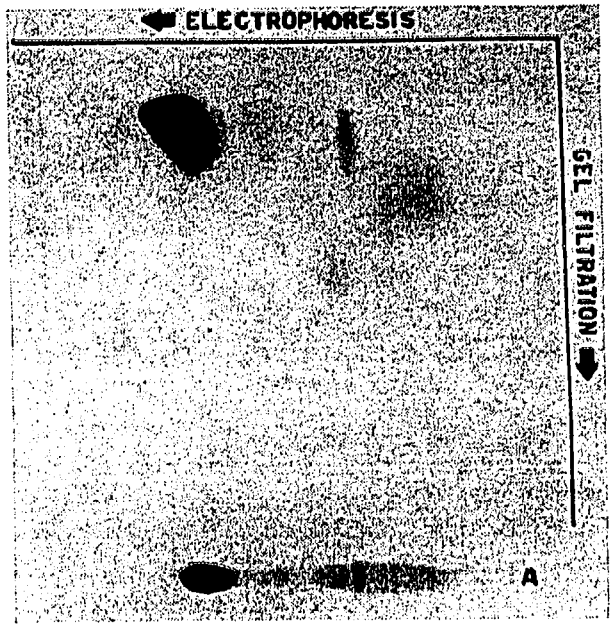
Electrogel filtration: A combination of thin-layer gel filtration on Sephadex G 200 superfine and electrophoresis on cellulose acetate

A fractionation method combining thin-layer gel filtration on Sephadex G 200 superfine with subsequent electrophoresis in a direction perpendicular to that of the filtration has proved of special interest for qualitative studies of serum proteins. It allows the characterization of serum proteins on the basis of their molecular size and electrophoretic mobility.

A recent attempt by JOHANSSON AND RYMO[1] along these lines, using Sephadex G 200, has shown, however, that with this technique the electrophoretic migration of some protein fractions of larger molecular dimensions is considerably delayed, presumably due to the Sephadex frictional effect. This phenomenon is most evident for the α_2M-globulins, which fall into electrophoretic position β. Even using veronal–lactate buffer, of which it is well known that it improves the fractionation of α_2-globulins, we have failed to obtain satisfactory results with JOHANSSON AND RYMO's technique.

In the method described in this paper the serum proteins fractionated by thin-layer gel filtration on Sephadex are transferred by imprinting on a cellulose acetate membrane. The electrophoretic fractionation is then carried out perpendicularly to the direction of the gel filtration, on the cellulose acetate membrane, so as to avoid the Sephadex frictional retardation.



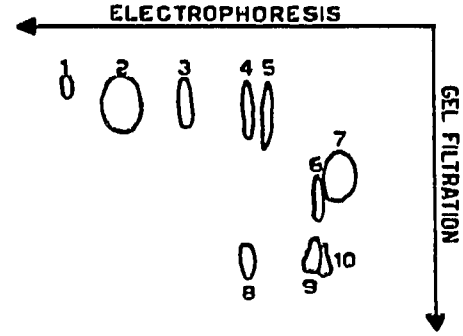


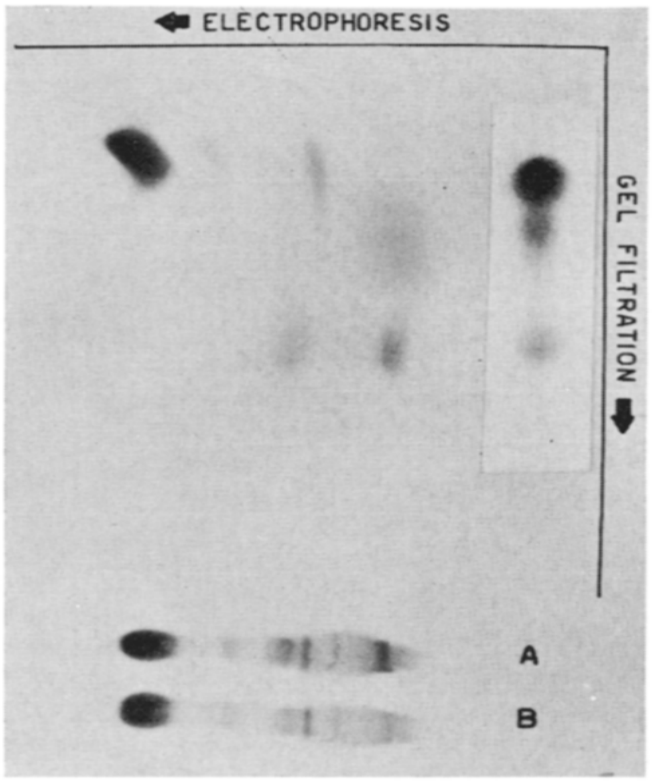
Fig. 1. Electrochromatographic pattern of a normal serum. The electrophoresis is carried out on a cellulose acetate membrane to which the serum proteins are transferred by imprinting after Sephadex gel filtration. A = Reference electrophoresis of the same serum.

