Zone Electrophoresis of Serum Proteins in Agar Gel

Although paper electrophoresis has been widely used, electrophoresis in agar gel has been rarely employed as a clinical method for the study of serum protein patterns. GORDON et al[1]) investigated the electrophoresis of proteins including those of normal human plasma in agar jelly. The method is mainly intended for the separation of proteins on preparative scale. It is obvious that it is not suitable for routine clinical work. GRABAR and WILLIAMS[2]) have described a method permitting the simultaneous study of electrophoretic and immunochemical properties of proteins in agar, using antigen-antibody reaction for locating the protein zones. Recently BASSARD[3]) applied agar electrophoresis to serum proteins. They used a thin film of plastic (polyvinyl chloride) coating on the agar gel to minimise the evaporation and air cooling during the passage of current. The separations achieved were not illustrated. A technique of agar electrophoresis of serum proteins which is in continuous use in this laboratory is described below. It is simple and avoids the use of any involved procedures described in the above methods.

Two glass plates (30 × 5·2 × 0·2 cm.) and two frames of marginal width (9 mm.) cut from a perspex sheet (1·2 mm. thickness) are used. One of the frames is placed over one of the glass plates. Two paper strips (2·4 × 8 cm.) of Whatman No. 3 are inserted at each end between the edges of the frame and the glass plate, both being kept well pressed by means of clips. One per cent agar gel containing veronal buffer p_H 8·6 is prepared by mixing equal volumes of 2% agar gel and veronal buffer (p_H 8·6) of ionic strength 0·1. Ten c.c. of the buffered agar gel while still warm are poured on to the surface of the glass plate by means of a 10 c.c. pipette into the space provided by the frame. The gel is allowed to cool and set at room temperature. Another perspex frame is placed over the one already kept on the glass plate after removing the clips. The glass plate with the frames is placed on the lips of the two electrode vessels of Kelab paper electrophoresis equipment (Model 5 K 51) with the ends of the paper dipping into the buffer solution (veronal buffer, p_H 8·6, ionic strength 0·05). 25 µl of serum is carefully applied to the surface of the gel by means of a special micro pipette, so as to form a narrow zone without spreading to the edges of the gel layer. Two samples of serum can be spotted on the same plate. The upper glass plate is then placed on top of the two perspex frames and the current is turned on. It has been found that about 180 to 200 volts and 7 to 9 m.A. for four hours are favourable for satisfactory separation of the proteins into four distinct bands. After the run the upper plate is removed and the bottom plate with the agar layer and the frames in tact is taken out of the cells and laid on a suitable support. The protein bands can be made visible[1]) by the formation of turbid zones after adding 5 c.c. of 10% trichloroacetic acid to the surface of the agar gel or[2]) by the usual staining methods used in paper electrophoresis. Naphthalene black 12 B 200 (Amidoschwarz) has been found to give good results. About 5 c.c. of the saturated solution of the dye in methanol containing 10 per cent acetic acid are added to the surface of the gel. After about 5 minutes the agar layer is washed with methanol-acetic acid solution, 200 c.c. of the solution being used for each washing. After three to four washings which take about 3 to 4 hours, the free dye is removed completely and the protein bands retain the colour against clear and transparent agar layer. Bromophenol blue also can be used for staining the protein bands. Typical normal and pathological patterns obtained by this technique are shown in Fig. 1. It may be seen from the patterns obtained that the proteins albumin, α, β and γ globulins separate into four distinct bands. The changes in the concentration of albumin and γ-globulin in infantile biliary cirrhosis and kalaazar are very well shown in the patterns. As visual examination of the patterns is adequate for clinical diagnosis, the technique is capable of wide application for routine examination of clinical samples of serum. It is obvious that the technique possesses certain advantages

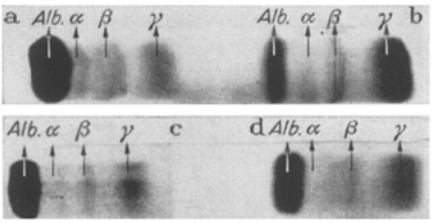

Fig. 1 a—d. Serum patterns obtained with agar electrophoresis. 200 volts; 9·0 mA; p_H 8·6; Ionic strength 0·05, 4 hrs. run. Alb. = albumin; α = α-globulin; β = β-globulin; γ = γ-globulin. a normal; b infantile biliary cirrhosis; c normal; d Kalaazar

over paper electrophoresis for the separation of enzymes and other biologically active proteins as agar exerts protective action on enzymes against inactivation. The quantitative evaluation of the patterns by densitometric measurements of the turbidity and colour of the bands is being investigated. Full details of the technique will be published elsewhere.

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[1]) GORDON, A. H., B. KEIL, K. SEBESTA, O. KNESSL and F. SORM: Coll. Czech. Chem. Communs. **15**, 1 (1950).

[2]) BRABAR, P., and C. A. WILLIAMS: Biochim. et Biophysica Acta **10**, 193 (1953).

[3]) BUSSARD, A.: C. R. hebd. Séances Acad. Sci. **239**, 1702 (1954).