Blut, Band 30, Seite 121–134 (1975)

German Red Cross Blood Transfusion Service Nordrhein-Westfalen
Hagen Institute (Director: Dr. W. Schneider)
Münster Institute (Director: Dr. H. Fiedler)

An Alternative Method of Large Scale Plasma Fractionation for the Isolation of Serum Albumin

Waldemar Schneider, Hans Lefèvre, Harald Fiedler and Lee J. McCarty

Summary

Human plasma may be separated into five fractions using the method described by *Cohn* in 1946. Although there are several drawbacks to alcohol precipitation, especially in albumin isolation, it is still used throughout the world. This paper describes an alternative procedure for albumin isolation from plasma or albumin-containing plasma fractions using a combined heat fractionation/polyethylene glycol precipitation method. No polyethylene glycol is detected in the final product which is immunoelectrophoretically 100% pure, salt poor, heat resistant during pasteurization, and stable during long-term room temperature storage. The yield is at least 90% of the original plasma albumin. In comparison with the Cohn method, fractionation time and expense are significantly reduced.

Zusammenfassung

Menschliches Plasma kann mittels der 1946 von *Cohn* und Mitarbeitern beschriebenen Methode in fünf Fraktionen zerlegt werden. Trotz zahlreicher Nachteile bei der Proteinpräzipitation mit Alkohol wird das Cohnsche Fraktionierungsverfahren noch heute in der ganzen Welt verwandt. Die vorliegende Arbeit beschreibt eine Alternative zur Isolierung von Albumin aus Plasma oder plasmahaltigen Fraktionen mittels einer kombinierten Hitze/PEG-Fraktionierungsmethode.

Im Endprodukt, das immunelektrophoretisch zu 100% aus Albumin besteht, salzarm und hitzestabil ist, ist PEG nicht nachweisbar. Die Ausbeute beträgt mindestens 90% des urprünglich vorhandenen Albumins. Im Vergleich zur Cohn-Methode sind Fraktionierungszeit und Kosten erheblich geringer.

Blood component therapy is finally coming into its own in modern medicine, replacing the less effective and more dangerous treatment with stored whole blood. In addition to being a waste of the basic material, whole blood has the highest risk of causing transfusion reactions, immunizations, and disease transfer, particularly hepatitis [*8,26,27,40*]. On the other hand, by administering blood components these risks are either completely avoided (e. g. human serum albumin), diminished (e. g. buffycoat free packed red cells), or, because of the highly increased therapeutic effectivity of some components, justified (e. g. pooled clotting factor concentrates).

The *Cohn* method developed in 1946 [*6,7*] has world-wide acceptance for the technical fractionation [*16,21*] of plasma proteins to be used for human therapy. Human plasma is separated into five fractions by using different concentrations of

---

Eingegangen am 10. 6. 1974/43.

ethanol, employed as a non-toxic protein precipitant, while simultaneously changing the pH with buffers of different ionic strengths. The concentration of alcohol increases from about 8% to 40%; the pH varies from 7.2 to 4.6. The ethanol can subsequently be easily removed by freeze-drying, but the disadvantage lies in the fact that it readily denatures proteins, especially when used in high concentrations.

This disadvantage may be reduced by lowering the temperature to between —3° and —7° C during fractionation. To accomplish this for large scale fractionation, either the entire process must take place in a cold room (an uncomfortable and unhealthy situation for the personnel), or self-cooling machinery must be used. In addition to this, all electrical equipment must be specially protected when working with large volumes of alcohol.

The first Cohn fractionation step removes mainly fibrinogen (Fract. I); the second-third, gammaglobulin (Fract. II-III); the fourth, alpha- and beta-globulins (Fract. IV). The remaining supernatant contains albumin which is precipitated with 40% ethanol as Fract. V. An additional purifying precipitation is required if high albumin concentrations (20%) are desired (Fract. VI).

Fract. V albumin paste (crude or refined) must be lyophilized to remove the ethanol. The powder is solved, the solution is cleared through filtration, and the pH, osmolality, and protein concentration are adjusted. Also, aliphatic carbonic acids (e. g. caprylic acid) must be added to protect the protein from heat denaturation during pasteurization [*1,3,4*]. Caprylic acid is most often used for stabilization usually in concentrations between 0.004 M (5% albumin) and 0.04 M (20% albumin).

With the exception of albumin, there are two other Cohn fractions which may be processed for patient administration: fibrinogen and gammaglobulin.

Fibrinogen prepared from large plasma pools should not be used because of the tremendously high risk of hepatitis transmission. Too, there are only rare indications for the administration of isolated clotting Factor I (fibrinogen). For all cases *where fibrinogen must be used, small pool products (e. g. cryoprecipitate,* which also has a high degree of Factor VIII activity) *should be given because there is much less danger of hepatitis transmission* [*2,14,17,32,38*].

With the exception of specific hyperimmune globulins, little demand presently exists for Cohn Fraction II gammaglobulin.

Generally speaking, then, the original five-step Cohn method isolates four useless fractions, thus making production of the fifth, albumin, more and unnecessarily expensive.

Consequently, less expensive methods for albumin fractionation have always being looked for. Cost reduction is minimal in the *Cohn* method by separating and discarding Fraction I-III in one step [*16*], as well as with automatic fractionation [*39*], for a great deal of the expense lies with the refrigeration systems which cannot be eliminated. Column fractionation has been tried [*11,20,23,36,37*], but, as well as causing pyrogenic problems, it is neither economic nor useful for large scale fractionation. In 1964 *Polsen* et al. [*24*] examined various protein precipitants and concluded that polyethylene glycol (PEG) was the optimal protein precipitant for preparation of products for human administration. In subsequent extensive studies PEG was proved to be non-toxic and non-pyrogenic [*5,15,25,33–35*]. As a result, PEG is now used in preparation of gammaglobulin [*24*] and clotting factor concentrates (e. g. high-purity Factor VIII) [*19*].

