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Note

Comparison of methods of determining the electrophoretic purity of albumin

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Stabilized 20% and 5% solutions of human albumin are widely used in clinical practice. They are administered in hypovolemia, shock, hypoalbuminemia and in the treatment of cerebral edema. Their transportation properties are also utilized.

As albumin production increases, so also does the requirement for electrophoretic determinations in interoperational and final controls of its production. Previous methods for the determination of the electrophoretic purity of albumin were laborious and time consuming. Tiselius free electrophoresis[1] was gradually replaced by electrophoresis on carriers[2].

Currently, the most popular method consists of the use of cellulose acetate membranes (CAMs)[3–7]. In its microzonal modification the effect of the carrier is reduced to minimum, and the time required for the analysis is sufficiently short. It must be remembered that the requirements for a control method for the determination of albumin purity are atypical compared with the conventional clinical analysis. The analysis is not of a complete human serum but of a particular fraction contaminated by traces of some other fractions. This results in the requirements of maximum sensitivity, accuracy and reproducibility (the maximum permissible error is $\pm 1\%$).

The purpose of this study was to compare suitable methods for the determination of electrophoretic purity of albumin preparations.

MATERIALS AND METHODS

Different types of CAMs, buffers and detection methods were compared. The experimental conditions are given in Table I.

Transparency was achieved using a 33% solution of cyclohexanone in ethanol or in transparent oil (Whitemore 120, Shandon, London, Great Britain), following the recommendations of membrane manufacturers.

On the basis of our experiments the following optimum method was developed. The membranes are saturated with buffer and tightened in an electrophoretic chamber. The sample or standard (human normal serum) is applied by means of a tube pen in amounts from 2 to 5 μl (according to protein concentration). The separation time is 30–40 min. Immediately after separation the membrane is placed into Ponceau S staining solution for 7 min. Excess staining solution is washed out with 5% acetic acid. The membrane is dehydrated for 1 min with 96% ethanol. The dehydrated membrane is firmly attached to a slide and rendered transparent by treatment for 3 min with dioxane and isobutanol solution or with cyclohexanone solution. Finally the membrane is dried for 10 min at 100°. The resulting transparent film is evaluated densitometrically.

TABLE I

EXPERIMENTAL CONDITIONS USED IN THE COMPARISON OF VARIOUS MEMBRANES

Cellulose acetate membrane (CAM)		*Detection*				*Buffers*		
Membrane	*Dimensions/mm*	*Fixation*	*Staining*	*Washing out*	*Dehydration*	*Buffer*	*pH*	*μ*
Sartorius Membranfolien für electrophorese (Göttingen, G.F.R.)	25 × 120	–	amido black 10B 0.5% solution in methanol acetic acid 9:1	methanol acetic acid 9:1	–	veronal acetate	8.6	0.1
Schleicher + Schüll Electrophorese folien CA 250/0 (Dassel, G.F.R.)	25 × 120	–	Ponceau S 0.2% in 3% acetic acid	acetic acid 5%	absolute alcohol	veronal barbituric acid	8.6	0.036
Cellogel strips Chemetron (Milan, Italy)	25 × 120	–	bromophenol blue 0.1% in 0.5% mercury chloride in alcohol	acetic acid 0.5%	absolute alcohol	veronal	9.95	0.04
Oxoid Electrophoresis strips (London, Great Britain)	25 × 120	sulphosalicylic acid	nigrosin 0.002% in 2% acetic acid	flowing water	absolute alcohol	veronal barbituric acid glycerol	8.8	0.04
						Tris	9.0	0.08

All experiments were performed in a 140 × 250 × 50 mm electrophoretic chamber, specially modified for our purposes from the standard immunoelectrophoretic chamber (product of the Institute of Sera and Vaccines). The Tesla TM 583-D stabilizer was used to supply power. The determinations were performed at a constant voltage (200 V), with the current varying in the range 20–25 mA. For densitometric evaluation of the membranes the ERI 65m extinction apparatus (Carl Zeiss Jena, Jena, G.D.R.) was employed.

RESULTS

Influence of the buffer employed on the separation of the individual fractions of human normal serum on different CAMs

Five different recommended buffers were used for all the membranes. All electrophoretic analyses were performed under identical conditions (amount of sample applied, time of run, voltage, staining, method of achieving transparency). The results are presented in Table II.

Influence of the detection method on the electrophoretic profile of human normal serum

Parallel electrophoresis of a human normal serum sample applied in the same amount on four membranes placed side by side was performed. After separation, each membrane was stained with a different staining solution. The results are presented in Table III.

Comparison of CAM electrophoresis with free electrophoresis

The results obtained by electrophoresis on CAMs were compared with those obtained by free electrophoresis. Electrophoretic purity of 20% human albumin was determined using both methods in parallel. An example of such a comparison is given in Table IV. The comparison was performed with 20 samples of 20% human albumin and a 100% albumin standard.

TABLE II

COMPARISON OF FRACTIONATION OF HUMAN NORMAL SERUM BY MEANS OF VARIOUS MEMBRANES AND VARIOUS BUFFERS

1, Albumin; 2, $\alpha_1\alpha_2$ globulins; 3, β-globulin; 4, γ-globulin. S, Start.