Fig. 2. Schematic representation of the normal serum proteins in the electrochromatogram. 1 = Prealbumin; 2 = albumin; 3 = α_1-glycoprotein; 4 = haptoglobin (?); 5 = hemopexin, transferrin; 6 = IgA; 7 = IgG; 8 = α_2-M; 9 = β-lipoprotein; 10 = IgM.

Experimental

Thin-layer gel filtration was performed according to the method of JOHANSSON AND RYMO[2], with slight modifications[3]. Sephadex G 200 superfine was swollen with veronal–lactate buffer, pH 8.6, added in a 1:25 ratio, having the following composition: veronal (diethylbarbituric acid) 1.11 g; sodium veronal 0.7 g; calcium lactate 1.02 g; distilled water to 1 l. The same buffer was also used as eluent for the chromatography. The latter was carried out on 20 × 30 cm plates inclined at 12°. The serum sample (10 μl) was deposited at a distance of 2.5 cm from the top edge of the plate and 8 cm from one of the side edges. Chromatography was continued until the fastest serum fraction had migrated 16 cm from the starting point: this took about 10 h. The speed of migration was adjusted using as marker serum conjugated with fluorescein according to MELLORS[4].

After stopping the run a cellulose acetate membrane of the same dimensions as the plate, previously soaked in the buffer used for the chromatography and dried between two sheets of filter paper, was applied to the gel. After 10 min the membrane was removed, carefully cleaned of any attached gel and laid in a horizontal electrophoretic chamber of suitable dimensions. The electrophoretic troughs contained the veronal–lactate buffer previously described, diluted 1:1.30. A current of 0.3 mA/cm was then applied. The migration was allowed to proceed for 4 h at 4°. At the end of



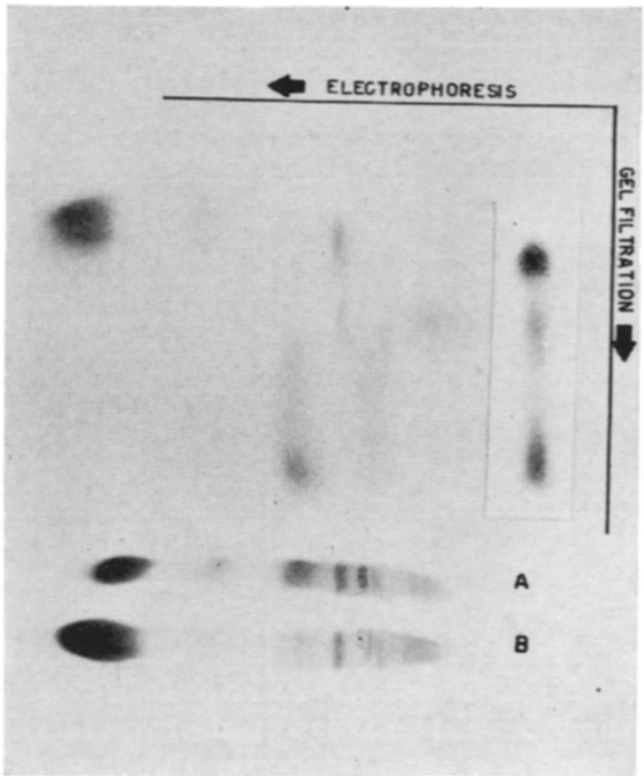
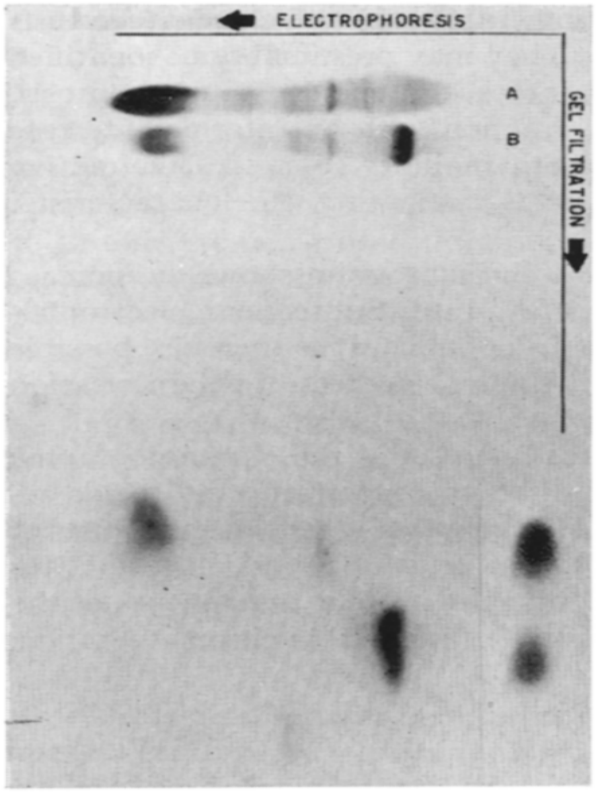


Fig. 3. Electrochromatographic pattern of serum proteins of a subject with macroglobulinemia. A = Electrophoresis of the same serum; B = electrophoresis of a normal serum. On the right the chromatogram after simple gel filtration is shown.

