- Ikeda, M., Conney, A. H. & Burns, J. J. (1968). *J. Pharmac. exp. Ther.* **162**, 338.
- Kato, R. & Gillette, J. R. (1965). *J. Pharmac. exp. Ther.* **150**, 279.
- Omura, T. & Sato, R. (1964). *J. biol. Chem.* **239**, 2370.

Some Modifications to the Technique of Two-Dimensional Immunoelectrophoresis

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The technique of two-dimensional immunoelectrophoresis as described by Minchin-Clarke & Freeman (1968) is very effective for the quantitative analysis of a mixture of proteins. The method consists of running the mixture first in a strip of agarose on a 10 cm × 10 cm glass plate, when a partial linear separation of the proteins occurs. The strip is then transferred to a second plate of similar size, the empty portion of the plate being filled with agarose containing an appropriate mixture of antibodies. The current is run at right-angles to the previous direction and the antigens are driven into the bed of antiserum. This results in the formation of antigen-antibody curves, the area under which is proportional to the amount of the protein present.

The choice of 10 cm × 10 cm glass plate appears to limit the use of the method, since it conditions the volumes of expensive reagents and the size of equipment. However, the size of the plate can be reduced to one-quarter of the original size without loss of clarity or detail. As a result, the electrophoretic tank can be completely redesigned to produce a more compact and flexible unit in which the volume of barbitone buffer is reduced to 10% of that originally used.

Redesigning the tank necessitates a change in the preparation of the plate, in that the two dimensions have to be prepared before commencing electrophoresis. This involved the construction of a new pattern of carrier and wick-electrode system.

A further improvement, which involved superimposition of plates one on top of the other, made it possible to increase the number of plates developed in a single run three- or four-fold.

Minchin-Clarke, H. G. & Freeman, T. (1968). *Clin. Sci.* **35**, 403.

The Low-Molecular-Weight Components of Bovine Skeletal Myosin

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Low-molecular-weight components (light components) released from rabbit skeletal myosin by chemical modification, urea and alkali treatment have been well characterized and more recently implicated in the functioning of the intact myosin molecule (Stracher, 1969). The presence of light components in myosins of other species has been demonstrated (Locker & Hagyard, 1967; Perrie, Perry & Stone, 1969) and an examination of those of bovine skeletal myosin was therefore made.

Myosin was prepared from muscle in rigor and was free of nucleoprotein and adenylate kinase activity. Low activities of adenylate deaminase present could be removed by batch treatment with DEAE-cellulose. No cathepsin-like proteolytic activity (Gordon & Zak, 1963) could be detected. On chromatography on DEAE-cellulose, Sephadex G-200 and Sephadex G-100 and on ultracentrifugation the preparation appeared as a single component. Amino acid analyses, molecular weight (540 000, by sedimentation-diffusion) and enzymic characteristics closely correspond to those of rabbit (J. Thomas, unpublished work).

Acetylation, maleylation or treatments with 0.1 M-Na₂CO₃, 1% sodium dodecyl sulphate or 8 M-urea were shown to release the light components. The acetylated and alkali-treated forms (best characterized in rabbit) and the maleylated form were examined in more detail. Light components obtained by acetylation and maleylation treatments were separated from the high-molecular-weight material (heavy components) by (NH₄)₂SO₄ fractionation, whereas the light components from alkali treatment were separated by dilution. In no case was the separation complete: the light components were contaminated by the heavy component. Further purification on Sephadex G-100 gave a component representing about 10% of the total protein that had consistent amino acid analyses and gave a single peak ($s_{20,w}^0$ 1.6S) on ultracentrifugation. The results of polyacrylamide-gel (7.5%) electrophoresis at pH 9.0 were complex, but in light components prepared with thiol groups protected by *N*-ethylmaleimide three major bands were detected.

The acetylated, maleylated and alkali-treated light components behaved differently on Sephadex G-100 calibrated for molecular weight and Stokes radius (the latter derived from diffusion coefficients of standard proteins): each gave a single peak, though at different elution volumes. In view of the sedimentation data and the work of Habeeb (1967)