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Editorial

IMMUNODISC ELECTROPHORESIS AND ITS APPLICATION

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Before Immunodisc Electrophoresis, a recent developed technic (4), is discussed. It will be worth-while to review shortly about electrophoresis, immunodiffusion, immunoelectrophoresis, and disc electrophoresis to get some ideas about the principles of each technique and how and when these techniques were developed.

1. **Electrophoresis.** Electrophoresis is a method for separating charged molecules in an electrical field. Each charge molecule will move depend upon the total charge of its molecule. If it has positive charge it will move to negative pole (cathode) and if it has negative charge it will move to positive pole (anode).
2. **Immunodiffusion.** Immunodiffusion is a method developed for antigen-antibody reaction in a medium, usually agar. When antigen was introduced into a well in a media (e. g. agar, polyacrylamide gel, starch, etc.) and the antibody in the other well. They will diffuse towards together. At the equivalence zone, a precipitin line will develop.
3. **Immunoelectrophoresis.** Immuncelectrophoresis is a technique developed by Grabar and Williams about twenty years ago. The principle of this technique is the combination of agar-precipitation and electrophoresis in a single experiment.
4. **Dics Electrophoresis.** Dics Electrophoresis was a technique developed by Ornstein and Davis about ten years ago. This technique is widely used to characterized and resolve complex mixtures of proteins such as bacterial toxins, mycotic protein, tissue extracts, purified hormones and enzymes (5). The high resolution of this technique is depend upon the molecular sieving and electrophoretic separation which operate simultaneously (3). Polyacry-

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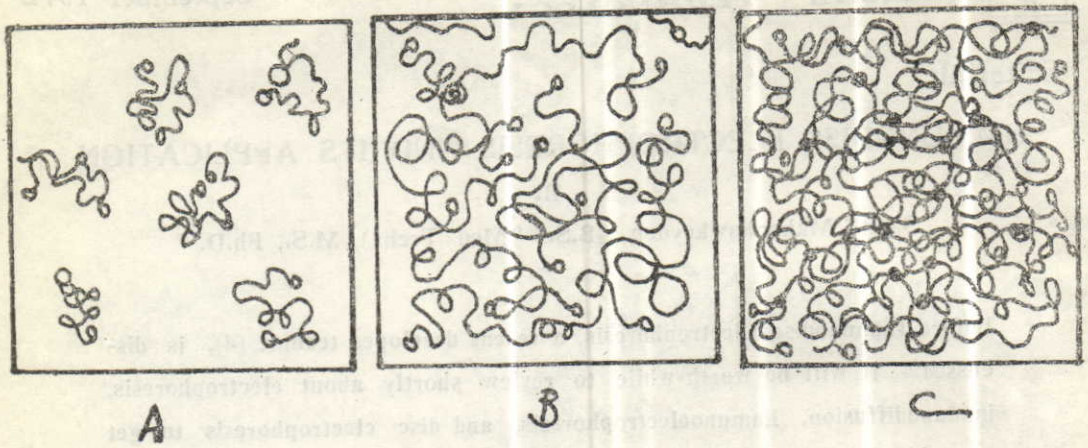


Figure I Schematic representation of the formation of polyacrylamide gel from random gel coils. Transition from a dilute polymer solution (A) through the concentrated solution (B) to the gel (C). -o- cross-linking agent; -o- tie-points.

lamide gel was used in dics electrophoresis because this gels are thermostable, transparent, strong, and relatively inert chemically and can be prepared with a large range of average pore sizes (6).

Polyacrylamide gel is the polymerization and cross-linking product of the monomer acrylamide, CH_2=CH-CO-NH_2, and a cross-linking comonomer, N,N'-methylene-bis-acrylamide (Bis), CH_2=CH-CO-NH-CH_2-NH-CO-CH=CH_2. The three-dimensional network of the gel is formed by cross-linking of polyacrylamide chains growing side-by-side by the mechanism of vinyl polymerization. This leads to the development of numerous, random polymer gel coils (Fig. IA) in which the polyacrylamide chains assume a state of maximum entropy i.e. the most irregular shape. The growing coils move together (Fig. IB) and are cross-linked by main valencies (Fig. IC), where bifunctional compounds, such as N,N'-methylene-bis acrylamide, are built into the polymer chains as cross-linking agents and can react with free functional groups at terminals of other chains. The chemical structure of the gel is shown in Fig. II.

