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APPLICATION OF ION-EXCHANGE CHROMATOGRAPHY FOR THE PRODUCTION OF HUMAN ALBUMIN

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SUMMARY

Albumin of high purity can be obtained from human plasma by ion-exchange chromatography. Most of the globulins were precipitated from human plasma with 150 g/l polyethylene glycol and pure albumin was obtained from the supernatant with QAE-Sephadex A-50. The purity of albumin was greater than 95%.

INTRODUCTION

The increasing demand for albumin has motivated many researchers to develop technically simple methods for producing sufficient pure albumin. At present the method most often used is the Cohn cold ethanol process[1,2] and modifications of this[3,4].

Several authors[5,6] have suggested the use of polyethylene glycol (PEG) for fractionation of plasma proteins. However, the disadvantage of this method is that PEG must be removed from the individual fractions. Precipitation with ammonium sulphate has been suggested for this purpose. Chromatography has also been employed for protein fractionation. Björling[7] used CM-Sephadex and DEAE-Sephadex for purifying albumin and IgG. Curling *et al.*[8] first treated plasma with PEG and then purified albumin by anion–cation exchange chromatography. Travis *et al.*[9] used Cibachron blue-Sephadex for producing pure albumin.

The aim of this work was to develop a one-step method for purifying albumin after precipitation of plasma with PEG. For this purpose QAE-Sephadex A-50 was used.

MATERIALS AND METHODS

Fractionation of crude albumin

Pooled human plasma was treated with PEG-4000 (Hoechst, Frankfurt/M, G.F.R.) according to the method described by Haskó and co-workers[6,10] and thus two main fractions were obtained (Fig. 1). After precipitation of IgG (pH 6.5, 150 g/l PEG), albumin was precipitated from the supernatant at pH 4.6 with 240 g/l PEG. The precipitate was dissolved in distilled water and brought to 80 g/l of protein.

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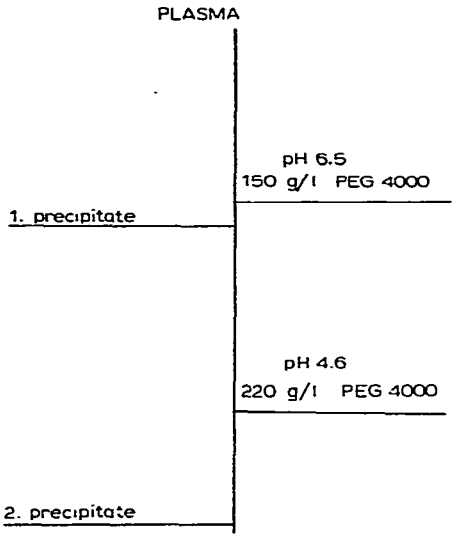

Fig. 1. Two-step fractionation.

Ion-exchange chromatography

Sodium acetate buffers were used for elution: A, ionic strength (I) = 0.05, pH = 5.2; B, I = 0.09, pH = 4.8; C, I = 0.15, pH = 4.0. QAE-Sephadex A-50 gel was swollen in 1.0 M sodium acetate, then washed and equilibrated with the starting buffer. A 30 × 1.5 cm column (Pharmacia, Uppsala, Sweden) was used. The crude albumin solution was adjusted to pH 6.5 with 0.1 N HCl. A 1-ml volume of gel was required to purify 30–35 mg crude albumin. Chromatography was performed at a rate of 1 ml/min. The fractions were collected by an Ultrorac 7000 collector and detected on a LKB Uvicord II photometer at 280 nm.

Batch procedure

QAE-Sephadex A-50 was treated as described above. A 3000-ml Büchner funnel was used for equilibration and elution. A 1200-ml volume of gel was added to 570 ml crude albumin solution containing 80 g/l protein, obtained from 2000 ml human plasma, and mixed at room temperature for 30 min. The gel suspension was drawn through a Büchner funnel. PEG and non-adsorbed proteins were eluted by three volumes of buffer A. The albumin (10 g/l) was eluted with buffer B, and the remaining proteins were eluted with three volumes of buffer C.

Concentration and pasteurization of proteins

The solution was concentrated by ultrafiltration or freeze-dried. Ultrafiltration was performed on a Bio-Fiber 80 hollow fibre ultra filter (Bio-Rad Labs., Richmond, CA, U.S.A.). A 200 g/l solution was made from the freeze-dried albumin with distilled water. The solution was pasteurized for 10 h in the presence of 0.05 M sodium caprilate in a water-bath at 60°C.

