THE USE OF CENTRIFUGALLY-ACCELERATED ULTRAFILTRATION FOR THE CONCENTRATION OF PROTEINS IN BIOLOGICAL FLUIDS

R. C. R. BARRETO AND D. B. MANO

Institute of Phthisiology and Pneumology, University of Brazil, Rio de Janeiro (Brazil) *

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INTRODUCTION

When biological fluids of low protein content, such as liquor, urine, saliva, etc., are subjected to paper electrophoresis, the samples must first be concentrated[1]. This has been done by precipitation[2,3], ultrafiltration[4,5], dialysis against colloidal suspensions[6,7] and water absorption by means of carboxymethyl-cellulose derivatives[8]. The time needed for such operations varies from a few hours[8] to several days[5].

We have used centrifugally-accelerated ultrafiltration to separate water-soluble metabolic products from blood samples (unpublished results). The technique proved to be rapid and easy to perform. Later on it was used for the concentration of biological fluids of low protein content, prior to paper electrophoresis, which is reported here.

MATERIALS AND METHODS

Preparation of the sample

The sample to be concentrated was placed in a dialysis bag, made of collodion, Parlodion (Mallinckrodt) or a similar material. The opening of the bag was tied up and the bag was wrapped in a square piece of gauze (15 × 15 cm). The gauze containing the bag with the sample, was then placed halfway down a 50 ml centrifuge

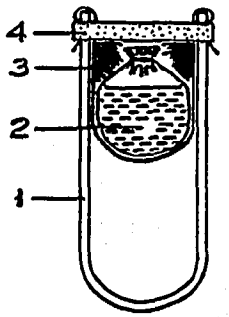

Fig. 1. Set-up for centrifugal ultrafiltration: 1 = 50 ml centrifuge tube; 2 = collodion bag with sample; 3 = gauze wrapping; 4 = rubber band.

* Inst. Tisiol. Pneumol., Universidade do Brasil, C. P. 4485, Rio de Janeiro, Brazil.

tube, the excess gauze being folded down over the outside of the neck of the tube. Rubber bands around the folded gauze held this set-up in position, as shown in Fig. 1. The best results were obtained when an empty space was left in the bag, as this prevented it from collapsing during centrifugation.

Preparation of the dialysis bag

A 4% solution (w/v) of collodion (or a similar material, such as Mallinckrodt's Parlodion) in ethanol–ether (1:3) was poured into a 50 ml centrifuge tube, which was then drained by turning it upside down (this is necessary in order to obtain a uniform distribution of the collodion film around the walls of the tube) and left to dry at room temperature.

These operations were repeated, as a fairly strong film is necessary to avoid the risk of the bag rupturing during centrifugation. The tube was then filled with water, and after 15 min the bag could be easily removed.

Centrifugal ultrafiltration

Centrifugation of the collodion bag led to ultrafiltration of the sample and consequent concentration of the protein material inside it.

RESULTS

As shown in Fig. 2, the rate of ultrafiltration depends on the speed of centrifugation and on the concentration of protein in the sample. We found that the risk of the

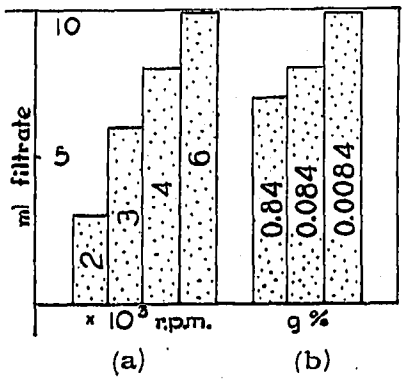

Fig. 2. Variation of the rate of ultrafiltration with (a) the speed of centrifugation (sample: water; initial volume: 10 ml; time: 10 min) and (b) the concentration of protein in the sample (sample: blood serum diluted with distilled water; initial volume: 10 ml; speed: 6,000 rev./min; time: 10 min).

collodion bag rupturing during centrifugation increases when speeds higher than 6,000 rev./min or volumes greater than 10 ml are used; hence these were considered as the safety limits.

Protein was never found in the filtrate, and there were no significant alterations in the composition of protein mixtures concentrated by centrifugal ultrafiltration (Table I).

