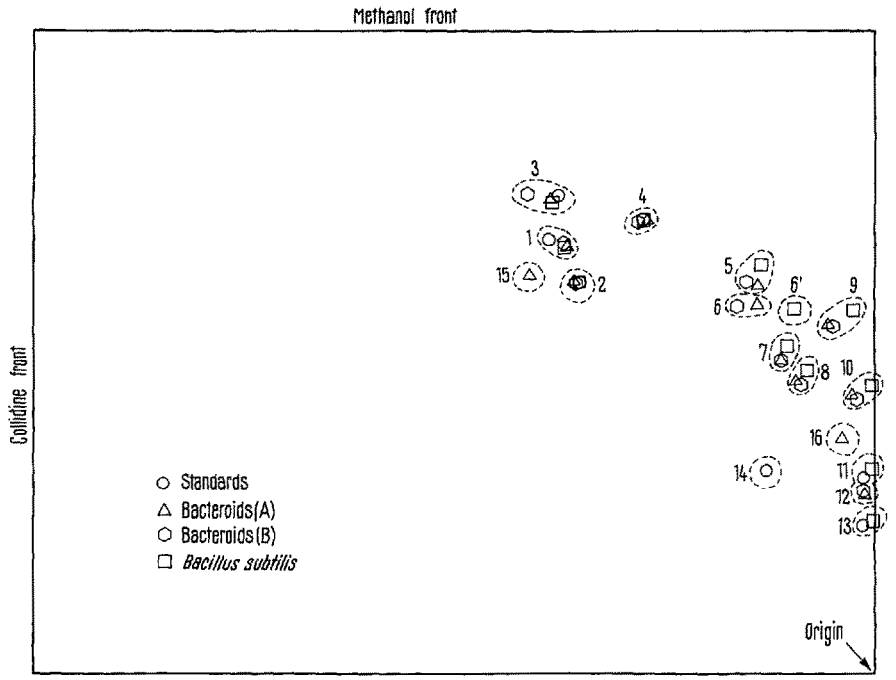

Fig. 3. 2-dimensional chromatograms of standards and bacteroid and *B. subtilis* hydrolysates superimposed here for purposes of comparison. (1) muramic acid, (2) glucosamine, (3) leucines, (4) valine, (11) LL-diaminopimelic acid, (13) meso-diaminopimelic acid, (14) cystine. Identity of 5–10, 12 and 15 is unknown.

bacteroids does not restrict them to the Eubacteria. However, considering these data together with studies of bacteroid fine structure, we are left with no doubt as to the bacterial nature of these cockroach symbiotes[21].

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Zusammenfassung. Papierchromatographisch wurde Murein in symbiontischen Bakteroiden der *Periplaneta americana* nachgewiesen. Die Murein-Komponente Glukosamin und der Murein-Anzeiger Muramic-Säure wurden mittels Bakteroid-Hydrolysaten identifiziert. Das Vorhandensein von α-ε-Diaminopimelic-Säure jedoch konnte nicht einwandfrei festgestellt werden.

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PRO EXPERIMENTIS

Methods for Increasing Sensitivity in Immunoelectrophoresis

Introduction. In the course of studies of the antigens of the vertebrate lens it was found advantageous to develop methods of immunoelectrophoresis giving increased resolution in 2 respects: firstly, the resolution of antigens whose arcs are superimposed, and secondly, the resolution of extracts available only in very small quantities. The superimposition of arcs in immunoelectrophoresis is due to a number of factors, primarily the possession of identical electrophoretic mobilities by different protein fractions and overlapping zones of optimal combination of several antigen/antibody complexes. Several methods of overcoming these difficulties and increasing the resolution of components in a mixture have been reported[1–9]. The majority of these methods rely on the use of an additional supporting medium for electrophoresis, which has the effect of gel filtration. However, this may so alter the position of antigens in the run that comparison with standard immunoelectrophoresis becomes difficult. In order to improve resolution but permit recognition by mobility, regions were cut from an electrophoresed gel and rerun at a different pH, thus resolving antigens whose electrophoretic mobility in the first run was similar.

Methods have also been devised for the immunoanalysis of small quantities of starting material, e.g. immunoelectrophoresis on cellulose acetate[10]. However, this is not ideal in that it is not possible to follow the progress of arc formation and it was considered that a method which would prevent lateral diffusion of the electrophoresing material would increase the likelihood

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of detection of minor components. Based on EDSTRÖM's use of rayon threads for electrophoresis of nucleotides[11], the method devised uses electrophoresis of the antigens on fine strips of cellulose acetate or rayon threads which are then embedded in agar in which the immuno-diffusion takes place.

