115	4	33
Saline Control	—	—	9	16

These results indicated the effectiveness of silica gel in a batchwise treatment to remove depressor substance from plasma protein.

Example 3

100 ml. each of Starting Material A was stirred with 1.0 g. and 2.0 g. silica gel (Aerosil 200) for 4 hours at room temperature. The treated solutions were then tested for depressor substance.

	Depressor Substance Method C				Method A
	Before	After	Av. Decrease	No. Detn.	Contraction, mm.
Starting Material A	148	78	70	2	33.5
Treated Product 1% Silica gel	138	98	40	2	4.0
Treated Product 2% Silica gel	152	115	37	2	0.0
Saline Control	—	—	14	14	
Bradykinin (1.0 mcg.)			69	2	34.0 (40 ng./ml.)

The results show a ratio of 1 to 5 of silica gel to protein is about as effective for removing depressor substance as a ratio of 1 to 2.5.

Example 4

A 100 ml. portion of heat-treated plasma protein solution (Starting Material B) was stirred gently with 2 g. of silica gel (Aerosil 200) at room temperature for 3 hours. The silica gel was removed and a portion of the solution was heated at 60° C. for 11 hours.

	Depressor Substance				
	Method A Contraction, mm.	Method C			
		Before	After	Av. Decrease	No. Detn.
Starting Material B	11.5	166	104	64	2
Silica gel treated product	1	167	139	28	2
Silica gel treated product (heated)	0	167	133	34	2

The results show depressor substance is effectively removed from plasma protein heated for relatively short periods of time and that prolonged heating of plasma protein which has been treated previously with silica gel to remove the depressor substance does not generate additional depressor substance.

Example 5

A 100 ml. of heat-treated plasma protein solution (Starting Material A) was stirred gently with 5 g. of a 2.6 percent suspension of aluminum hydroxide for four hours at 25° C. The mixture was centrifuged, then filtered, and the clear solution was tested for depressant substance. The composition of the protein after aluminum hydroxide treatment was essentially unchanged from that of the starting material with respect to albumin, alpha and beta globulins as determined electrophoretically.

	Depressor Substance - Method C Av. Decrease
Starting Material A	52
Product of Example 5	28

The results show the effectiveness of aluminum hydroxide in removing depressor substance from plasma protein.

Example 6

100 ml. portions of plasma protein solution (Starting Material A) were treated as follows with the indicated results:

Sample	Silica Gel Adsorbent	Amount	Time	Temp., °C.	Method C Av. Decrease
A	Merck H	3 g.	45 min.	5	22
B	Aerosil 200	2 g.	45 min.	5	25
C	Aerosil 200	2 g.	4 hours	25	26
Starting Material A					52

The results show Merck H Silica gel to be as effective as Aerosil 200 in removing depressor substance and that time or temperature do not materially affect the removal of depressor substance by silica gel.

B. Cation Exchanger

Example 7

A 5 percent protein solution (Starting Material C) was allowed to pass through a column of carboxymethyl Sephadex which had previously been equilibrated with 0.05 M phosphate buffer containing 0.1 M sodium chloride. The flow rate was between 50–150 ml./hr./cm^2. The column was then washed with the same buffer-saline solution and the effluent and wash solution were combined. About 90 percent of the protein was recovered as determined by optical density at 280 nm. on this mixture.

	Depressor Substance	
	Method A	Method B
Starting Material C	Strong Contraction	Large Depression
Product of Example 7	No Contraction	No Depression

The results show a weak cation exchanger effectively removes depressor substance from plasma protein.

Example 8

100 ml. of a 5 percent plasma protein solution (Starting Material A) was stirred gently with 5 g. of moist Dowex 50-X2 (freshly regenerated with dil. NaOH, then dil. HCl) for 3 hours at room temperature at pH 7.2. The resin was filtered off and the solution showed 85 percent recovery of protein as determined by optical density at 280 nm.

	Depressor Substance - Method C			
	Before Infusion	After Infusion	No. Determinations	Av. Decrease
Starting Material A	161	78	7	83
Product of Example 8	161	110	3	51
Saline Control	—	—	9	16

The results show depressor substance is removed from plasma protein by treatment with a strong cation exchange resin.

C. Ultrafiltration

Example 9

A 5 percent plasma protein solution (Starting Material C) was ultrafiltered with a UM10 ultrafiltration membrane (Amicon) until essentially all the low molecular weight substances had passed into the filtrate. The material which did not pass through the membrane represented a recovery of about 98.9 to 99.4 percent of the protein.

	Depressor Substance Method A	Method B
Starting Material C	Strong Contraction	19.1
Product of Example 9	No Contraction	0.0

The results indicate ultrafiltration of plasma protein solution effectively removes depressor substance. The filtrate obtained in the above experiment was tested by Method A and produced strong contractions, indicating the depressor substance had a molecular weight below 10,000.

D. Gel Filtration

Example 10

A column 5 × 50 cm. filled with Sephadex G-50 was charged with 70 ml. of a plasma protein solution (Starting Material C) and eluted with 0.5 M sodium chloride solution at a rate of 4 ml./min. Ten milliliter fractions were collected and assayed for protein content by optical density at 280 nm. The first 300 ml. of eluate contained essentially all the protein. This 300 ml. portion was concentrated to 5 percent protein solution, additional N-acetyl-tryptophan and sodium caprylate were added to bring the respective concentration of each to 0.004 M, and the solution was heated at 60° C. for 10 hours. When tested by Method A, this final solution showed no depressor substance whereas the eluates collected from 580 to 730 ml. contained all the depressor substance. The Starting Material C produced strong contractions of the uterine strip comparable to that produced by 10 ng. of Bradykinin. This experiment demonstrates that depressor substance can be removed from plasma protein by gel filtration.