Although PEG could logically be used in albumin fractionation also, problems arise because the optimal precipitation with PEG occurs at room temperature [*24*]. In contrast to prothrombin complex and Factor VIII preparation methods which take only a few hours, conventional cold ethanol albumin fractionation takes approximately one week. If alcohol were replaced by PEG as a precipitant at room temperature in an otherwise unchanged Cohn method, one would run a great risk of additional bacterial growth in the fractionation material, resulting in pyrogenic products. This may be one of the reasons PEG has not replaced ethanol in Cohn albumin fractionation.

Our alternative to the Cohn method

In our search for an alternative to the Cohn method, we were looking for a procedure which filled the following requirements: minimum initial investment in equipment, low operating costs, minimum of time required for production, greatest purity of the product, highest yield of the initial plasma albumin, and maximum comfort and safety for our personnel.

Helpful to us was the observation of *Gabr* et al. [*9*] that caprylate added to plasma protects albumin during heating up to +70° C, while most of the globulins are denatured and precipitated.

Principle

The principle behind our fractionation method may be outlined:

1. *Plasma fractionation:* Plasma (or albumin-containing plasma fractions) protected by sodium caprylate is heated. During this step the globulins are precipitated while the albumin remains in solution.
2. *Fraction separation:* The precipitated globulins are sedimented by centrifugation.
3. *Albumin precipitation:* Albumin remaining in solution is concentrated by PEG precipitation; salt content is greatly reduced.

Equipment

A. Pressure proved stainless steel container which is directly heated electrically, thermostatically controlled, and equipped with a magnetic stirrer (Kniese, Marburg),
B. Double-walled pressure proved stainless steel containers each equipped with an electric stirrer (Seitz, Bad Kreuznach),
C. Digital pH-meter connected to an automatic print-out (WTW, München),
D. Seitz filters: Pilot and EF 30,
E. Continuous flow centrifuges (C 61 H, Padberg, Lahr),
F. Peristaltic pumps,
G. Membrane filter (293, Sartorius, Göttingen),
H. Laminar flow sterile cabinet (CEAG, Dortmund),
I. Pasteurizing water bath (Coesfeld, Dortmund),
J. (Pan) freeze-dryer for high concentration albumin preparation (Kniese, Marburg),
[K. Ultra-Cryostat UK 40 DL (Meßgerätewerk, Lauda/Tauber)].

Reagents

Sodium caprylate (Roth, Karlsruhe); PEG 4000 (Union Carbide); Glucose, pyrogen free (Roth, Karlsruhe); 0.5N HCl; 0.2N NaOH; 96% Ethanol; Distilled water, pyrogen free.

Procedure

Plasma fractionation

For albumin fractionation we use plasma which has had the clotting factors previously removed: Factor VIII/fibrinogen by cryo- (3%) ethanol precipitation (Factor VIII later isolated, fibrinogen discarded), and prothrombin complex by DEAE cellulose adsorption [*26, 29*]. The initial plasma is HBAg negative, has normal transaminase values, and contains

no visible hemoglobin. For albumin fractionation only, we also use plasma from HBAg positive units, units with pathological transaminases, and slightly hemolytic plasma.

Sodium caprylate is added to the plasma until a concentration of 0.004M is obtained. This caprylate-stabilized plasma is heated in the Kniese container together with ethanol (final plasma alcohol concentration is 9%) after the pH has been adjusted to 6.5 with 0.5N HCl. The plasma is continually stirred during the heating process. The heating is thermostatically controlled, and when a plasma temperature of +68° C is reached, the plasma is transferred by compressed air to a Seitz container which is connected to a cooling system. The plasma is stirred until the temperature is brought down to the desired +10° C, at which time the pH is lowered to 4.4 with HCl. This acidified plasma may now be stored overnight (at +10° C).

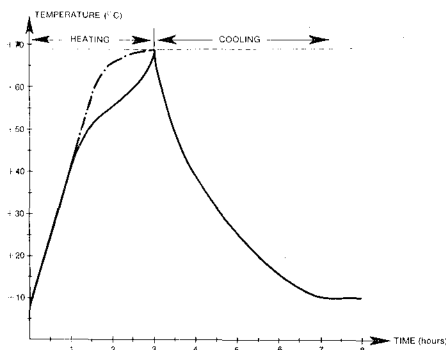

Table 1: Temperature curve of the heating and cooling procress.

Table 1 shows the heating and cooling process temperature curve. Approximately three hours are needed for the plasma to reach a temperature of +68° C, regulating the thermostat in the manner we do. During heating there are two different ways of doing this so that +68° C is arrived at the shortest time, without albumin denaturation. Cooling takes about another four hours using our cooling system. We regulate the temperature in both the cooling and heating process such that the time between +30 and +40° C is as short as possible, thus giving bacteria little chance to grow. (Note: If there is no opportunity or equipment for the rapid cooling of the plasma, one should lower the pH after heating to pH 4.4 while the plasma temperature is higher than +40° C: there should be no bacterial growth at this low pH.)

Tables 2 and 3 show the results of studies designed to determine at which pH and temperature albumin recovery and purity were optimal, using 0.4M sodium caprylate as a basis.

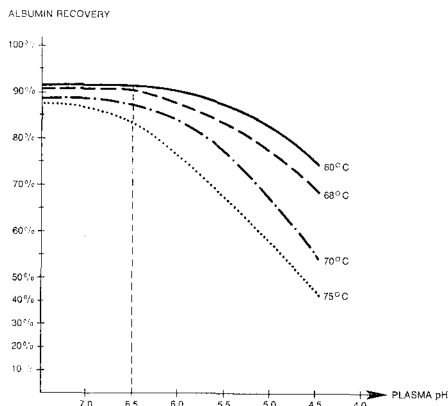

Table 2: Relation of plasma temperature and pH to albumin recovery.