Methods. (a) Two-dimensional immunoelectrophoresis. Electrophoresis in the first dimension is carried out on a standard microscope slide in 1.5% agar dissolved in the first buffer. The origin is a slot 2 mm · 12 mm. After the electrophoretic separation the agar is cut into strips 2 mm wide, parallel to the direction of electrophoresis. One of these strips is used as the origin for the second electrophoresis by transferring it to a glass plate 8 cm · 8 cm and then covering the plate with 1.5% agar dissolved in the second buffer. Electrophoresis is then carried out at right angles to the length of the embedded strip. After the second electrophoretic separation the agar is cut into strips 2 mm wide parallel to the direction of the electrophoresis. The strips are then transferred to microscope slides, two being placed on each slide 11 mm apart. 1.5% agar dissolved in phosphate-buffered saline, pH 7.2, is poured round the strips and a slot 2 mm · 60 mm is cut in the agar between and equidistant from the embedded strips. The slot is filled with antiserum and immuno-diffusion is allowed to proceed. The method is illustrated in Figure 1. After precipitin line formation is complete the slides can be washed, dried and stained. Strips obtained from the first run can be used in a direct immuno-diffusion test of the separation obtained at the first pH.

(b) Micro-immunoelectrophoresis. The electrophoresis apparatus consists of an external chamber, slotted to

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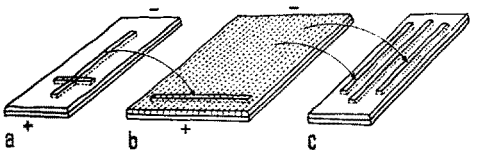

Fig. 1. Illustration of two-dimensional immunoelectrophoresis. Material to be separated is placed in slot shown in (a). After the run the strip is cut as indicated, transferred to the plate and surrounded by agar at second pH as shown in (b). After the second run at right angles to the first the agar on the plate is cut into strips as indicated which are transferred in pairs to microscope slides. Each pair is surrounded by agar at pH 7.2 and a slot is cut in the agar between the strips as shown in (c). The slot is filled with antiserum.

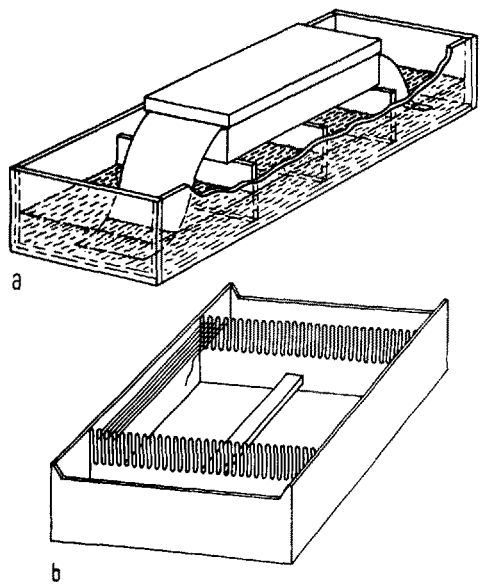

Fig. 2. Apparatus used for micro-immunoelectrophoresis. In (a) the apparatus is shown connected by wicks to the electrode compartment of the tank. In (b) the arrangement of the supporting frame in the box is seen and the position of the cellulose acetate threads is indicated.

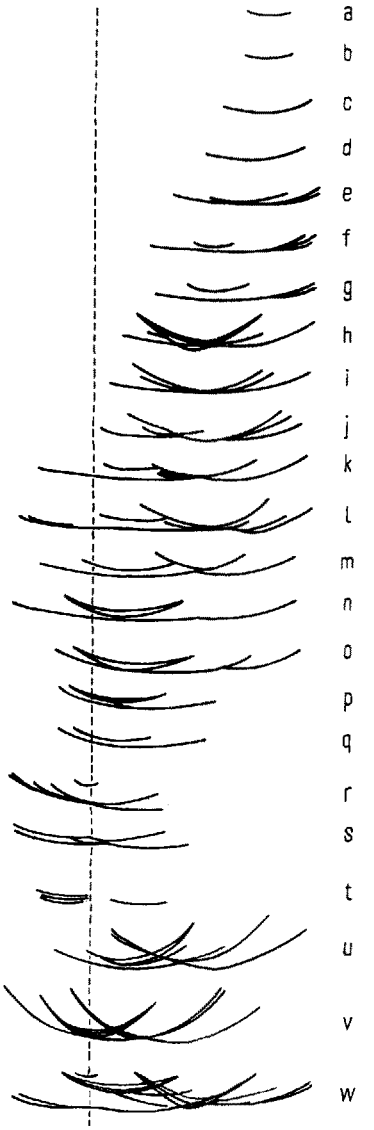

Fig. 3. Diagram of results of two-dimensional immunoelectrophoresis of chick lens extract. First separation at pH 7.9 and second separation at pH 8.9. Antigens allowed to react with antiserum to adult chick lens. Diagrams (a-t) represent immuno-precipitin lines after second run. The cathode end of the first run is at (a) and anode end at (t). Diagram (u) illustrates immuno-precipitin lines obtained after running at pH 7.9 only and diagram (v) lines obtained after running at pH 8.9 only. Diagram (w) is a composite picture of lines found in diagrams (a–t).

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.

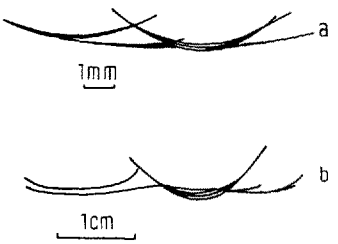

Fig. 4. Diagram of results of micro-immunoelectrophoresis of chick lens extract (a) compared with the results obtained by the method of SCHEIDEGGER (b). Note the difference of scale. The anode was to the left in both cases.

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.

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CONGRESSUS

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