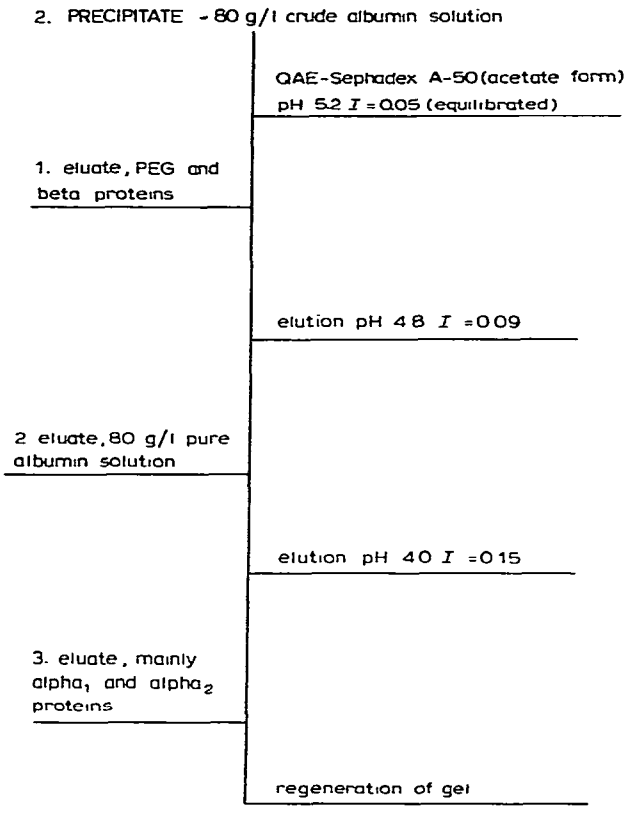

Fig. 2. Elution of albumin.

Analytical methods

The protein content of the sample was assayed by the biuret method. The albumin purity was determined by electrophoresis densitometry on cellulose acetate membranes (Gelman, Ann Arbor, MI, U.S.A.), and by the Scheidegger micro-immunoelectrophoresis method[11]. A polyvalent anti-human horse serum (Humán, Gödöllő-Bp., Hungary) was used for the latter.

PEG remaining in the protein solutions was detected with Nessler–Winkler reagent. Quantitations were performed as described by Skoog[12]. Flame photometry was used for determining the sodium and potassium contents. Sterility tests were performed according to ref. 13.

Pyrogenicity tests were performed on rabbits. Three rabbits were intravenously injected with 15 g/l solution (10 ml/kg). Toxicity tests were performed on mice, 0.5 ml 200 g/l albumin solution being injected intravenously.

RESULTS

The crude 80 g/l albumin solution obtained by PEG fractionation was purified by QAE-Sephadex chromatography. Fig. 3 displays the chromatogram obtained with

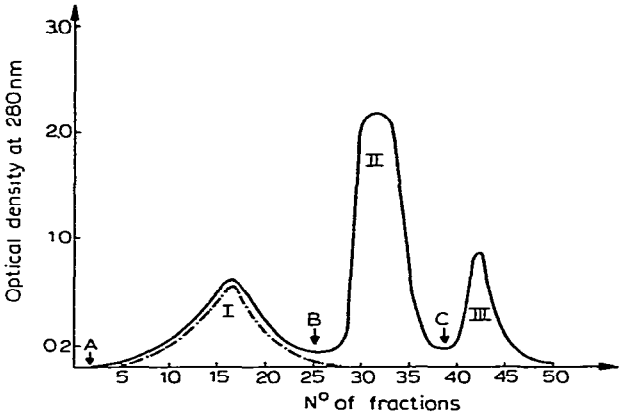

Fig. 3. Chromatogram of crude albumin precipitated by PEG and gradient elution on QAE-Sephadex A-50. Column: Pharmacia K 15/30: bed volume 40 ml. Sample volume: 30 ml. Buffers A–C as in Experimental. Peaks: I = beta-globulin fraction and PEG (—·—); II = albumin fraction; III = mainly $alpha_1$ and $alpha_2$ proteins.

gradient elution. The first peak (buffer A) contains beta proteins; PEG was eluted at this stage. The second peak (buffer B) contains pure albumin (20–25 g/l). The third peak (buffer C) contains mainly $alpha_1$ and $alpha_2$ proteins.