Fig. 4. Electrochromatography of the serum proteins of a subject with nephrosis. A = Electrophoresis of the same serum; B = electrophoresis of a normal serum. On the right the chromatogram after simple gel filtration is shown.



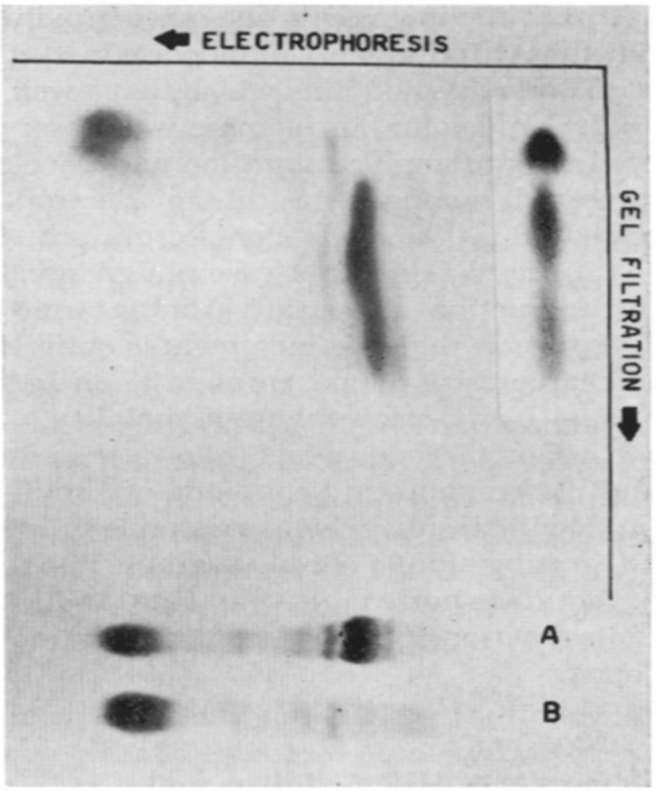


Fig. 5. Electrochromatographic pattern of the serum proteins of a subject with IgG myeloma. A = Electrophoresis of the same serum; B = electrophoresis of a normal serum. On the right the chromatogram after simple gel filtration is shown.

Fig. 6. Electrochromatographic pattern of the serum proteins of a subject with IgA myeloma. A = Electrophoresis of the same serum; B = electrophoresis of a normal serum. On the right the chromatogram after simple gel filtration is shown.

the run the membrane was immersed for 20 min in a 3 % solution of sulphosalicylic acid, to fix the proteins, and stained with Lissamine Green S.F. 150 according to LAURENT[5].

Results and discussion

Electrochromatographic fractionation of a normal serum resolved 10 components (Fig. 1). These appear, arranged in three groups, corresponding from top to bottom to the three fractions—slow, intermediate, fast—(4.5 S, 7 S and 19 S) obtained by thin-layer gel filtration on Sephadex. The first group comprises 5 fractions: starting from the one nearest the anode, these consist of prealbumin (not always visible), albumin, α_1-glycoprotein, haptoglobin and transferrin. The second group, always proceeding from anode to cathode, comprises IgA and IgG fractions. The third group, which corresponds to the most rapidly migrating fraction and hence to that having the largest molecular size, consists of α_2M-globulins, lipoproteins and IgM globulins (Fig. 2). Fig. 3 refers to a macroglobulinemic serum: it shows clearly an increase in a rapidly migrating chromatographic fraction, failing into a β_2 electrophoretic position, corresponding to the homogeneous component present in the electrophoretic pattern of the same serum. It is justifiable to consider this fraction as consisting of IgM immunoglobulins. In a case of nephrosis (Fig. 4) it may be obser-

ved that the increase in the most rapidly migrating fraction in the chromatogram is chiefly attributable to an increase in α_2-globulins. They may presumably be identified with the α_2M-globulins which, as is well known, increase in the course of nephrosis. In IgG myeloma an increase was observed in a fraction with an intermediate rate of chromatographic migration and a γ electrophoretic mobility (Fig. 5). This fraction appeared homogeneous in an electrophoretic field, presenting the characteristic appearance of a single-clone serum protein.

Fig. 6 refers to a case of IgA myeloma. The chromatogram shows an increase in the fractions migrating at an intermediate and fast rate. Subsequent electrophoresis reveals that this increment is entirely due to β_2-globulins. It is therefore possible to characterize the paraprotein as an IgA, having different degrees of polymerization and with a β_2-electrophoretic mobility.

Our method, which allows the simultaneous analysis of the chromatographic and electrophoretic behaviour of serum proteins is easy to perform and yields reproducible results which are simple to interpret. It has proved a very useful means of integrating simple chromatographic analysis on a thin layer of Sephadex: the latter in fact does not enable us to detect which of the different protein components of the three fractions obtained by gel filtration is responsible for possible chromatographic changes.

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