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Note

Immunoreactive precipitation of protein components after isoelectric focusing

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Reaction with specific antibody is extensively used to detect specific components of a protein mixture separated by electrophoresis[1] and by isoelectric focusing[2–5]. Alternatively, antigens have been used to detect specific antibodies in a complex mixture of proteins after similar fractionation procedures[5,6]. We have found that none of these procedures is conveniently simple when all the components of an isoelectric focusing separation are to be tested against a variety of reagents (antibodies in our specific case). We describe here a modification of previous procedures based on the use of strips of paper soaked with antibody (or antigen) applied onto the gel plates at the end of the electrophoretic fractionation. The main advantage of the modification is its simplicity and the fact that several antisera can be tested *in situ* and simultaneously against all the fractionated components of a single sample after separation by isoelectric focusing. This easily allows both location and characterisation of individual bands which may be critical in some experiments[7].

In our experiments isoelectric focusing was carried out in polyacrylamide gels (6%) in the pH range 3–10 (ref. 7).

The sample (100 μl) to be analysed was applied to a piece of Whatman No. 1 paper (1 × 3 cm) which was then laid on the electrofocusing slab (15 × 20 cm) near the anode (Fig. 1). Focusing was for 1 h at 250 V followed by 21 h at 400 V at 4° in a humidified atmosphere. The paper was then removed and strips of Whatman No. 3MM paper (0.4 × 18 cm) impregnated with different antisera placed on the gel parallel to its long side, thus intersecting the position of sample application and separated bands at right angles (Fig. 1). Antisera were diluted 1 in 10 (unless stated otherwise) in 0.1 *M* KPO_4 pH 7 and 0.25 ml was applied to each strip. The plate was then incubated at 37° in a humidified atmosphere for 24 h (plastic sandwich box moistened with saturated paper tissue in the base).

The strips of paper were then removed and the plate directly observed (see Fig. 2, sample 3) or, in the case of radioactive samples, dried without washing, under a domestic fan at room temperature. Autoradiographs were obtained by exposing the side of the plate containing the dry gel onto autoradiographic film, Blue Brand BB54 (Kodak). In the experiments described in Fig. 2 (samples 1 and 2) where immunoglobulins secreted by myeloma cells were labelled with [^{14}C]lysine[7,8] the

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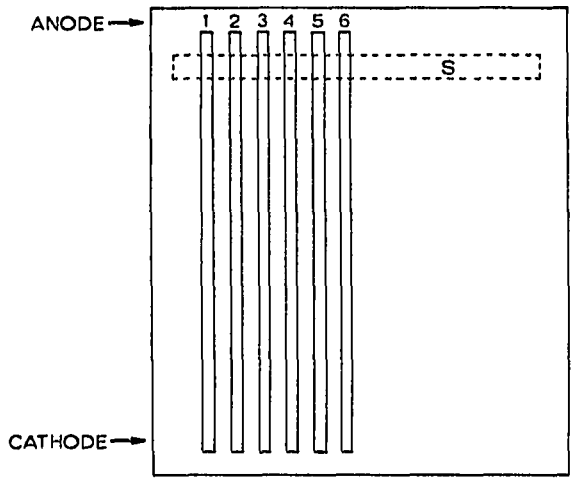

Fig. 1. Layout of gel slab for immunofixing experiments. S indicates the position where the sample was applied prior to electrofocusing. The numbers refer to several strips impregnated with antiserum applied at the end of electrofocusing.

