AGAR ELECTROPHORESIS

Part IV. Circular Agar Electrophoresis

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ABSTRACT

A technique of circular agar electrophoresis is described which possesses the advantages of analysing large number of serum samples simultaneously on the same plate; resolution of the protein components into compact zones and evaluation of the components by colorimetric determination of the stained zones after elution with alkali.

The agar electrophoresis technique (Giri, 1956, *a*, *b*, *c*) developed in this laboratory has been adapted to the resolution of serum proteins by circular technique, by which the protein components can be separated into compact zones in the form of arcs round the centre of a circular agar plate. In the present communication the apparatus and technique for carrying out electrophoresis are described.

MATERIALS AND METHODS

The apparatus is illustrated diagrammatically in Figs. 1 and 2. It comprises the agar plate unit (Fig. 2) and electrode vessels with platinum wire electrodes connected to D.C. voltage power supply unit. The Kelab Electrophoresis equipment is used in the present investigation as source of D.C. supply.

Circular agar plate Unit.—It consists of two circular plate glasses (19 cm. diam.) one of which having a hole (7 mm. diam.) at the centre and the other is used as cover plate. Two 'perspex' rings of the same diameter as the plate glasses with a marginal width of 7 mm. are used to provide space for the agar gel. Whatman No. 1 filter-paper cut to the size of the plate glass with six V-shaped wedges round the paper at equidistant points as shown in Fig. 2 is used to maintain contact between the buffer in the anode vessel and the agar gel. The inner diameter of the filter-paper disc should be slightly less than that of the perspex ring, so that about 0·5 cm. width of the filter-paper extends towards the inside space enclosed by the 'perspex' ring to establish contact with the agar gel. A cellophane sheet cut into a disc of diameter about 1 cm. more than that of the plate glass is soaked in water and placed over the plate glass with the hole, and pressed gently with a filter-paper to remove the moisture, taking care to see that no air bubbles are formed between the cellophane film and plate glass and a smooth surface is obtained. The edge of the cellophane is pressed down the plate glass. By this manipulation the entry of buffer solution between the plate glass and cellophane by capillary attraction will be prevented. The Whatman No. 1 filter-paper with the V-shaped wedges is placed over the cellophane film. One of the 'perspex' rings is kept over the

filterpaper. The 'perspex' ring and the plate glass with the paper are kept well clamped by means of spring clips. A hole is made at the centre of the cellophane film for inserting a paper wick through the hole into the buffer solution in the cathode vessel.

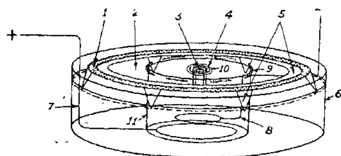

FIG. 1.

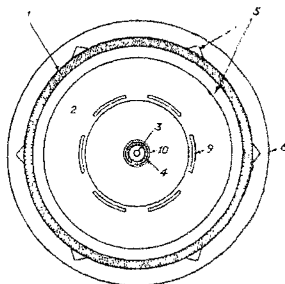

FIG. 2.

FIG. 1. Experimental arrangement for Circular Agar Electrophoresis.

FIG. 2. Plan of Circular Agar Plate Unit.

1. 'Perspex' ring; 2. Agar gel layered on cellophane; 3. Central paper wick; 4. Central 'perspex' ring; 5. Circular paper ring cut to the size of the circular plate glass with six V-shaped wedges; 6. Glass or 'perspex' basin (Anode vessel); 7 & 8. Platinum wire electrodes; 9. Paper strip (Whatman No. 1) containing serum; 10. Small Whatman No. 3 paper disc; and 11. Cathode vessel.

The whole unit is placed over the cathode vessel containing about 400 ml. of buffer (veronal-acetate buffer, pH 8·6 and ionic strength 0·025) and arranged for proper levelling. A small spirit level can be used to adjust it to a horizontal position. This step is important as it ensures uniform thickness of the agar gel layer. A small Whatman No. 3 filter-paper disc (2·7 cm. diam.) is placed at the centre, over which a small 'perspex' ring (inner diameter 1·5 cm., with marginal width of 0·5 cm.) is kept. Whatman No. 1 paper strip (4×3 cm.) rolled into the form of a wick is inserted at the centre through the filter-paper and the cellophane into the buffer solution contained in the cathode vessel. Another Whatman No. 3 filter-paper disc (1·4 cm. diam.) is placed at the centre inside the small 'perspex' ring and pressed gently to make contact with the head of the paper wick. About 30 ml. of agar gel, containing 1·0% agar and veronal-acetate buffer of pH 8·6 and ionic strength 0·025, while still warm is poured carefully with a pipette on the cellophate into the space provided by the large 'perspex' ring leaving the central portion occupied by the small paper disc. There should be a contact between the gel and the outer edge of the central filter paper disc and the inner edge of the filter-paper with the V wedges. The gel is allowed to cool and set at room temperature.