Table I lists the average yields and purities of albumin preparations obtained by column chromatography and by the batch procedure. Column chromatography results in a 95% yield of albumin and a purity above 99%. Albumin produced by the batch procedure is somewhat less pure and the yield is also lower; the relatively simple and inexpensive equipment used in this method is however an advantage.

TABLE I

COMPARISON OF THE AVERAGE YIELDS AND PURITIES OF ALBUMIN PREPARATIONS OBTAINED BY COLUMN CHROMATOGRAPHY AND BATCH PROCEDURE FROM CRUDE ALBUMIN FRACTION

Procedure	*Albumin*			
	Crude (g)	*Pure (g)*	*Yield (%)*	*Purity (%)*
Column chromatography (n = 15)	0.79 ± 0.02	0.75 ± 0.01	95 ± 2	99 ± 1
Batch procedure (n = 10)	30.1 ± 0.61	27.6 ± 0.56	91.6 ± 2	95 ± 1

Fig. 4 shows the immunoelectropherogram of albumin purified by column chromatography. Albumin obtained by our method showed a higher purity compared to commercially available albumin preparations for therapeutic purposes. Besides albumin, only beta-globulin is present in our preparation.

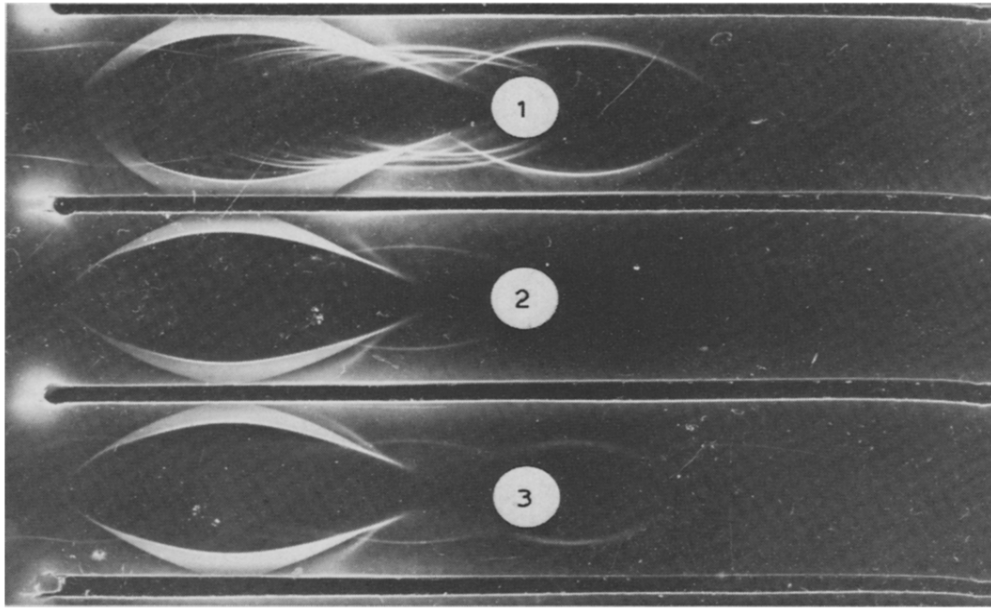

Fig. 4. Immunoelectropherogram of fractions obtained by QAE-Sephadex A-50 column chromatography. Fractions: 1 = crude albumin; 2 = purified albumin; 3 = standard albumin. Antibody = polyvalent anti-human horse serum.

Fig. 5 shows the immunoelectropherogram of fractions obtained by the QAE-Sephadex A-50 batch procedure. Besides albumin, the preparation contains alpha- and beta-globulins. In the albumin end-product obtained by column chromatography the PEG concentration was 0.012 g/l. In albumin produced by the batch procedure the PEG concentration was 3 g/l (Table II).

