CHROM. 4340

A simplified method for the preparation of immunoglobulin G by gel filtration–centrifugation

There is an extensive bibliography on the methods used in the preparation of γ-globulin. SCHULTZE AND HEREMANS[1] presented an up-to-date review. KISTLER AND NITSCHMANN[2] emphasized the preparation of γ-globulin for therapeutic purposes. Due to the high degree of purification of the finished product, each of these methods requires the application of accurate techniques or results in poor efficiency (see also BAUMSTARK *et al.*[3]).

Here a simplified technique for the rapid preparation in the laboratory of an immunoelectrophoretically almost pure γ-globulin G is described.

Euglobulin was precipitated from human serum by saturating one-third of the serum with ammonium sulphate. The precipitate was washed with a 30% solution of ammonium sulphate and then dissolved in aqua dest. The euglobulin solution was centrifuged with Sephadex G-200 (40–120 μ) which had been equilibrated in distilled water with a specific conduction of $\sigma = 2.693 \cdot 10^{-7}$ ($1/\Omega$ cm) (For conductivity measurements see Table I.)

TABLE I

RESULTS OF CONDUCTIVITY MEASUREMENTS

For the measurements a Wheatstone bridge of up to 1000 c/sec was employed.

Material	*Conductivity ($1/\Omega cm$)*
Human serum pool	$6.640 \cdot 10^{-3}$
F_1	$0.168 \cdot 10^{-3}$
F_2	$2.048 \cdot 10^{-3}$
F_3	$5.760 \cdot 10^{-3}$

Ten millilitres of human serum pooled from clinically healthy donors were mixed with 5 ml of a saturated solution of ammonium sulphate, and the precipitate was washed twice in a 30% solution of ammonium sulphate. (Sufficient centrifugation is necessary for adequate separation of the proteins in solution.) After washing, the precipitate was collected in 3 ml of aqua dest. or in a 1% solution of NaCl.

The serum of patients with γ G-myeloma was used in other tests. For the results of these tests see Fig. 2.

For centrifugation, the upper part of the centrifugation tube (see Fig. 1) was filled to the brim with a dense Sephadex gel and centrifuged for 5–6 min at a velocity of 500–600 r.p.m. (swing-out centrifuge; radius, 20 cm). Too many revolutions or the use of other types of gel may produce separation of gel surface.

After the first centrifugation of the Sephadex gel, the euglobulin solution was placed on the gel surface and then centrifuged again at a velocity of 500 r.p.m. for 5 min. The filtrate obtained was designated F_1. In order to obtain a complete elution of proteins, 3 ml of distilled water were added to the gel surface, and centrifuged for

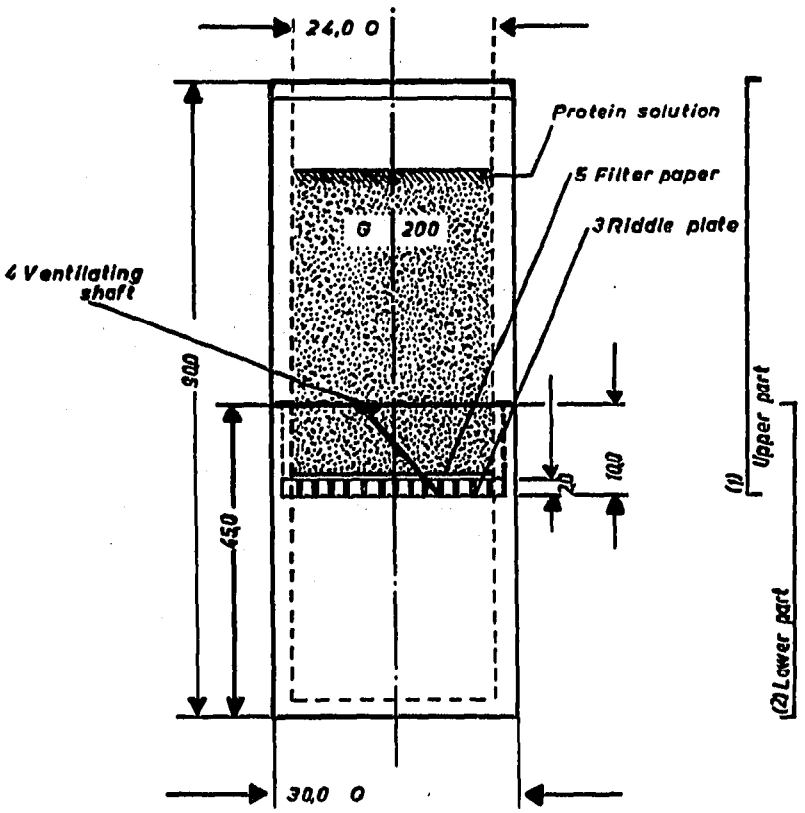

Fig. 1. Centrifugation tube with riddle plate. (1) Upper part with a fixed riddle plate (3); (2) lower part with an air-balanced channel (4); (5) paper filter on the riddle plate. The diameter of the riddle holes is 1 mm. The data are expressed in mm. Material: plexiglass.