After the gel is set, the spring clips are removed and another ' perspex ' ring is placed over the one already kept on the plate glass.

Ten μ 1 of serum is applied with a mecropipette to a small strip of Whatman No. 1 (3 cm. long, 1 mm. width) and placed gently with a foreceps on the surface of the gel at points about 3·5 cm. distance from the centre of the plate. Six to eight strips can be placed depending on the size of the paper strip. A filter-paper disc of diameter 7·5 cm. with a hole 2 cm. diam. cut at the centre and marked with a pencil dividing the surface into six sectors is kept on the bottom surface of the plate glass after moistening with water. It serves as guide for placing the serum paper strips at points equidistant from the centre. Similar paper strips (3×1 mm.) soaked in 0·2% aqueous Amidoschwarz dye solution are superimposed on the serum paper strips. The dye serves as marker for the albumin zone and facilitates the observation of the movement of the albumin on the gel during electrophoresis. It is then covered with the other plate glass. The V-shaped wedges are dipped into the outer electrode vessel containing about 800 c.c. of the buffer, which serves as the anode compartment. The current is switched on and electrophoresis is carried out at 200 volts, 8–8·5 mA for 7–8 hours. During electrophoresis moisture collects at the centre of the bottom surface of the cover plate glass which can be removed to wipe off the moisture and electrophoresis is continued after replacing the plate. Occasionally, drops of water collect on the surface of the gel, which can be removed by means of filter-paper by gently touching the drop with the pointed end of the paper.

After electrophoresis the plate is dried, stained and washed as described in earlier publications. After final washing, the cellophane film can be peeled off from the plate glass and washed again for 30–60 minutes in fresh methanol-acetic acid solvent to wash off the free dye completely and dried at room temperature.

Results and Discussion

The electrophoretic patterns of serum proteins of normal and pathological patients are shown in Fig. 3, which illustrates the separation of serum proteins into five distinct zones—albumin, α_1, α_2, β and γ-globulins. In the case of normal serum, the α_1 component is not clearly visible in the photograph. The patterns show the characteristic changes in the concentration of the protein components in tuberculosis, cirrhosis, cancer and nephritis. The lowering of the albumin in all these cases, the increase in γ-globulin in cirrhosis and tuberculosis and increase in α_2 component in cancer and tuberculosis are clearly shown in the patterns.

The circular technique confers the following advantages:

1. Six to eight samples of serum can be examined simultaneously on the same plate, thereby facilitating the comparison of the patterns obtained under exactly similar experimental conditions.

2. The radial distribution of the protein zones ensures more compact bands without diffusion.

3. The stained protein zones obtained on cellophane can be cut and eluted with alkali for quantitative colorimetric evaluation of the protein components (Giri, 1956 *c*).

The technique can be used generally for examination of normal and pathological samples of sera.

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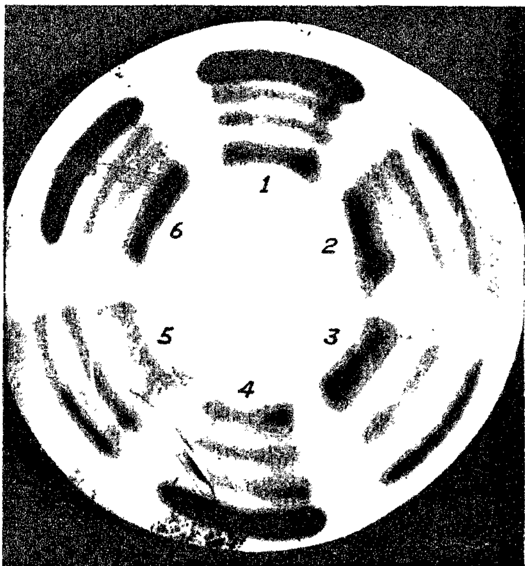

FIG. 3. Circular agar electrophoresis patterns of serum proteins.
1 and 4 Normal; 2. Cancer (Cervix); 3. Cirrhosis; 5. Nephritis; 6. Tuberculosis.