The maximum yield with 100% purity was obtained when heating the plasma to +68° C at pH 6.5. With pH and temperature thus constant, the minimum sodium caprylate concentration giving the maximum protection and therefore the greatest albumin recovery was determined. As seen in Table 4, this concentration is 0.004M.

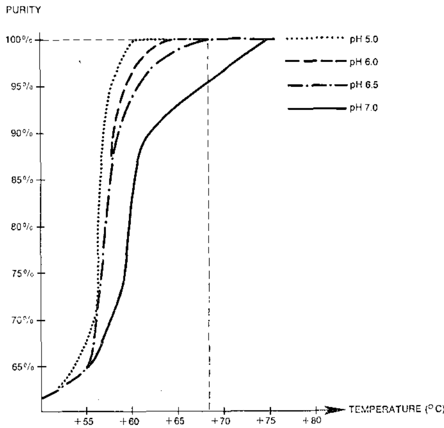

Table 3: Relation of plasma temperature and pH to albumin purity.

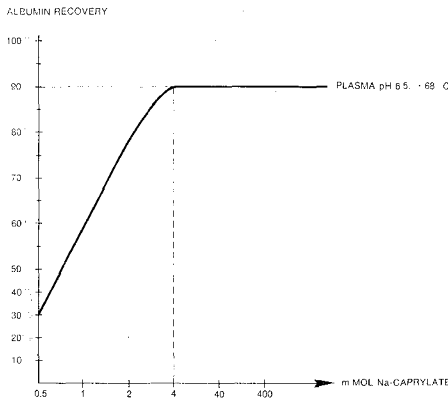

Table 4: Relation of sodium caprylate concentration to albumin recovery when heating plasma to+68°C at pH 6.5.

Fraction separation

The cooled acidified plasma is transferred to continuous flow centrifuges using silicon tubes and peristaltic pumps. The precipitated proteins are sedimented in the rotors, while the albumin remains in the supernatant as an approximately 2.5% solution. This supernatant is collected from the centrifuges in separate containers. The yield at this point in the procedure is about 70% of the albumin available in the original plasma. By eluting the sediment with distilled water at pH 4.8 and recentrifuging, another 20% albumin can be recovered.

Albumin precipitation

The supernatant (pH 4.8) is passed through SSK filters (Seitz-Pilot) to remove remaining lipids and denatured proteins before the precipitant is added. We use PEG 4000 in a concentration of 22%. Instead of PEG any other suitable protein precipitant may be used. It is added directly to the supernatant which now has a temperature of about +16° C. After the PEG is in solution, we find 30 min. to be adequate for albumin precipitation.

Table 5 shows the relationship between PEG concentration and albumin precipitation at different pH at a temperature of +15° C. One sees the minimum PEG concentration needed for 100% albumin precipitation is 20% at pH 4.8.

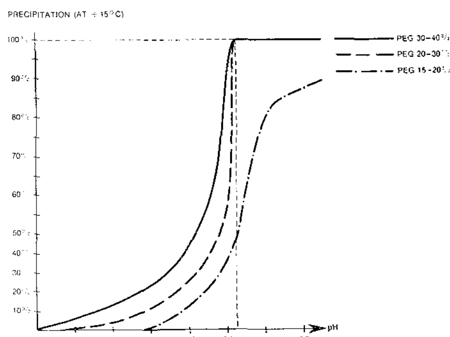

Table 5: Percent albumin precipitation obtained by using different PEG concentrations at different pH.

The PEG/albumin suspension is transferred by peristaltic pumps to continuous flow centrifuges. While PEG and salts remain in the protein-free supernatant, the albumin collects in the rotors as a paste. This paste is transferred to a container where it is resolved in pyrogen free distilled water (four kilograms of water added for each kilogram of paste) giving an approximate 8% albumin concentration. Resolving takes between two and three hours.

The pH is then raised with 0.2N NaOH to pH 6.8, and a four-step Seitz filtration takes place using EF 30 filters in the following order: SSK, EK, EKS, and EKS-1. The albumin may now be processed into low (5%) or high (20%) concentrations.

For the 5% solution, the concentration is corrected with pyrogen free distilled water, and the osmolality is adjusted to approximately 300 mosm with pyrogen free glucose. Under a laminar flow cabinet, the albumin is bottled directly from the sterile filtration (Sartorius membrane filter, pore size 0.22 μ). The bottled product is pasteurized for ten hours in a +60° C water bath.

One would perhaps assume the 20% solution could be made by merely solving the albumin paste in less water. However, we have found this to be technically undesirable. Therefore, for high albumin concentrations we freeze-dry the solved albumin after the four-step filtration. (Note: In this case, the last filtration step, EKS-1, may be omitted.) The lyophilized powder is easily reconstituted, and we filter this solution through a Seitz EF 30 EKS-1, after which the concentration is corrected to 20%. Sterile filtration, bottling, and pasteurization follow, as for the 5% preparations.

Table 6 is a flow chart of our entire fractionation process.

Laboratory controls

While the tests 1 to 3 were done for research purposes during the development of our method, tests 4 to 12 are run on all finished products.

1) HBAg by radioimmunoassay (RIA),
2) Polyacrylamide gel electrophoresis (PAA),
3) Final PEG concentration with Nessler's reagent,
4) Protein (Biuret), Digital spectral photometer (Leitz, Wetzlar),
5) Electrolytes: Na, K, Ca, Digital atomic absorption photometer (Philips, Hamburg),
6) Glucose (o-toluidine), Digital spectral photometer (Leitz, Wetzlar),
7) Osmolality, Osmometer (Knauer, Berlin),
8) Cellulose-acetate electrophoresis (CAE),
9) Immunoelectrophoresis (IEP),
10) Sterility,
11) Pyrogenicity (Dr. M. Kienholz, Aschaffenburg, Assoc. Prof., University of Giessen),
12) Stability (nephelometric density), Digital spectral photometer (Leitz, Wetzlar).