5 min (500 r.p.m.). This filtrate was designated F_2. Filtrates F_3 and F_4 were obtained in the same way.

Immunoelectrophoresis was performed in agar Difco-Noble and Veronal buffers (pH 8.2). The filtrates were placed directly in the start position (in the other tests after concentration). Horse antihuman serum (see BUNDSCHUH *et al.*[4] with respect to the immunization plan) as well as rabbit antihuman serum were used as immune sera.

As shown in Fig. 2, the first filtrate contains immunoelectrophoretically pure

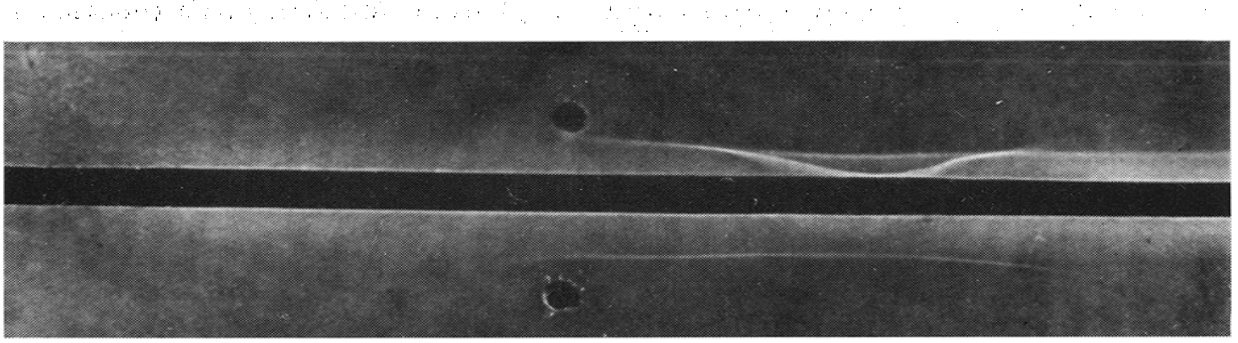

Fig. 2. Immunoelectrophoretic analysis of F_1 filtrate after centrifugation with Sephadex G-200. Above: F_1 from γG-myeloma serum. Below: F_1 from normal serum. Horse antihuman serum, see text. The F_1 filtrate of the myeloma serum shows the paraprotein (precipitation arc near the antibody channel) as well as the normal γ-globulin G.

γ-globulin G. In consecutive filtrates γ-globulin G is concentrated; however, other euglobulin traces as well as albumin traces can be found.

The results of the tests permit us to conclude that a part of the γ-globulin G is still soluble in a dilute salt medium. The conductivity of filtrates F_1 and F_2 is usually less than that of the initial material. The other euglobulin components are considered similar to a protein insoluble in the small amount of salt present in the Sephadex gel. According to HEIMBURGER AND SCHMIDTBERGER[5], these components can be obtained by means of an increase in the salt gradients during the elution phase.

Contrary to the usual filtration procedure of Sephadex gel, the basis of separation of the protein components by the method described here does not depend on the size of the molecule of the different proteins but on the protein being soluble in a medium poor in salt.

The paraprotein may be obtained from the γ G-myeloma serum in relation to normal γ-globulin G (see Fig. 2) by the method described.

In a further communication we shall report on the purity of γ-globulin, obtained by this method, which is determined by means of the hyperimmunization of rabbits as well as by the quantitative efficiency of the material obtained.

We wish to thank Dr. S. MÜLLER, Institute of Physics and Chemistry, Humboldt University, Berlin, at present at the National Center for Scientific Investigations, Havana, Cuba, for the conductivity measurements.

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1 H. E. SCHULTZE AND J. F. HEREMANS, *Molecular Biology of Human Proteins*, Vol. 1, Elsevier, Amsterdam, 1966.
2 P. KISTLER AND H. NITSCHMANN, *Helv. Med. Acta*, 26 (1959) 131.
3 J. S. BAUMSTARK, R. J. LAFFIN AND W. A. BARDAWIL, *Arch. Biochem. Biophys.*, 108 (1964) 514.
4 G. BUNDSCHUH, M. SCHÄFER, A. KUNTZE AND A. MIX, *Z. Immunforsch.*, 130 (1966) 483.
5 N. HEIMBURGER AND R. SCHMIDTBERGER, *Behringwerk-Mitt.*, 43 (1964) 83.

Received August 25th, 1969

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