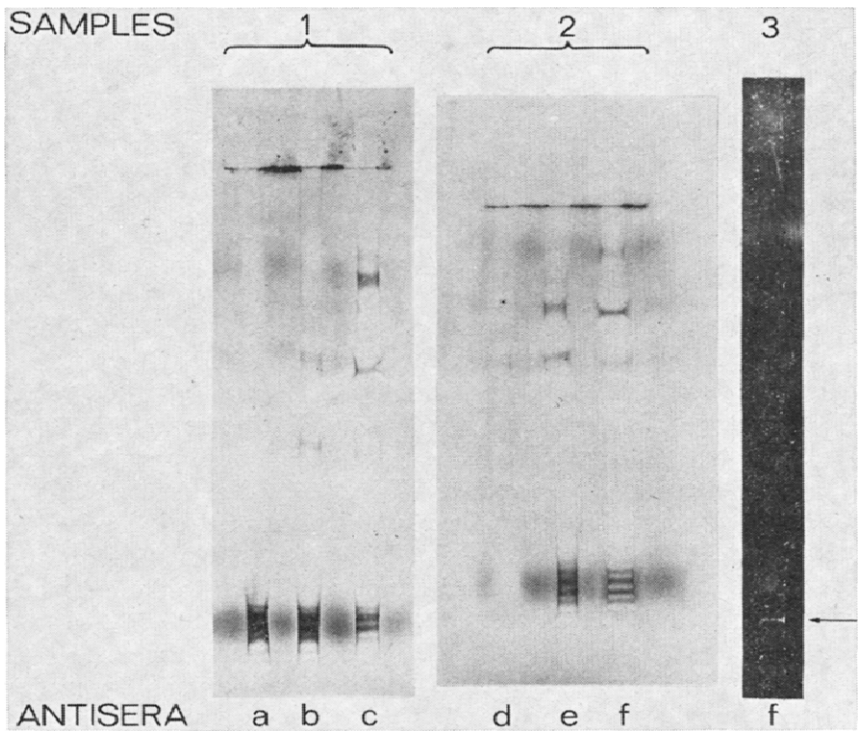

Fig. 2. Analysis of components separated by isoelectric focusing using different antisera. Samples 1 and 2 are unpurified [^{14}C]lysine-labelled, secreted myeloma protein of a mouse-rat hybrid clone (HYIII-2)[7]. Sample 2 is serum (10 μl) from a Balb/C mouse carrying an MOPC 21 tumour mixed with 90 μl Dulbecco's medium[8]. The antisera are: (a) antimouse heavy chains; (b) antimouse $\varkappa$ light chain; (c) antirat (210) light chains; (d–f) antimouse heavy and light chains diluted 1 in 100, 1 in 10, and undiluted, respectively.

exposure time was 1–2 days. Where the antisera reacted with the separated bands under the paper they were fixed in the position established by focusing. When the quantity of protein was sufficient, the actual precipitated band could be seen directly over a dark background (Fig. 2, sample 3, marked with an arrow). The plate can be stained with protein dyes after extensive washings as is conventionally done to visualize immunoprecipitin reactions. When radioactive samples were used, and as the plate was not washed, the diffused bands not under the paper could still be seen at either side of the paper strip (Fig. 2, samples 1 and 2). Where the antisera did not react with focused bands these diffused into the paper and were removed with it. Thus a negative reaction is indicated by decrease of the radioactivity in the region which had been under the paper (*e.g.* upper bands, strip a). Thus both positive and negative interactions are emphasized by the diffuse radioactivity of regions at either side of the position of antiserum strip.

The effect of antiserum concentrations on the fixing of bands was tested (Fig. 2). It can be seen that in our experiments the bands are more effectively fixed with undiluted serum. Sometimes (Fig. 2, sample 2f), precipitin arcs form having their apex at the edge of the fixed band. This arc can be used to obtain some indication of the ratio of antibody to antigen. Thus when lower concentrations of antisera are used the arcs form a continuous line parallel to the side of the paper and the actual band becomes more diffuse.

Although this procedure was used for isoelectric focusing it is believed it is also applicable for zone electrophoretic separation. The technique is particularly useful when a variety of antisera are to be compared simultaneously after a single fractionation.

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REFERENCES

1 M. D. Poulik, *Ann. N.Y. Acad. Sci.*, 121 (1964) 470.
2 R. F. Riley and M. K. Coleman, *J. Lab. Clin. Med.*, 72 (1968) 714.
3 N. Catsimpoolas, *Clin. Chim. Acta*, 23 (1969) 237.
4 S. Carrel, L. Theilkaes, S. Skvaril and S. Barandun, *J. Chromatogr.*, 45 (1969) 483.
5 A. H. Good and B. B. Ceverha, *J. Immunol.*, 106 (1971) 1677.
6 A. R. Williamson, *Eur. J. Immunol.*, 1 (1971) 390.
7 R. G. H. Cotton and C. Milstein, *Nature (London)*, 244 (1973) 42.
8 R. G. H. Cotton, D. S. Secher and C. Milstein, *Eur. J. Immunol.*, 3 (1973) 135.