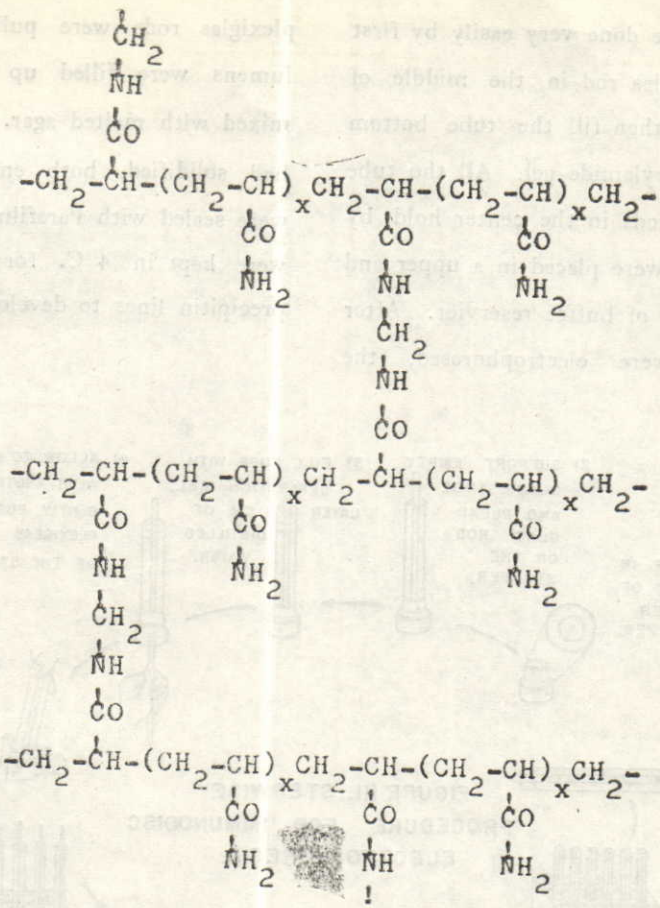
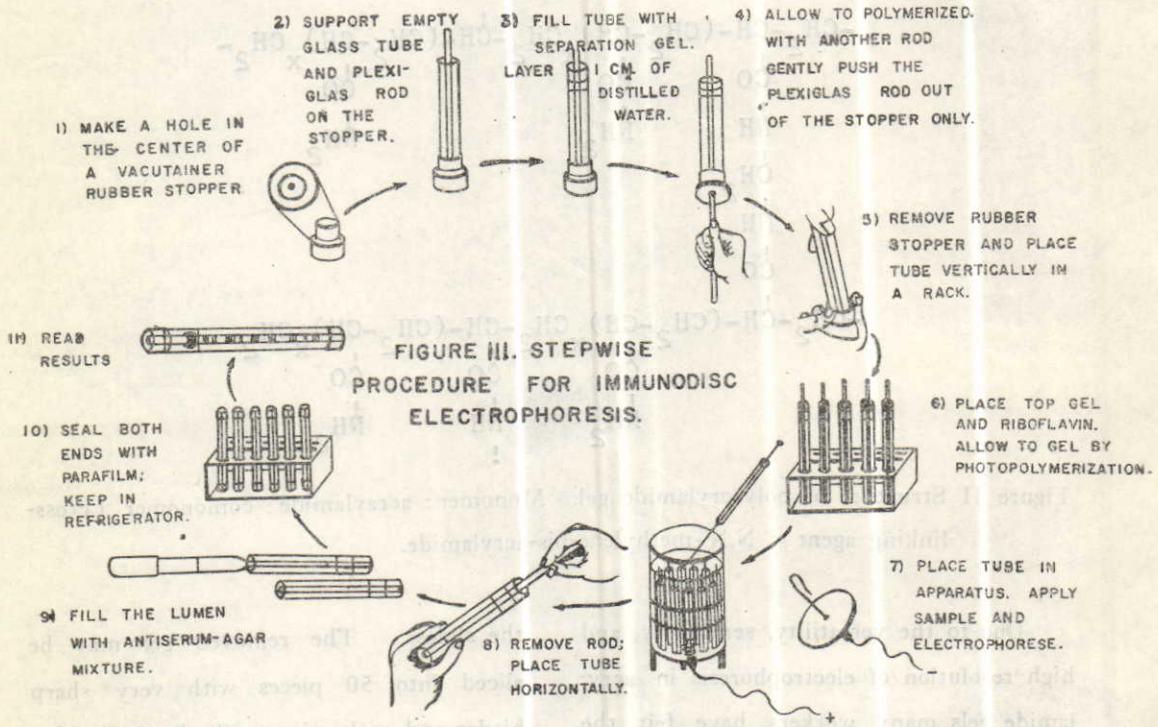


