(time required, 200 min). After the chromatogram had been dried (2 h at 40°) two parallel lines were cut in the substrate; one along the film-junction to isolate the resin band, and the other about 10 mm from the opposite edge of the cellulose to isolate the band of dirt in the front.

Third elution: with *tert.*-butanol–acetic acid–water (5:1:1) in a direction at right angles to the first elution, for 160 mm (time required, 210 min). After the substrate had been dried, it was sprayed with a solution of ninhydrin (0.2 % in butanol–acetic acid (4:1)) and the plate was stored in the dark for some hours to allow full development of the colour.

One of us (M.N.C.) thanks the International Wool Secretariat for financial assistance.

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1 J. A. BERGER, G. MEYNIEL, P. BLANQUET AND J. PETIT, *Compt. Rend.*, 257 (1963) 1534; *Bull Soc. Chim. France*, (1963) 2662.
2 D. C. ABBOTT AND J. THOMSON, *Chem. Ind. (London)*, (1965) 301.
3 D. C. ABBOTT, J. A. BUNTING AND J. THOMSON, *Analyst*, 91 (1966) 94.
4 A. MUSGRAVE, *J. Chromatog.*, 36 (1968) 388.
5 F. KRAFFCZYK AND R. HELGER, *Z. Anal. Chem.*, 243 (1968) 536.
6 P. J. WAKELYN, private communication.
7 P. F. LOFTS AND E. V. TRUTER, *J. Textile Inst.*, 60 (1969) 46.

Received September 26th, 1969

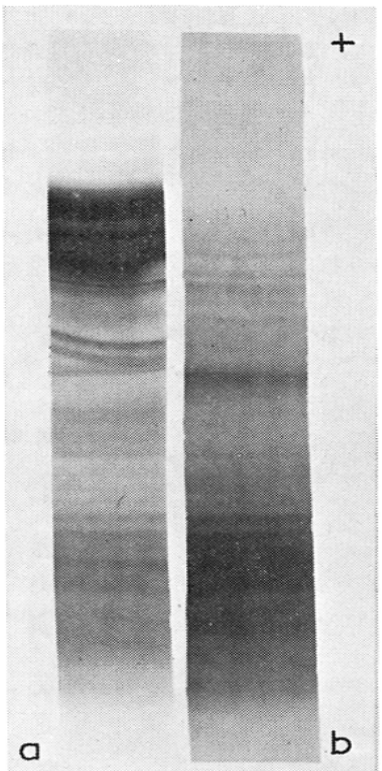
CHROM. 4389

Gel-electrofocusing in combination with immunodiffusion

With gel-electrofocusing[1–5] being used increasingly in protein chemistry as a new purity criterion for isolated proteins, the question of identification of the different zones focused becomes of great importance.

This communication describes a procedure where isoelectric separation of the proteins in polyacrylamide gel is used and the proteins are then identified by a method based on immunodiffusion with specific antisera[6–8]. Electrofocusing was carried out in tubes according to WRIGLY[4] using a conventional disc-electrophoresis apparatus, and on polyacrylamide plates (18 × 8 cm) using an apparatus described by AWDEH *et al.*[5].

Stock solutions for electrofocusing gels were prepared as follows: Acrylamide solution: 30 g acrylamide; 0.8 g N,N′-methylenebisacrylamide; water to 100 ml. Catalyst solution: 1.0 ml N,N,N′,N′-tetramethyl ethylene diamine; 14 mg riboflavine; water to 100 ml.



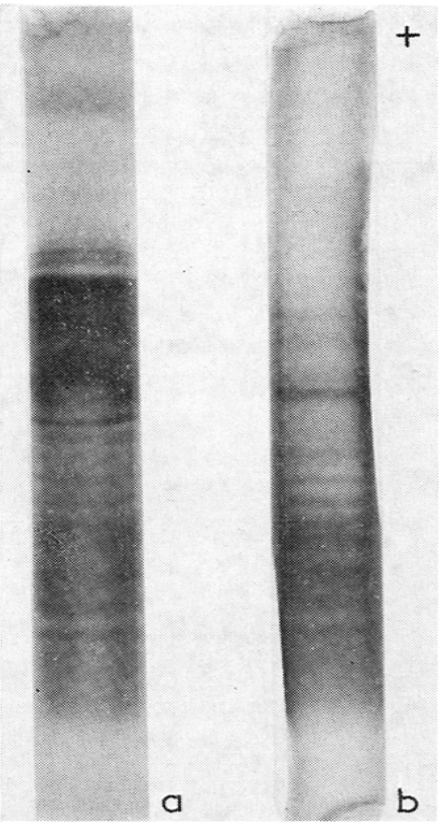


Fig. 1. Isoelectrofocusing and immunodiffusion of normal human serum on polyacrylamide gel plates. (a) isoelectric separation; (b) identical separation followed by incubation with absorbed anti-IgG serum and staining after elution of non-precipitated proteins.