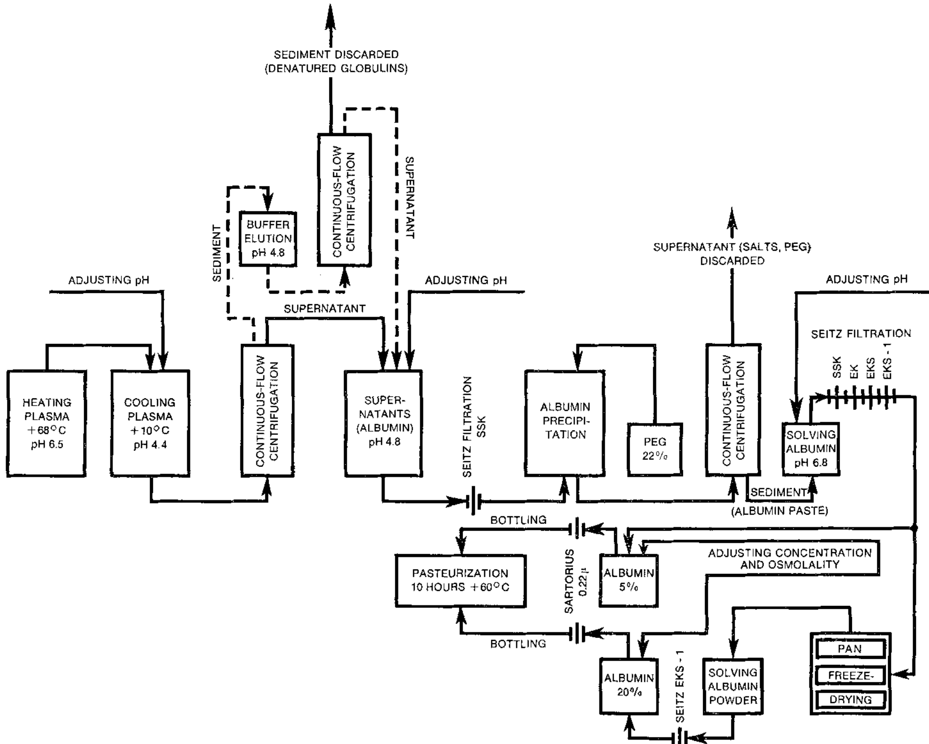

Table 6: Flow chart of the combined heat fractionation/PEG precipitation method. Entire process from initial heating to final pasteurized albumin.

Results

It has been proven that HBAg can be detected in all Cohn fractions with the exception of Fract. II (gammaglobulin) [*12,13,31*]. There has been no case reported, however, where a post-transfusion hepatitis has occurred as the result of (pasteurized) albumin administration: during the heating process the infective agent must be inactivated [*10,18,22*].

During the fractionation of a 200 l batch of plasma from HBAg positive donors, all steps of our fractionation process were tested for HBAg using RIA to follow the course of the antigen and to determine if the antigen remains in our final product. The results of this study will be reported [*28*], but we would like to state here that as in Cohn-fractionated albumin, HBAg was demonstrated in the final product.

Various preparations were examined by PAA electrophoresis. In all samples tested only one band was detected with the identical position of normal human serum albumin.

As the concentration of PEG in the final albumin preparations is calculated to be 0.00007 gram PEG per gram albumin, we were unable to detect any using Nessler's reagent.

While in the Cohn procedure caprylate is usually added in concentrations between 0.04 and 0.004 M at the end of the fractionation after resolving the albumin paste, in our method it is added to the starting plasma in a concentration of 0.004 M only. During our fractionation process, a loss of caprylate is to be expected, thus increasing the binding capacity of the albumin.

The nephelometric density is estimated to prove the stability of the product by comparing the results of samples run both before and after pasteurization, as well as after long-term storage. Excellent stability has always been observed in both the 5% and the 20% solutions, and they remain stable during room temperature storage.

There are no sterility problems using a membrane filter with a 0.22 μ pore size.

Up to the present time 98 batches have been produced with pyrogenicity never having been demonstrated. The temperature rises produced by rabbits after having been injected with our albumin may be seen in Table 7.

Albumin batches	1	2	3	4	5	6	7	8	9	10
5%	0,18	0,09	0,38	0,01	0,29	0,20	0,25	0,25	0,21	0,01
20%	0,00	0,13	0,00	0,00	0,14	0,14	0,05	0,26	0,12	0,20
Albumin batches	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
5%	0,01	0,34	0,09	0,16	0,00	0,20	0,41	0,29	0,26	0,10
20%	0,15	0,00	0,01	0,02	0,12	0,00	0,25	0,12	0,00	0,10
Albumin batches	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
5%	0,00	0,12	0,21	0,02	0,05	0,02	0,09	0,12	0,00	0,21
20%	0,21	0,05	0,20	0,16	0,12	0,08	0,12	0,04	0,16	0,30

Table 7: Temperature rises produced by injecting rabbits with several batches of our albumin (dosage = 3 ml albumin/kg rabbit). Each value is the sum of the squares of the temperature rises in three rabbits.

Test	Albumin solutions 5%	20%
Protein	5% (± 0.05)	20% (± 0.02)
Osmolality	300 mosm (± 10)	450 mosm (± 10)
Glucose	3%	
Electrolytes		
Na+	26.0 mVal/L (± 2.5)	
K+	0.17 mVal/L (± 0.06)	
Ca++	0.68 mVal/L (± 0.1)	
Purity		
CAE	100%	
IEP	100%	

Table 8: Laboratory Control Test Results.

Results of the other routine laboratory tests are summarized in Table 8. One should note especially the very low electrolyte values.

