Agar Electrophoresis on Cellophane and Polyester Films

While the agar electrophoresis technique[1]) can be adapted to densitometric evaluation of the stained components of proteins or other constituents separated on the gel, it cannot be adapted easily to radioactive tracer studies by counting technique, as in the case of paper electrophoresis, without further improvement. Although the quantitative evaluation of the protein components can also be made by scraping off the stained bands with a razor blade from the glass into a test tube and extracting the colour with alkali for colorimetric measurement, the elution technique used in paper electrophoresis by cutting the paper strips and extracting the dye for colorimetric measurements, cannot be adapted to agar

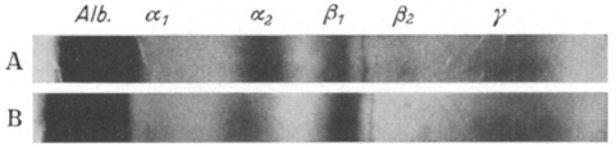

Fig. 1. Agar electrophoresis patterns of normal serum proteins obtained on cellophane and "Mylar" polyester film. (200 volts, 9·5 mA, 0·05 ionic strength, 8·6 p_H; 4 hrs. run.) — A "Mylar" Polyester film; B Cellophane.

electrophoresis carried on glass. There is need, therefore, for a simple procedure in which the above limitations inherent in the agar electrophoresis technique are eliminated. A thin film of cellophane or any other suitable material, which is transparent, uniform in thickness, easily amenable to cutting into small strips and does not exhibit pronounced self-absorption of the radiation in the material used for supporting the gel, will fulfill the requirements.

Cellophane (0·0009 inch thickness) or "Mylar" polyester film *) (0·00025 inch thickness), cut to the size of the plate glass are used as support for the agar gel. The cellophane sheet is soaked in water and placed over the plate glass. The excess moisture is removed by means of a filter paper and the cellophane sheet is pressed gently and evenly on the plate, so that it is placed on the glass without forming any wrinkles on the surface of the film. The perspex frame is then placed on cellophane, the paper strips are introduced at each end and the agar gel containing the puffer is layered on the cellophane sheet. The electrophoresis is carried out in the same manner as described before[1]). After the run, the plate glass with the cellophane containing the agar gel is taken out, dried and treated with the naphthalene black dye solution according to the procedure described in the earlier communication[1b]). The washing of the free dye takes about 30 to 45 minutes. The cellophane sheet containing the agar with the stained electrophoretic pattern can be peeled off from the glass, washed with fresh methanol-acetic acid solvent and dried. Similar procedure can be adopted for the "Mylar" polyester film. The time taken for the washing of the free dye in this case is, however, short (3 to 4 minutes). The polyester film being insensitive to moisture, the thin agar film can be peeled off from the film after drying and the agar film can be cut and the colour of the bands can be eluted with alkali for the quantitative colorimetric measurement in the usual manner. The same film can be used for subsequent experiments. Densitometric evaluation of the patterns obtained on the films can be made with the scanning instruments used for direct photometry of paper electropherograms. It is more convenient to carry out the routine analysis of serum proteins on cellophane sheets instead of glass, as they are readily available, less expensive and the electropherograms can be preserved for future reference. It is obvious that the technique can be used for investigation of radioactive compounds as the radioactivity of the compounds separated on the thin film of agar can be easily determined. It can be used in any continuous radioactive scanning device used for paper strips. Typical agar electrophoresis patterns of sera obtained on cellophane and "Mylar" polyester films are illustrated in Fig. 1. It can be seen that the patterns obtained are well defined for quantitative evalution by any of the above methods. Full details of this technique will be published elsewhere.

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*) Manufactured by E. I. Du Pont de Nemours & Co. (Ind.) Wilmington 98, Delaware, U.S.A.

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