Fig. 2. Isoelectrofocusing and immunodiffusion of normal human serum on polyacrylamide gel tubes. (a) isoelectric separation; (b) identical separation followed by incubation with absorbed anti-IgG serum and staining after elution of non-precipitated proteins.

Plates of polyacrylamide gel (18 × 8 cm) were prepared by polymerizing 4.4 ml normal human serum (diluted 1:50), 17.6 ml water and 11 ml gel mixture between two glass plates, 3 mm apart, by photoactivation for 1 h at 4°. (The gel mixture consisted of 2.4 ml catalyst solution, 0.9 ml ampholyte solution (40 %), pH 3–10, 9 ml acrylamide solution). The starting current was about 200 V and 10 mA. As soon as the pH gradient of carrier ampholytes was established, the current was increased to 300 V and kept constant for 36 h. The gel was then cut in two parts (length ways). One part was immediately immersed in 5 % trichloroacetic acid (TCA) and washed for 12 h to remove the ampholytes, and then stained with Coomassie Brilliant Blue R (ref. 9, Fig. 1a). A 2 mm thick layer of agar was placed on top of the other part of the gel. This agar had been obtained by mixing 50 ml of a 2 % agar solution (veronal buffer, ionic strength 0.025; pH 8.6) with 5 ml of an anti-IgG serum absorbed by using our own specific immunoadsorbent[10] (both solutions being heated to 50° before mixing); the absorbed antiserum reacts with the k and l light chains and the Fc portion of the gamma heavy chain, when tested by immunoelectrophoresis. The gel plus agar was then left at room temperature for 48 h in order that immunodiffusion

could take place. The polyacrylamide gel was then freed from the agar layer and washed for 3 days in physiological saline and a further day with 5 % TCA, before staining with Coomassie Brilliant Blue R (Fig. 1b); the TCA pretreatment improves the staining.

In the electrofocusing experiments, 0.5 ml gel mixture, 0.1 ml ampholyte (40 %) pH 3–10, and 0.9 ml of normal human serum (diluted 1:50) were polymerized by photoactivation for 1 h at 4° in 1.5 ml glass tubes (6 mm diam.). (Gel mixture: 3.0 ml acrylamide solution, 0.8 ml catalyst solution.) A current was then passed through the gel column for 3 h, starting with 1.5 mA for each gel. Twelve tubes were run simultaneously in each experiment. After focusing, the gels were removed from the tubes and treated as follows: two were immersed in 5 % TCA, washed for 12 h and stained with Coomassie Brilliant Blue R (Fig. 2a); the other ten gels were divided into pairs and immunodiffusion was carried out. Each pair was treated with 2 ml of a different specific antiserum in an 8 mm diameter tube and incubated for 24 h. The five antisera used were as follows: anti-IgG, anti-IgG/γ, anti-IgA, anti-IgM and anti-human serum albumin (HSA). Anti-IgG serum was the same as described above, the other antisera against immunoglobulins reacted only with the corresponding heavy chains, *i.e.* γ, α and μ respectively. After subsequent washing for 3 days in physiological saline and another day in 5 % TCA, they were stained with Coomassie Brilliant Blue R.