Immunoelectrophoresis (IEP) results may be seen in Fig. 1. Antisera in all cases were polyvalent anti-human sera produced in horses.

While Fig. 1 a and 1 c are samples of the basic starting plasma in each case, Fig. 1 b and 1 d show IEP of our 5% and 20% solutions, respectively. One sees clearly the products are 100% pure albumin.

The solution after albumin-globulin separation/SSK filtration, and the supernatant after PEG precipitation are seen in Fig. 1 e and 1 f. Note the albumin is already pure after SSK filtration. The PEG supernatant is protein free.

Fig. 1 g and 1 h illustrate commercial albumin from two different firms: in addition to albumin, alpha- and betaglobulins are also present. As far as we know, Cohn fractionation was used in producing both.

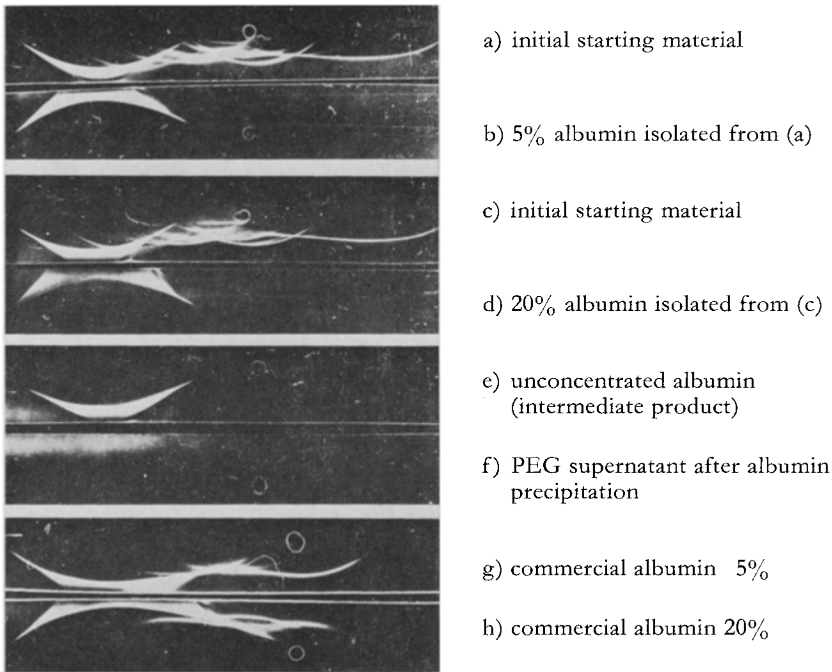

Fig. 1: Immunoelectrophoresis patterns of various materials run against polyvalent anti-human serum (horse).

Discussion

In comparison with Cohn fractionation, our method has the following advantages leading to significant cost reduction:

1) There is no necessity for cooling equipment
2) Working conditions for personnel are improved
3) The lack of protein denaturation results in an increased yield

4) Freeze-drying is only necessary in production of high concentrations
5) Over-all production time is greatly reduced

Although we use cooled containers to reduce the temperature after initial heating, they are not necessary, but serve only to shorten the cooling time, and therefore the fractionation process. As the entire fractionation process may be done at room temperature, there is no need for cold fractionation rooms or refrigerated continuous flow centrifuges, which always present a problem since a constant temperature cannot be maintained throughout the procedure. It is not only working under cold room conditions, but also handling the cold tools and equipment (i. e. rotors) that causes personnel discomfort when fractionating following the Cohn method.

Even under cold conditions, there is still some alcohol protein denaturation which contributes to reducing the Cohn albumin yield to only 60% to 70%. There is virtually no loss of albumin due to denaturation in our process. Our final albumin recovery is approximately 90% using an additional elution step, but the yield may be theoretically increased to 100%. This requires, however, further elutions, which become less effective with each additional step.

The addition of 9% ethanol before heating must be discussed, as we know alcohol is capable of denaturing proteins. We have found that the alcohol added in this concentration at this step does not reduce albumin recovery, but helps to pack the globulin sediment, thus reducing filtration problems.

Freeze-drying or vacuum distillation is an unavoidable step in the Cohn method, while it is necessary in our procedure only when preparing albumin in concentrations higher than 15%. In addition, when preparing albumin in high concentrations (20% to 25%), the second precipitation step for refining the albumin paste required in Cohn fractionations (which decreases the yield still further) is not necessary using our method.

With our present method and facilities, a batch of plasma takes three days to fractionate from the starting material to the bottled product:

Day 1: Plasma (or albumin-containing plasma fractions) heating, cooling, and acidifying to pH 4.4
Day 2: Separation of globulins, precipitation of albumin, and solving the albumin paste
Day 3: Filtration and bottling; pasteurization

By working on a shift basis, the production time can be significantly reduced. As seen in Tables 9 and 10, our entire fractionation procedure takes about half the time the Cohn method does. Also, Cohn fractionation requires many (necessarily complicated) changes of temperature, pH, and ethanol concentration, while our method is much less envolved, needing only two changes of temperature and pH and only one addition of the precipitant PEG (Table 11):

Although the combined heat fractionation/PEG precipitation method is economically optimal for the production of albumin, one must consider that no gammaglobulin can be prepared by this procedure.

