A Simple Agar-Electrophoresis Apparatus and Technique

In an earlier communication[1]), a technique was described for the separation of serum proteins using electrophoresis on agar gel. The present communication describes a simple and inexpensive electrophoresis equipment which is capable of obtaining electrophoretic patterns of serum proteins. The procedure described before[1]) has been improved for obtaining consistently uniform patterns with better resolution of the components. The method of application of the serum sample has been improved by the technique of filter paper strip application. By drying the agar gel after electrophoresis and using aqueous dye solution [500 mg. of naphthalene black 12 B 200 (Amidoschwarz) in 100 ml. of the solvent mixture of the composition methanol-acetic acid-water (40:10:50)] for staining, the time of washing off the free dye has been considerably reduced from 4 to 5 hours to about two minutes. The unit can be assembled at small cost with the materials ordinarily available in hospitals and small laboratories.

A schematic diagram of the apparatus is shown in Fig. 1. It consists of the following component parts: *A* = Two plate glasses of the dimensions described before, one of the plate glass is used as support for the agar gel and the other as cover.

B = Two perspex frames — one for enclosing the agar gel and the other for supporting the plate glass cover. C = Two electrode vessels (glass jars), each holding 300 cc. of veronal buffer (p_H 8·6; 0·05 ionic strength). D = Two platinum wire electrodes connected through a labyrinth system to perspex sheets. E = A sensitive milliammeter which is placed in the circuit in series with the agar gel plate. F = A constant voltage transformer with a rectifier or a series of 45 volt radio B batteries serve as a source of potential. Any voltage between 180 to 300 can be used.

The procedure for carrying out the electrophoresis is exactly similar to that described in the earlier communication, with the following modifications introduced for obtaining

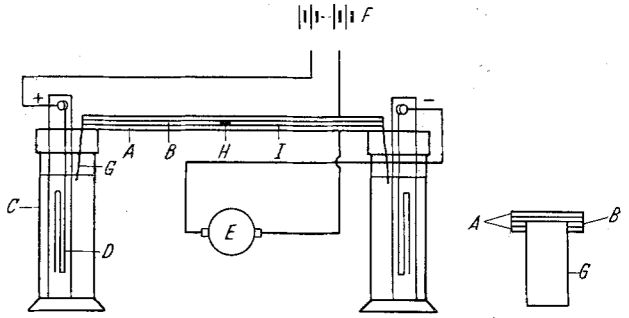

Fig. 1. Schematic diagram of the general layout of apparatus. *A* Plate glasses; *B* Perspex frames; *C* Electrode vessels containing buffer solution; *D* Platinum electrodes; *E* Milliammeter; *F* Source of potential; *G* Filter paper (Whatman No. 3) connecting the electrode vessel and the agar plate; *H* Position of filter paper strip for sample insertion; *I* Layer of agar gel

well defined zones with better resolution of the components. Instead of applying the serum directly on the plate, it (10 µl.) is carefully applied to a small Whatman No. 1 filter paper strip (20 × 2 mm.) by means of a 10 µl. micropipette. The paper strip is placed carefully at the centre of the agar gel layer with the help of forceps. The electrophoresis is carried out at room temperature. Using this equipment, the current varies between 4 to 8 milliamps under the experimental conditions used. After 3 hours run at about 300 volts or 4 hours at 180 to 200 volts, the top plate glass is removed carefully without the drops of moisture collected on the plate falling on the surface of the agar gel. The filter paper strip on the agar plate is removed carefully by means of forceps without disturbing the surface of the gel. The agar plate together with the perspex frame kept intact by means of two rubber bands at each end is allowed to dry over night (12 to 16 hrs.) at room temperature. After complete drying of the agar gel layer, it is immersed for about 30 minutes in the dye bath contained in a measuring jar of one litre capacity. The plate is then removed from the dye bath and washed with methanol acetic acid (9:1) solvent, for 1 to 2 minutes. The solvent mixture used before can be used again for the first washing. The plate is again washed a second time for 1 to 2 minutes in a fresh solvent mixture. The washings can be conveniently carried out in enamelled trays. After the second washing, the free dye will be removed completely leaving a transparent background and the dye bound to the proteins is retained. The protein bands appear as blue bands against clear and transparent background. Fig. 2 shows the typical patterns of sera obtained by this technique.

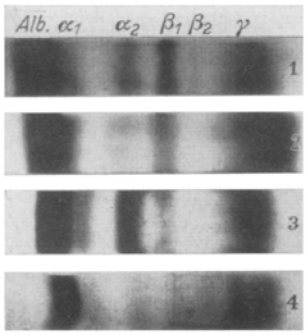

Fig. 2. Typical serum patterns obtained with agar electrophoresis. 1 Normal serum; 2 Cirrhosis (γ-globulin increased and albumin decreased); 3 Pulmonary Tuberculosis (α_2- and γ-globulin increased); 4 Nephritis (Albumin decreased; diffused γ-globulin resolved into two components γ_1 and γ_2)

As many as two or three electrophoresis cells can be used in parallel from the same power supply. Optimum separation is usually obtained between 3 to 4 hours. This inexpensive and simple unit has given satisfactory results comparable to those obtained by using commercial and expensive instruments. The technique is simple enough for introduction into hospital laboratories for routine examination of serum protein patterns. It is obvious that this simple technique should be useful in research on many types of substances as enzymes, antibiotics, alkaloids, hormones and in immuno-electrophoretic analysis.

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[1]) Giri, K.V.: Naturwiss. **43**, 36 (1956).