TABLE I

PROTEIN COMPOSITION OF RABBIT SERUM

A sample of serum was diluted a hundredfold with distilled water and taken to the initial concentration (total protein = 7.6 g %) by means of centrifugally accelerated ultrafiltration (6,000 rev./min for 20 min).

Sample	*Protein, % concentration*			
	Alb.	*α-Glob.*	*β-Glob.*	*γ-Glob.*
Serum	34.47	21.20	23.72	20.61
Concentrate	35.21	20.59	22.64	21.54

Different procedures were established for the concentration of the various biological fluids, prior to paper electrophoresis:

Liquor. 5-ml samples of liquor were mixed with equal portions of a suitable buffer (the same to be used in the electrophoretic separation), placed in the collodion bag, as described, and centrifuged at 6,000 rev./min for 10 min or more, until a volume of 0.1 ml was obtained.

Urine. 40-ml samples of urine, previously centrifuged (6,000 rev./min for 10 min), were placed in collodion bags (10 ml in each) and centrifuged at 4,000 rev./min for 10 min. The concentrates (about 1 ml per bag) were transferred to a single bag and the volume was adjusted to 10 ml with a suitable buffer solution. The centrifugation of these concentrates (6,000 rev./min for 10 min) yielded residues of about 20μl, containing sufficient protein for direct densitometry of the paper electrophoresis strips.

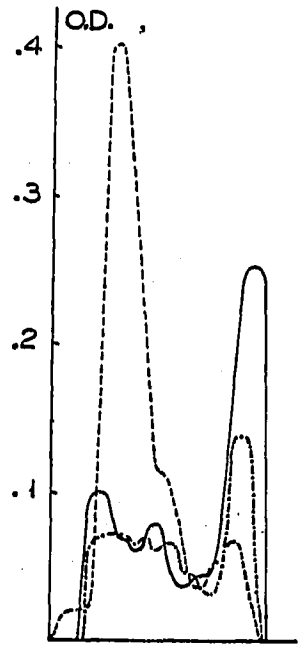

Fig. 3. Paper electrophoresis of protein from human liquor (———), urine (– . – . –) and saliva (– – –), concentrated by means of centrifugal ultrafiltration. Samples collected from normal subjects and separated in veronal–acetate buffer, pH 9.0 (300 V; 5 h).

Saliva. 10-ml samples of saliva were concentrated a hundred times by centrifugal ultrafiltration at 6,000 rev./min for 20 min.

Fig. 3 shows the results obtained after electrophoretic separation of the proteins of some biological fluids, concentrated as described. Paper electrophoresis was carried out in veronal–acetate buffer, pH 9.0 (300 V; 5 h), by the hanging strip technique. The proteins were evaluated by direct densitometry of the strips stained with bromophenol blue. The following volumes of concentrate were applied to the paper: liquor, 10 μl; urine, 20 μl; and saliva, 40 μl.

DISCUSSION

Centrifugal ultrafiltration was found to be a rapid and reliable technique for the concentration of proteins in biological fluids. Working with volumes of 10 ml and using speeds of 6,000 rev./min hundredfold concentrations were obtainable after 10 to 20 min. There is no loss of protein material during the process, and no changes were found in the electrophoretic behaviour of protein mixtures concentrated by centrifugal ultrafiltration.

The application of this technique to samples of liquor, urine and saliva yielded protein concentrates that could be readily studied by paper electrophoresis, since they were rich enough for direct densitometric evaluation of the stained strips.

The extremely short time required for the concentration, as well as the fact that centrifugation can be carried out at low temperatures, minimizes the risk of protein denaturation. Further advantages of the technique are that there is practically no delay between the collection of the sample and its examination, and that no special equipment or installations are needed.

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SUMMARY

The concentration of biological fluids of low protein content by means of centrifugally-accelerated ultrafiltration was studied. The samples were placed in collodion bags, which were suspended inside centrifuge tubes by means of gauze wrappings, and centrifuged.

Working with volumes of 10 ml and using speeds of 6,000 rev./min hundredfold concentrations were obtainable in 10 to 20 min. The short time required for centrifugal ultrafiltration minimizes the risk of protein denaturation and allows rapid examination of the sample. Another advantage of the technique is that no special equipment or installations are needed.

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