However, if it is also desirable to isolate the gammaglobulin, one possibility we have tried is to separate the fibrinogen and gammaglobulin with PEG in the beginning [*30*]. To avoid heating a supernatant containing PEG, all the remaining protein

in the supernatant must be precipitated by increasing the existing PEG concentration. After this, the paste must be resolved to a concentration of about 6% protein. The material may then be fractionated as described. It is also possible to combine equal

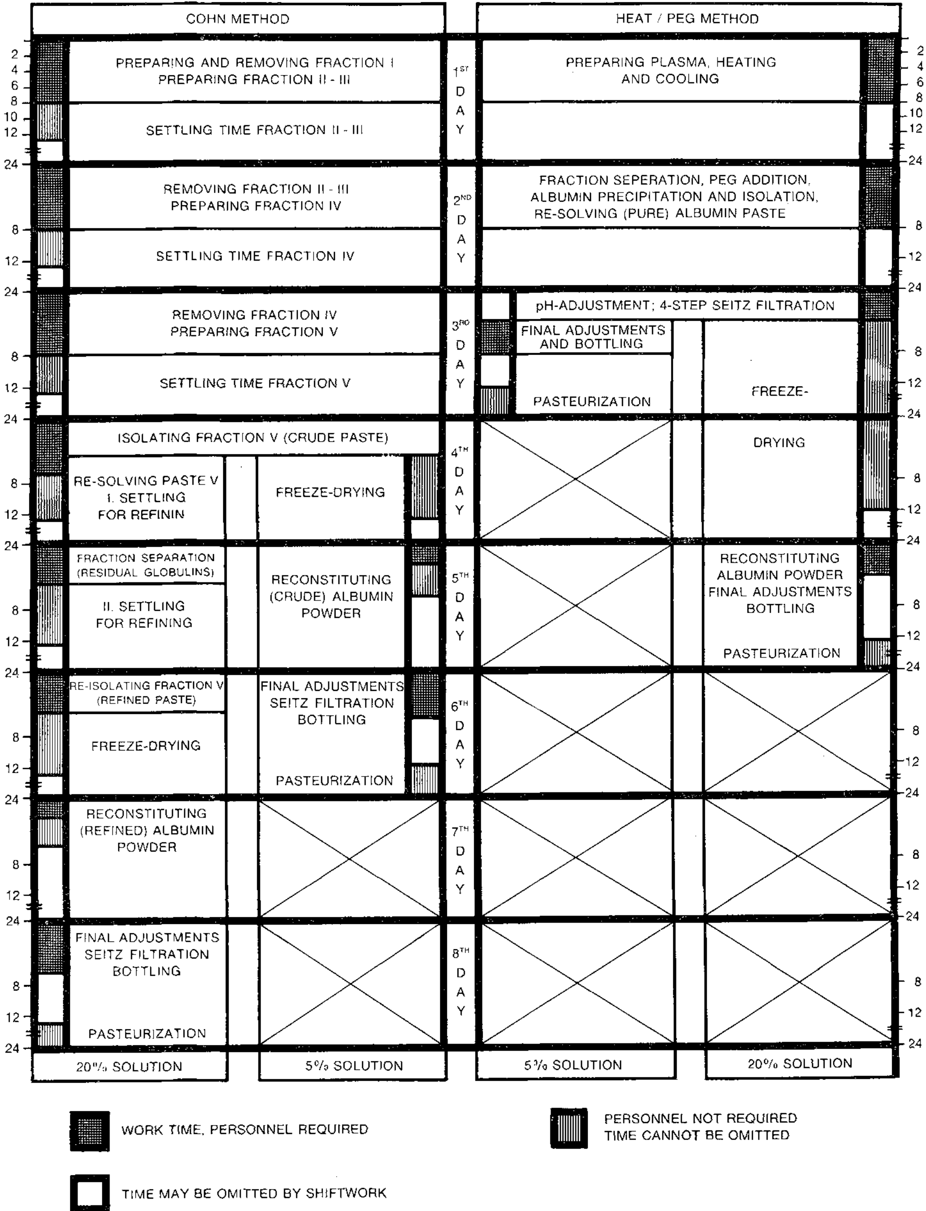

Table 9: Days (8-hours shifts) needed for 5% and 20% albumin production using cold alcohol fractionation and combined heat fractionation/PEG precipitation.

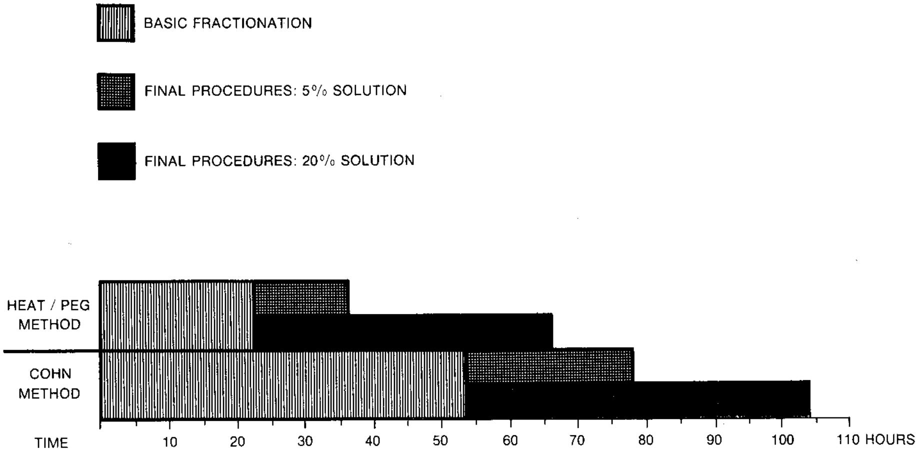

Table 10: Hours required for 5% and 20% albumin production using Cohn and heat/PEG methods.