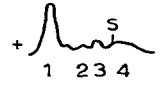
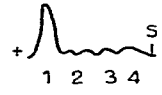
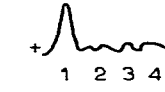
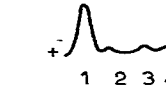
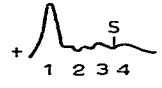
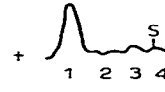
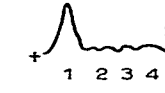
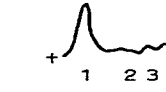
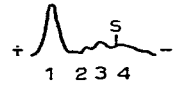
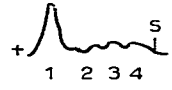
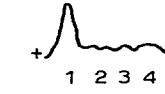
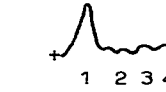
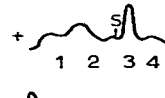
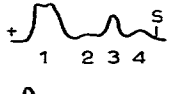
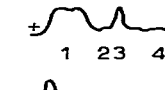
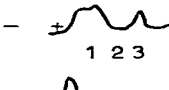
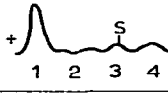
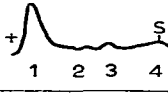
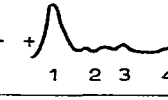
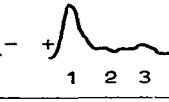
Buffer	*pH*	*μ*	*Oxoid*	*Cellogel*	*Sch + Sch*	*Sartorius*
Veronal acetate	8.6	0.1				
Veronal barbituric acid	8.6	0.036				
Veronal	9.95	0.04				
Tris	9.0	0.08				
Veronal barbituric acid glycerol	8.8	0.04				

TABLE III

COMPARISON OF STAINING SOLUTIONS

0, Prealbumin; 1, albumin; 2_1, α_1-globulin; 2_2, α_2-globulin; 3, β-globulin; 4, γ-globulin.

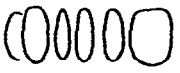
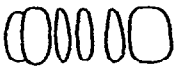
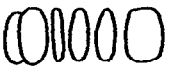
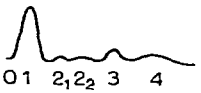
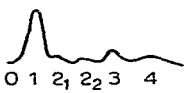
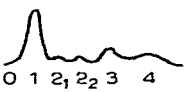
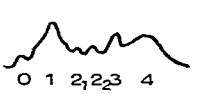
	Amido black 10B	*Ponceau S*	*Bromphenol blue*	*Nigrosin*
Membrane				
Densitometric record				
Quantitative checking of densitometric record (%)	0 1.25 1 63.75 2_1 3.0 2_2 7.25 3 8.75 4 15.75	0 0.75 1 62.25 2_1 3.0 2_2 8.25 3 8.75 4 17.0	0 0.75 1 62.5 2_1 2.5 2_2 7.25 3 10.0 4 17.0	0 2.0 1 20.5 2_1 5.5 2_2 15.0 3 17.5 4 39.5

TABLE IV

COMPARISON OF PURITY DETERMINATION BY MEANS OF TISELIUS FREE ELECTROPHORESIS AND CELLULOSE ACETATE MEMBRANE ELECTROPHORESIS TECHNIQUE OF 20% HUMAN SERUM ALBUMIN

	CAM electrophoresis	*Free electrophoresis*
Record		
Checking (%)	albumin 94.9 impurities 5.1	albumin 95.2 impurities 4.8

Reproducibility of the method

Altogether 12 determinations of the same sample of a 20% human albumin were performed. From the results the standard deviation for the individual determination (S.D.$_i$) was calculated using the relationship

$$\text{S.D.}_i = \sqrt{\frac{1}{n-1}\sum(\bar{x} - x_i)^2}$$

The mean standard deviation (S.D.$_m$) was also found, using the relationship

$$\text{S.D.}_m = \sqrt{\frac{1}{n(n-1)}\sum(\bar{x} - x_i)^2}$$

In both formulae, n is the number of samples, $\bar{x}$ is the average value and x_i is the estimated value. The results are as follows: S.D. = 0.19%; for n = 12, S.D.$_m$ = 0.054%, the maximal deviation from the mean value is 0.423% and the difference between marginal values is 0.7%.

DISCUSSION

Of various electromigration methods, electrophoresis on CAMs appears to be the best for routine checks during the manufacture of albumin solutions. The advantages are the abolition of tailing, a clear background and fast separation with sufficient accuracy.

The most popular membrane types, recommended buffers and detection methods were compared. From the tested membranes the types Sartorius, Sch + Sch and Cellogel satisfy the criteria as far as the separation itself is concerned. The main differences, however, can be seen during the subsequent handling of the membranes. In the case of Cellogel the staining and manipulation in 30% methanol are somewhat more difficult. On the Oxoid membranes the γ-globulin fraction migrated to the cathode, *i.e.* towards the opposite side from the other fractions. In this case the start line must be placed in the middle of the cellulose membrane, which may cause some problems during evaluation. Transparency of the Oxoid membranes can be achieved only by means of a special transparent oil, which means a considerably lengthier and more laborious process than using the solvents needed for the other membranes.

The course of blood plasma separation is similar in all types of veronal buffer. The only marked difference is found when tris buffer is used, when plasma is separated quite atypically into approximately nine components, and its albumin fraction is divided into two zones. The tris buffer is unsuitable for the purity of albumin preparations, but it finds use in some structural studies[8–10]. The detection sensitivity is almost the same with amido black 10B, Ponceau S and bromophenol blue[9,11,12], as is the mutual percentual relation of the individual fractions in densitometric evaluation. If nigrosin solution is used, the albumin, as well as the α-, β- and γ-globulin zones are overstained, and the densitometric evaluation is distorted. The prealbumin zone appears as a slight trace only if other staining solutions are used. This confirms that nigrosin can be advantageously used for the detection of small and trace amounts of proteins. Better detection of the trace impurity zones that in free electrophoresis often merge with the basic curve justifies of the CAM method, as does the demonstrated reproducibility of the method (standard mean deviation 0.054%, standard deviation of the individual determination 0.19%).

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