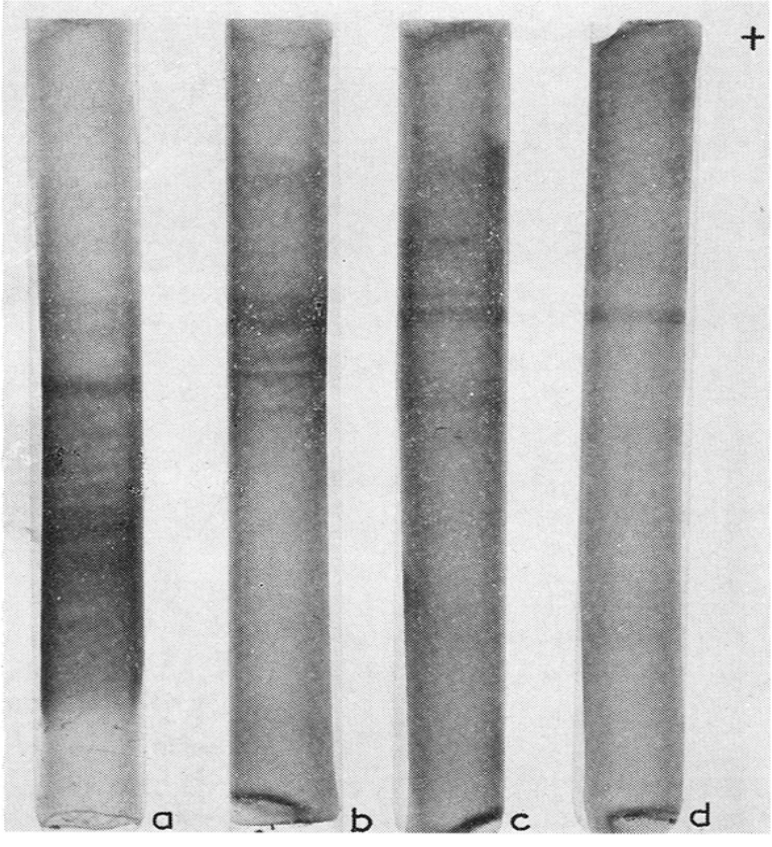


Fig. 3. Isoelectrofocusing of normal human serum and immunodiffusion with different antisera on polyacrylamide gel tubes. (a) incubation with anti-IgG/γ serum; (b) incubation with anti-IgA serum; (c) incubation with anti-IgM serum; (d) incubation with anti-human serum albumin.

Immunoelectrofocusing patterns of IgG in normal human serum on the plates, developed with anti-IgG antibodies are shown in Fig. 1b in comparison to all serum proteins detected by TCA precipitation in Fig. 1a. Comparable results were obtained when the same system was applied to polyacrylamide tubes (Fig. 2a, b). However, it must be borne in mind that in both these cases not only the IgG but also other immunoglobulins are detected by the antiserum used which also possesses antibodies against common light chain moieties.

Precipitation bands corresponding exclusively to IgG, IgA and IgM immunoglobulins, respectively, were developed with specific anti-heavy chain sera (Fig. 3a, b, c); when these three patterns are overlapped in a logical sequence the same picture is obtained as that shown in Fig. 2b. A distinct heterogeneity was found not only in all three main immunoglobulins, but it can be shown that HSA can be separated into at least four fractions under same conditions (Fig. 3d).

The heterogeneity of human IgG immunoglobulins has also been studied using the electrofocusing method[11]; in this case the IgG was separated from the serum and electrofocused after further purification. The detection of IgG micro fractions in whole normal serum, without isolation, has been made possible by the above method, and avoids the possible alteration of the IgG molecules during the treatment. The above findings have demonstrated the microheterogeneity of all the proteins studied. The occurrence of numerous IgG immunoprecipitation bands is of particular interest. It is expected that this method will permit further identification of separated protein fractions. The above method and some results will be published in detail elsewhere.

This work was supported by the Swiss National Foundation for Scientific Research.

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1 J. S. FAWCETT, *FEBS Letters*, 50 (1968) 81.
2 D. H. LEABACK AND A. C. RUTTER, *Biochem. J.*, 108 (1968) 19 p.
3 G. DALE AND A. L. LATNER, *Lancet*, No 7547 (1968) 847.
4 C. WRIGLY, *Sci. Tools*, 15 (1968) 2.
5 Z. L. AWDEH, A. R. WILLIAMSON AND B. A. ASKONAS, *Nature*, 219 (1968) 66.
6 P. GRABAR AND C. A. WILLIAMS, *Biochim. Biophys. Acta*, 10 (1953) 193.
7 O. ZWISLER AND H. BIEL, *Behringwerk-Mitt.*, 46 (1966) 129.
8 N. CATSIMPOOLAS, *Immunochem.*, 6 (1969) 501.
9 J. URIEL, *Bull. Soc. Chim. Biol.*, 48 (1966) 969.
10 S. CARREL, H. GERBER AND S. BARANDUN, *Nature*, 221 (1969) 385.
11 E. VALMET, *Sci. Tools*, 15 (1968) 1.

Received September 24th, 1969