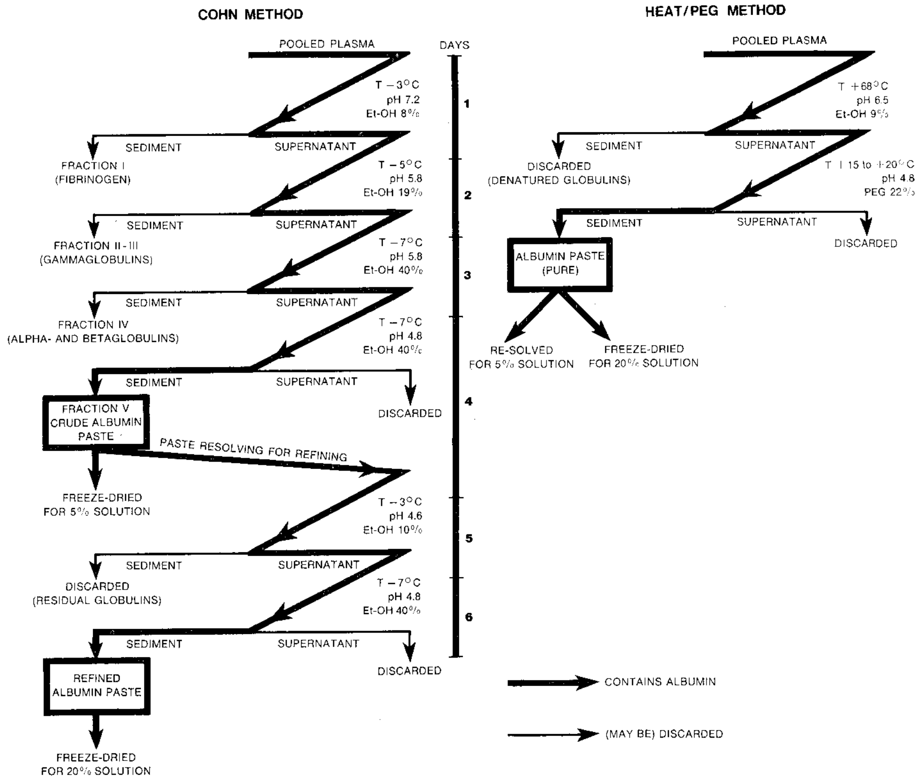

Table 11: Comparison of temperature, pH, and precipitant concentration variations required, as well as the time and different steps needed in Cohn (left) and heat/PEG (right) fractionation

amounts of Cohn II-III supernatant (~18% ethanol) with ethanol free plasma and heat-fractionate the resulting 9% alcohol solution.

In conclusion, this fractionation method, while keeping costs to a minimum, gives a maximum albumin yield; a very important fact when one considers that the basic raw material is not only expensive but also very limited in supply.

We hope that with combined heat fractionation/precipitation method, non-profit transfusion services, heterefore unable to produce albumin, will now have the opportunity for fractionating their own plasma themselves at a low cost to the patient.

We wish to thank Dr. D. *Wolter* and Dr. Ch. *Fröhlich* for their excellent advice, and our technologists C. *Gottschlich*, K. *Jürgens* and M. *Schneider* for their valuable assistance.

References: 1 Allary, M. et J. Saint-Blancard: Utilisation du caprylate de sodium pour fractionner les proteines plasmatiques. Ann. Pharm. Franc. *31*, 513–520 (1973). — 2 Andrassy, K., E. Ritz und R. Sanwald: Australia-Antigen-Nachweis in Fibrinogenkonzentraten und anderen gerinnungsaktiven Proteinen. Deutsch. Med. Wschr. *95*, 2467–2469 (1970). — 3 Ballou, G. A., P. D. Boyer, J. M. Luck and F. G. Lum: Chemical, clinical, and immunological studies on the products of human plasma fractionation. V. The influence of non-polar anions on the thermal stability of serum albumin. J. Clin. Invest. *23*, 454–457 (1944). — 4 Ballou, G. A., P. D. Boyer, J. M. Luck and F. G. Lum: The heat coagulation of human serum albumin. J. Biol. Chem. *153*, 589–605 (1944). — 5 Carpenter, C. P., M. D. Woodside, E. R. Kinkead, J. M. King and L. J. Sullivan: Response of dogs to repeated intravenous injection of polyethylene glycol 4000 with notes on excretion and sensitization. J. Toxicol. Appl. Pharm. *18*, 35–42 (1970). — 6 Cohn, E. J., L. E. Strong, W. L. Hughes jr., D. J. Mulford, J. N. Ashworth, M. Melin and H. L. Taylor: Preparation and properties of serum and plasma proteins. IV. A system for the separation into fractions of the protein and Lipoprotein components of biological tissues and fluids. J. Amer. Chem. Soc. *68*, 459–475 (1946). — 7 Cohn, E. J., F. R. N. Gurd, D. M. Surgenor, B. A. Barnes, R. K. Brown, G. Derouaux, J. M. Gillespie, F. W. Kahnt, W. F. Lever, C. H. Liu, D. Mittelman, R. F. Mouton, K. Schmid and E. Uroma: A system for the separation of the components of human blood: quantitative procedures for the separation of the protein components of human plasma. J. Amer. Chem. Soc. *72*, 465–474 (1950). — 8 Fiedler, H.: Probleme der Posttransfusionellen Hepatitis. Münch. Med. Wschr. *114*, 549–552 (1972). — 9 Gabr, Y., M. H. Soliman, S. Dawoud, A. El-Molla and El S. Amin: Studies on stabilized human plasma protein solution. Acta Biol. Med. Germ. *27*, 341–349 (1971). — 10 Gellis, S. S., J. R. Neefe, J. Stokes jr., L. E. Strong, C. A. Janeway and G. Scatchard: Chemical, clinical and immunological studies on the products of human plasma fractionation. XXXVI. Inactivation of the virus of homologous serum hepatitis in solutions of normal human serum albumin by means of heat. J. Clin. Invest. *27*, 239–244 (1948). — 11 Hess, B. und S. J. Walter: Chromatographische Serumeiweißfraktionierung und ihre klinische Anwendung. Verh. Dtsch. Ges. Inn. Med. *66*, 639–646 (1960). — 12 Holland, P., H. Alter, R. Purcell and J. T. Sgouris: Hepatitis-associated antigen and antibody in cold ethanol fractionates of human blood. Vox Sang. *20*, 464–465 (1971). — 13 Holland, P. V., H. J. Alter, R. H. Purcell, J. J. Lander, J. T. Sgouris and P. J. Schmidt: Hepatitis B antigen (HB Ag) and antibody (anti-HB Ag) in cold ethanol fractions of human plasma. Transfusion *12*, 363–370 (1972). — 14 Janeway, C. A.: Clinical use of blood derivatives. J. Amer. Med. Ass. *138*, 859–865 (1948). — 15 Johnson, A. J., M. H. Karpatkin and J. Newman: Clinical investigation of intermediate- and high-purity antihaemophilic factor (factor VIII) concentrates. Brit. J. Haemat. *21*, 21–41 (1971). — 16 Kistler, P. and Hs. Nitschmann: Large scale production of human plasma fractions. Vox Sang. *7*, 414–424 (1962). — 17 Ledermann, K. L.: Zur Frage der Hepatitisübertragung durch menschliche Plasmafraktionen. Praxis *49*, 273–278