DISCUSSION

The samples obtained by the column or batch procedure passed the sterility, pyrogenicity and toxicity tests. Our experiments indicate that crude albumin obtained

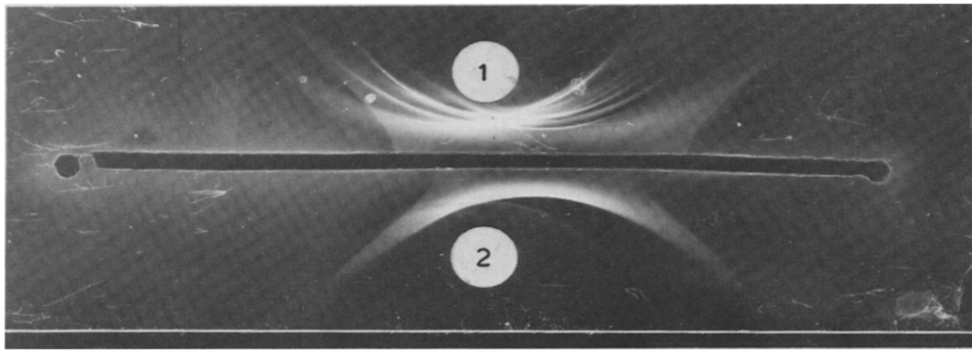

Fig. 5. Immunoelectropherogram of fractions obtained by QAE-Sephadex A-50 batch procedure. Fractions: 1 = crude albumin; 2 = purified albumin. Antibody = polyvalent horse serum.

TABLE II

COMPARISON OF THE AVERAGE OF PEG CONCENTRATION IN CRUDE ALBUMIN SOLUTIONS AND ALBUMIN END-PRODUCTS OBTAINED BY COLUMN CHROMATOGRAPHY AND BATCH PROCEDURE

Procedure	*PEG (%)*	
	In crude albumin solution	*In albumin end-product*
Column chromatography	2.5	0.0012
Batch procedure	2.5	0.3

by fractionation with PEG can be purified with QAE-Sephadex so as to pass requirements for therapeutic substances[14].

Our method is a one-step technique which is an advantage compared to Curling's method. No toxic KSCN solution is necessary for elution as in the method using Cibachron blue-Sephadex[9]. The ionic solutes can be removed by dialysis or ultrafiltration and at the same time the solution can be concentrated. The end-product of 40–200 g/l albumin solution can be pasteurized at 60°C for 10 h without appearance of any turbidity. The end-product is non-toxic and pyrogen-free. The PEG can be removed by buffer A together with β-globulins.

The advantage of the batch procedure is that it is very inexpensive, and employs simple equipment. The purity and yield of albumin obtained are somewhat lower compared to albumin produced by column chromatography.

REFERENCES

1 E. J. Cohn, F. R. N. Gurd, D. M. Surpenor, 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, *J. Amer. Chem. Soc.*, 72 (1950) 465–474.
2 E. J. Cohn, L. E. Strong, W. L. Hughes Jr., D. J. Mulford, J. N. Ashworth, M. Melin and H. L. Taylor, *J. Amer. Chem. Soc.*, 72 (1946) 459–475.
3 P. Kistler and H. S. Nischmann, *Vox Sang.*, 7 (1962) 414–424.
4 J. G. Watt, J. K. Smith, W. Grant and C. Turnbull, *Proc. R. Soc. Edinburgh*, 71, Suppl. (1972) 2–34.
5 A. Polson and C. Ruiz-Bravo, *Vox Sang.*, 23 (1972) 167–118.
6 F. Haskó, *First European Congress on Biotechnology, Interlaken, Switzerland, September 25–29, 1978*, Dechema, Frankfurt/M., 1978.
7 H. Björling, *Vox Sang.*, 23 (1972) 18–25.
8 J. M. Curling, J. Berglöf, L. O. Lindquist and S. Eriksson, *Vox Sang.*, 33 (1977) 97–107.
9 J. Travis, J. Bowen, D. Tewksbury, D. Johnson and R. Pannel, *Biochem. J.*, 157 (1976) 301–306.
10 F. Haskó and R. Vasileva, *Haematologia*, 14 (1981) 199–206.
11 J. J. Scheidegger, *Int. Arch. Allergy*, 7 (1955) 103.
12 B. Skoog, *Vox Sang.*, 37 (1979) 345–349.
13 *Pharmacopeia Hungarica*, Medicina, Budapest, 6th, 1967.
14 *WHO Tech. Rep. Ser. No. 530*, WHO, Geneva, 1973.