The preceding examples can be repeated with similar success by substituting the generically or specifically described reactants and/or operating conditions of this invention for those used in the preceding examples.

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

What is claimed is:

1. A sterile aqueous solution of an electrophoretically non-homogeneous, human, heat-stable plasma protein fraction suitable for rapid intravenous infusion, substantially free of depressor effects.

2. The solution of claim **1**, stabilized with a stabilizer selected from the group consisting of sodium acetyltryptophanate, N-acetyltryptophan and sodium caprylate.

3. The solution of claim 1 wherein the protein concentration is about 5 percent.

4. The solution of claim **3** wherein the protein comprises at least 83 percent albumin and no more than 17 percent of alpha globulin and beta globulin.

5. The solution of claim **1** wherein the plasma protein fraction consists essentially of a mixture of 85–92 percent albumin, 4–10 percent globulin and 2–7 percent beta globulin.

6. The solution according to claim **5**, heat-treated until free of infective hepatitis virus.

7. A process for the production of an electrophoretically non-homogeneous plasma protein solution which is substantially free of depressor substance so that the solution may be rapidly infused intravenously without causing a significant fall in blood pressure, which comprises the step of contacting a solution of an electrophoretically non-homogeneous human plasma protein containing depressor substance with a material selected from the group consisting of surface active adsorbents, cation exchangers, ultrafiltration membranes and gel filtration molecular sieves which removes the depressor substance from the protein. membranes and gel molecular sieves.

8. The process of claim **7** wherein the proteins of the starting protein solution are a mixture consisting of at least 83 percent albumin and not more than 17 percent alpha-globulin and beta-globulin.

9. The process of claim **8** comprising the step of heating the starting stable protein solution prior to contacting the protein solution with the material which removes the depressor.

10. The process of claim **9** wherein the heating step is conducted for about 1 to 4 hours.

11. The process of claim **10** comprising the step of heating the protein solution after the removal of the depressor therefrom at about 60° C. for a period of time of at least 10 hours effective to destroy any hepatitis virus therein.

12. The process of claim **7** wherein the depressor is removed by contacting the starting solution of nonhomogeneous human plasma protein containing a depressor with a surface active adsorbent.

13. The process of claim **12** wherein the surface active adsorbent is selected from the group consisting of silica gel and aluminum hydroxide gel.

14. The process of claim **13** wherein the starting plasma solution containing the depressor is a heat-treated mixture of at least 83 percent albumin and not more than 17 percent alpha-globulin and beta-globulin.

15. The process of claim **13** wherein the surface active adsorbent and the solution containing the depressor are mixed in a batchwise operation.

16. The process of claim **13** wherein the plasma solution containing the depressor substance is passed through a column of the surface active adsorbent.

17. The process of claim **15** further including the step of separating the surface active adsorbent from the mixture by centrifugation or filtration.

18. The process of claim **7** wherein the depressor is removed by contacting the starting solution of nonhomogeneous human plasma protein containing a depressor with a cation exchanger.

19. The process of claim **18** wherein the cation exchanger is a member of the group consisting of sulfonated polystyrene cross-linked with divinylbenzene, carboxymethyl cellulose, and cross-linked dextran having terminal carboxymethyl groups.

20. The process of claim **7** wherein the depressor is removed by contacting the starting solution of nonhomogeneous human plasma protein containing a depressor with gel filtration molecular sieve.

21. The process of claim **20** wherein the gel filtration particles are selected from the group consisting of cross-linked dextrans and agarose gels having the capacity for entrapping substances with molecular weights below about 10,000.

22. The process of claim 7 wherein the depressor is removed by filtering the starting solution of non-homogeneous human plasma protein containing a depressor with an ultrafiltration membrane which will allow the depressor substance to pass through the membrane into the filtrate but prevents the passage of substantially all of the desired plasma proteins.

23. The process of claim 7 which comprises the steps of

a. heating a solution of a non-homogeneous stable human plasma protein fraction at about 60° C.,
b. contacting the heated solution with a material selected from the group consisting of surface active adsorbents, cation exchangers, ultrafiltration membranes and gel filtration molecular sieves.

24. The process of claim 23 comprising the step of cooling the solution prior to step b).

25. The process of claim 24 comprising the step of separating any precipitate from the cooled solution.

26. The process of claim 25 comprising heating the solution in step a) for about 1 to 4 hours and again heating the cooled solution, for about 10 hours at about 60° C.

27. The process of claim 26 wherein in Step b) the material is a surface active agent selected from the group consisting of silica gel and aluminum hydroxide gel.

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UNITED STATES PATENT OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 3,876,775
DATED : April 8, 1975
INVENTOR(S) : Izaka et al

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

Column 4, line 41, change "the" to --The--.

Column 7, line 12, change "leadidng" to --leading--.

Column 11, line 64, change "4-10 percent globulin" to --4-10 percent alpha globulin--.

Column 12, lines 12 and 13, after the period, delete "membranes and gel molecular sieves."

Signed and sealed this 15th day of July 1975.

(SEAL)
Attest:

RUTH C. MASON
Attesting Officer

C. MARSHALL DANN
Commissioner of Patents
and Trademarks