(1960). — 18 Murray, R. and W. C. L. Diefenbach: Effect of heat on the agent of homologous serum hepatitis. Proc. Soc. Exp. Biol. *84*, 230–231 (1953). — 19 Newman, J., A. J. Johnson, M. H. Karpatkin and S. Puszkin: Methods for the production of clinically effective intermediate- and high-purity factor-VIII concentrates. Brit. J. Haemat. *21*, 1–20 (1971). — 20 Nitschmann, Hs. und P. Kistler: Eine pasteurisierbare humane Plasmaproteinlösung (PPL), erhalten durch Entsalzung von Plasma mittels Ionenaustauschern. Helv. Chim. Acta *37*, 1767–1778 (1954). — 21 Nitschmann, Hs., P. Kistler und W. Lergier: Vereinfachtes Verfahren zur Gewinnung von humanem Albumin und γ-Globulin aus Blutplasma mittels Alkoholfällung. Helv. Chim. Acta *37*, 866–876 (1954). — 22 Paine, R. S. and C. A. Janeway: Human albumin infusions and homologous serum jaundice. J. Amer. Med. Ass. *150*, 199–202 (1952). — 23 Peterson, E. A. and H. A. Sober: Chromatography of proteins. I. Cellulose ion-exchange adsorbents. J. Amer. Chem. Soc. *78*, 751–755 (1956). — 24 Polson, A., G. M. Potgieter, J. F. Largier, G. E. F. Mears and F. J. Joubert: The fractionation of protein mixtures by linear polymers of high molecular weight. Biochim. Biophys. Acta *82*, 463–475 (1964). — 25 Polson, A. and C. Ruiz-Bravo: Fractionation of plasma with polyethylene glycol. Vox Sang. *23*, 107–118 (1972). — 26 Schneider, W., K. Glaßner, Ch. Fröhlich and L. J. McCarty: Multi-channel preparation of fresh blood. An economic therapy with blood components. Blut *28*, 100–108 (1974). — 27 Schneider, W. und K. Glaßner: Heilmittel aus menschlichem Blut. Diagnostik 7 325-329 (1974). — 28 Schneider, W., D. Wolter, H. Fiedler, H. Lefèvre und Ch. Fröhlich: HB Ag in menschlichen Plasmafraktionen unter Berücksichtigung neuer Methoden der Plasmafraktionierung. (in print). — 29 Schneider, W., D. Wolter and L. J. McCarty: Large scale fractionation of high purity factor VIII concentrate and prothrombin complex. (in print). — 30 Schneider, W., D. Wolter and L. J. McCarty: Multi-channel fractionation of plasma: Alternative methods in view of new procedures. (in print). — 31 Schroeder, D. D. and M. M. Mozen: Australia antigen: Distribution during Cohn Ethanol fractionation of human plasma. Science *168*, 1462–1463 (1970). — 32 Shaw, A. E., P. Schiff and P. A. Castaldi: Hepatitis following the use of fibrinogen. Med. J. Australia *2*, 1308 (1971). — 33 Smyth Jr., H. F., C. P. Carpenter and C. B. Shatter: The toxicity of high molecular weight polyethylene glycols; chronic oral and parenteral administration. J. Amer. Pharm. Ass. *36*, 157–162 (1947). — 34 Smyth Jr., H. F., C. P. Carpenter and C. S. Weil: The toxicology of the polyethylene glycols. J. Amer. Pharm. Ass. *39*, 349–354 (1950). — 35 Smyth Jr., H. F., C. P. Carpenter and C. S. Weil: The chronic oral toxicology of the polyethylene glycols. J. Amer. Pharm. Ass. *44*, 27–30 (1955). — 36 Sober, H. A., F. J. Gutter, M. Wyckhoff and E. A. Peterson: Chromatography of proteins. II. Fractionation of serum protein on anion-exchange cellulose. J. Amer. Chem. Soc. *78*, 756–763 (1956). — 37 Sober, H. A. and E. A. Peterson: Protein chromatography on ion exchange cellulose. Fed. Proc. *17*, 1116–1122 (1958). — 38 Spurling, N., J. Shone and J. Vaughan: The incidence, incubation period, and symptomatology of homologous serum jaundice. Brit. Med. J. *1*, 409–412 (1946). — 39 Watt, J. G.: Automatic fractionation of plasma proteins. Vox Sang. *23*, 126–134 (1972). — 40 Westphal, R. G.: Rational alternatives to the use of whole blood. Ann. Intern. Med. *76*, 987–990 (1972).

Authors' addresses: Hagen Institute, Dr. Waldemar Schneider and Lee J. McCarty, MT (ASCP). D-5800 Hagen, Feithstr. 180–184.
Münster Institute, Dr. Harald Fiedler and Dr. Hans Lefèvre. D-4400 Münster, Sperlichstr. 15.