permit passage of wicks, and inside which is a supporting frame to hold the threads. It was found that a plastic cover-slip box, 70 mm · 43 mm · 22 mm made a convenient external chamber and the supporting frame was made from pieces of dust-comb held at the correct distance by a piece of Perspex. The apparatus is illustrated in Figure 2. Fine threads can be cut from moist sheets of cellulose acetate with a sharp scalpel. For the electrophoretic separation the threads, after soaking in buffer, are slotted into the supporting frame and the ends are held in place by a small amount of 1.5% agar in the appropriate buffer, which also serves as a bridge between the threads and the wicks which dip into the electrode compartments. The most satisfactory wicks were found to be pieces of muslin coated with 1.5% agar in buffer. The whole apparatus is set up in a Shandon electrophoresis tank, which serves as buffer vessel and humidity chamber. The sample is applied to the threads from a fine capillary pipette, about 0.01 µl being an adequate volume. After the run the threads are transferred to microscope slides covered with 2 ml of 1.5% agar in phosphate-buffered saline, pH 7.2. Similar threads soaked in antiserum are placed on the agar parallel to the antigen threads at a distance of 2.5 mm and immunodiffusion is allowed to take place.

Results. Both of these techniques have been applied to the study of the soluble antigens of the chick lens. In the case of the two-dimensional electrophoresis the buffers used were the high resolution buffer of ARONSSON and GRÖNWALL[12] at pH 8.9 and a similar buffer adjusted to pH 7.9 by the addition of HCl. The first electrophoresis was at pH 7.9 for 16 h at 2.1 v/cm, the low voltage being necessary to avoid excess current. The second electrophoresis was at pH 8.9 for 2 h at 6.0 v/cm. The antigens were allowed to react with antiserum to adult chick lens. The results obtained are shown diagrammatically in Figure 3, a–t. The results of one dimensional immunoelectrophoresis at each of the 2 pH values are shown in Figure 3, u and v. By combining the results of the two-dimensional immunoelectrophoreses it is possible to construct a diagram (Figure 3, w) in which 14 immunoprecipitin lines can be clearly resolved, whereas the single-dimensional immunoelectrophoreses show only 8–9 lines distinctly. Essentially similar results were obtained when the 2 buffers were used in the opposite order.

With the micro-immunoelectrophoresis the pH 8.9 buffer system used was as described above[12]. The sample applied contained 2.8 µg of lens proteins and the separation was for 2 h at 10 v/cm. The antigens were allowed to react with antiserum to adult chick lens. The results are illustrated in Figure 4 which shows a comparison with results obtained by the normal method of immunoelectrophoresis devised by SCHEIDEGGER[13] which uses 20 µg of starting material. It can be seen that the micro-method clearly demonstrates the major components of the antigen system.

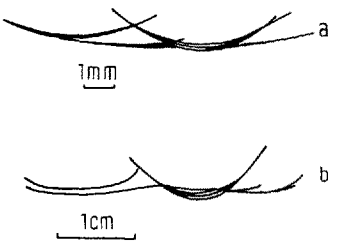

Fig. 4. Diagram of results of micro-immunoelectrophoresis of chick lens extract (a) compared with the results obtained by the method of SCHEIDEGGER (b). Note the difference of scale. The anode was to the left in both cases.

Résumé. On décrit 2 modifications apportées à la méthode d'immunoélectrophorèse. L'une d'elles comprend l'électrophorèse dans 2 sens, aux pH divers, en milieu gélifié suivie par l'analyse immunologique, permettant une définition plus avantageuse. L'autre comprend l'électrophorèse sur un fil de rayonne ou d'acétate de cellulose suivie par l'analyse immunologique en milieu gélifié, permettant l'analyse des quantités d'antigène à partir de 3 µg.

D. E. S. TRUMAN, R. M. CLAYTON, and J. C. CAMPBELL

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