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Towbin H, Staehelin T & Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Nat. Acad. Sci. USA* 76:4350-4, 1979.
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A method for transferring proteins from polyacrylamide slab gels onto nitrocellulose membranes by transverse electrophoresis is described. The protein pattern is faithfully preserved, and the proteins adhere firmly to the membrane, where they remain readily accessible to antibodies or other ligands. [The *SCI*® indicates that this paper has been cited in over 7,440 publications, making it the most-cited paper from this journal.]

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I came to Julian Gordon's laboratory at the Friedrich Miescher Institute as a postdoctoral fellow to raise antibodies against eukaryotic ribosomal proteins: initially by conventional methods in goats and later by the hybridoma technique (which at that time had started to spread from the pure immunologists' realm to other fields of research). The hybridoma technique was suggested by Theo Staehelin, who was with Hoffmann-La Roche at the time. He also introduced us "newcomers" to other modern immunological techniques. I was happy that I did not have to inject and bleed the lovely goats—a rare black-and-white breed from the Alps.

The hybridoma technique offered us the prospects of immunizing with impure material and still obtaining an antibody specific for 1 of approximately 70 different ribosomal proteins. However, we still needed an efficient means of characterizing the monoclonal antibodies that we would get. Because they lack function when dissociated from the ribosome, ribosomal proteins had been defined by their position on two-dimensional gels, and it seemed most straightforward to allow the antibodies to react with such two-dimensionally separated proteins on the gel. The matrix of these gels was, however, very dense, and we did not expect that antibodies would readily diffuse into and out of such gels.

The idea of using replicas of gels to avoid these problems was certainly inspired by the example of DNA-blotting introduced by E.M. Southern.[1] In addition, from binding assays used in ribosome research, we knew that polypeptides would readily bind to nitrocellulose filters. We discussed various methods of obtaining replicas, and the idea of some electrophoretic elution transpired. In Gordon's laboratory an electophoretic destainer was used for destaining our numerous polyacrylamide gels. Since protein stains are charged, the excess dye moves out of the gel in the electric field maintained by the apparatus. Hence, the gel clears in a matter of minutes, as compared to hours by simple diffusion.

This impressive acceleration gave me the idea of also trying to elute proteins by transverse electrophoresis. The apparatus was very much in use and quite dirty from the residual Coomassie Blue. Still, one day I cleaned it and assembled gel and nitrocellulose into a sandwich supported by a frame constructed from the ubiquitous plastic waste material generated in all cellular laboratories. We were quite excited at how efficiently this electrophoretic elution worked for our proteins. More importantly, transferred proteins reacted with our antibodies. Among the various methods that we tested to visualize the bound antibody, enzyme amplification was particularly pleasing since colored bands developed in a manner reminiscent of a photographic process proceeding in daylight.

Methods for preparing protein replicas from gels have been independently developed by several groups and were published in 1979 and 1980.[2] The rapid acceptance of our procedure and its frequent citation may be due to its technical simplicity, its publication in a widely read journal, and the "snowball" effect provided by important publications quoting our method. Methods papers in immunochemistry are of interest to an extremely wide range of potential users, reflecting the spread of immunochemical techniques to all areas of the medical and biological sciences.

It is rewarding that the principle outlined in the original paper has been retained in the many modifications later introduced to adapt particular problems. It is even more rewarding to see the method being extended to unanticipated fields, such as the use of blotted proteins for sequencing, immunizations, or antibody purification. Several reviews covering the classical applications as well as novel uses have been published.[3-5]

1. **Southern E M.** Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* 98:503-17, 1975. (Cited 13,150 times.)
2. **Towbin H & Gordon J.** Immunoblotting and dot immunobinding—current status and outlook. *J. Immunol. Method.* 72:313-40, 1984. (Cited 120 times.)
3. **Beisiegel U.** Protein blotting. *Electrophoresis* 7:1-18, 1986.
4. **Bers G & Garfin D.** Protein and nucleic acid blotting and immunobiochemical detection. *BioTechniques* 3:276-88, 1985.
5. **Gershoni J M & Palade G E.** Protein blotting: principles and applications. *Anal. Biochem.* 131:1-15, 1983. (Cited 245 times.)