Figure II Structure of polyacrylamide gel. Monomer: acraylamide; comonomer (cross-linking agent): N,N'-methylene-bis-acrylamide.

Due to the versatility, sensitivity, and high resolution of electrophoresis in acrylamide gels many workers have felt the need to extend its use to immunoprecipitation analysis (1,2). The procedures developed so far have required that either the gel be removed from the tube and subsequently embedded in agar to allow the fractions to diffuse and react with the antiserum or the electrophoresis be done in acrylamide gel polymerized directly on the slides. The removed gel may be sliced into 50 pieces with very sharp blades and each piece will be embedded into the agar. Removal of the gel from the tube suffers from the hazard of breakage while the polymerization of the crylamide on the slides requires incubation under carbon dioxide. Therefore, a new technique, called immunodisc electrophoresis, was developed in order to circumvents all such complications. This

technique can be done very easily by first putting a plexiglas rod in the middle of the glass tube, then fill the tube bottom up with polyacrylamide gel. All the tube with plexiglas rods in the center hold by acrylamide gel were placed in a upper and lower chambers of buffer reservior. After the samples were electrophoresed, the plexiglas rods were pulled out and the lumens were filled up with antiserum mixed with melted agar. When the agar had solidified, both ends of the tubes were sealed with Parafilm and the tubes were kept in 4 C. for 5—10 days for precipitin lines to develop (Fig. III).



The immunodisc electrophoresis technique reported here circumvents all such complications as it allows for the precipitin reaction to occur in the electrophoresis tube itself. The technique also has a high resolving power as it yields a large number of precipitin bands than the other techniques (see below). Furthermore since the bands are not in the form of overlaping arcs but appear as discs they are easy to enumerate. These precipitin discs appear in the inner agar column rather than in acrylamide gel. This finding was surprising but it may

be due to the very small pore size of 7.5 per cent polyacrylamide gel which Ornstein (6) reported to be of the order of 50 A.

The use of a hollow cylinder of acrylamide gel as described in this technique makes the disc electrophoresis technique very versatile as it opens a multitude of possibilities for analyzing the samples. For instance, in simple disc electrophoresis the gel can now be stained directly in the tube by filling the lumen with the stain, thus avoiding the need of removing the gel from the tube. The lumen can also be filled with various enzyme substrates contained in agar and the enzymic activities of the isolated fractions can thus be visualized. Presently the technique is based on simple one-way immunodiffusion analogous to Oudin's method. The technique can be easily modified and used for double-diffusion studies if desired. When compare immunodisc electrophoresis, immunoelectrophoresis and disc electrophoresis using the same samples antigen and antiserum. Immunodisc electrophoresis is about 3 times more sensitive than immunoelectrophosis. This method combines the resolving power of disc electrophoresis with the immunological identification of immunoelectrophoresis. It should be very useful to identify antigenic composition of bacteria, fungus, biological materials and especially viruses. When any viral components were isolate, they can be proved to be antigenic of not by this new method. This method should, therefore, find wide application in the antigenic analysis of biologically complex mixtures.

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