of thalassaemia minor with an increased haemoglobin A₂ fraction.

The electrophoresis was carried out for 16 hours by the hanging strip method, using Whatman No. 3 paper for chromatography at 160 v., with a strip 8.5 cm. wide and 36.5 cm. long. The current per strip was 3.5 mA.

The slight but important modification of the TRIS buffer as given by Aronsson and Grönwall (1957) consists in using it more dilute, and the recommended preparation of the TRIS buffer is as follows:

Trishydroxymethylaminomethane	..	..	50.4 g.
Ethylenediaminetetracetic acid	..	..	5.0 g.
Boric acid	..	..	3.8 g.
Dissolve to a final volume of 1,000 ml. in distilled water			

REFERENCES

- Aronsson, T., and Grönwall, A. (1957). *Scand. J. clin. lab. Invest.*, **9**, 338.
 Derrien, Y. (1959). In *Abnormal Haemoglobins*, p. 63, a Symposium organized by the Council for International Organizations of Medical Sciences, edited by Jonxis, J. H. P., and Delafresnaye, J. F. Blackwell Scientific Publications, Oxford.

A Technique for Electrophoresis in Agar Gel

R. BIRD AND DOROTHY JACKSON

From the Department of Endocrinology, New End Hospital, Hampstead, London

(RECEIVED FOR PUBLICATION OCTOBER 3, 1958)

Gordon, Keil, and Sebesta (1949) described the electrophoretic separation of proteins in agar gel. Grabar and Williams (1955) developed the method for the immuno-electrophoretic analysis of proteins in agar, and that technique is finding increasing application in the characterization of proteins. Uriel (1958a) has described how the use of electrophoresis in agar combined with immunological and chemical reactions allows a triple characterization of constituents of biological fluids to be determined, namely, electrophoretic mobility, immuno-chemical specificity, and chemical nature. Recently Uriel (1958b) has described the quantitative analysis of serum proteins after separation in agar gel. This paper describes how an electrophoresis box designed for free horizontal paper electrophoresis may be easily adapted for electrophoresis in agar gel.

Method

Buffer.—The buffer is pH 8.6 barbitone buffer ionic strength 0.05; sodium barbitone 5 g., hydrated sodium acetate 3.33 g., 0.1 N sulphuric acid, 34.2 ml., and water to 1 litre.

Agar Gel.—One per cent. agar (Davis Standard) was made up in buffer solution by heating in a water bath. While still hot the resulting solution was filtered through glass wool, poured on to a perspex plate and allowed to cool. The thickness of the agar sheet was 3–4 mm. The gel was cut into strips 25 cm. long by 4.5 cm. wide. For convenience several strips were prepared at a time and stored in a flat-bottomed enamel tray, under buffer solution, until required. On being removed from the tray and blotted, the strips were ready for immediate use. Uniform runs were obtained in gels stored in this way. It was usual to prepare a week's supply at a time.

Adaptation of the Electrophoresis Box.—A bridge of perspex was made by cutting a section from a sheet of perspex (6 mm. thickness), and this rested on the sides of the buffer solution compartments.

Gel: Buffer Solution Contact.—To eliminate the difficulties encountered in the various paper or gauze wick contacts between the agar gel and the buffer solution, it was so arranged that the gel itself dipped into the buffer solution, as shown in Fig. 1. This procedure was easily carried out and was found to provide a good gel:buffer contact. The agar gel is placed on a piece of celluloid (used x-ray film), folded

FIG. 1.—Simplified diagram of the apparatus. Electrode compartments are not shown. AA', Buffer solution compartments. B, Perspex bridge. C, Celluloid film. D, agar gel.

as indicated. This rests on the bridge and prevents any break where the gel dips into the buffer compartment. A second piece of film, lightly smeared with mineral oil, is placed on the surface of the agar gel while electrophoresis is in progress to prevent evaporation from the surface of the gel. This greatly reduces condensation and the formation of water droplets on the glass lid of the box. Without this precaution drops of water were liable to fall on to the gel during electrophoresis.

Application of Serum and Voltage for Electrophoresis.—A trough is cut in the centre of the gel strip. The serum is mixed with an equal volume of buffered 2% agar. While still warm the liquid is placed in the trough and allowed to set. Up to 0.1 ml. of serum may be applied in this way.

It was convenient to run electrophoresis overnight (17 or 18 hours) at 2 volts per cm. length of the agar strip in order to obtain a good separation of serum proteins. The current remained practically constant throughout the run. Fig. 2 shows a typical serum

FIG. 2.—The serum protein pattern obtained after electrophoresis in agar gel at pH 8.6.

protein separation. Particularly good separation between the α_1 and α_2 globulins and between the β and γ globulins was obtained. When 0.1 ml. of serum was used, there was very little visible protein trailing between the various protein bands. The deeply stained α_2 globulin region appears to be partly due to lipoproteins in that region (Pezold, 1958).

Staining and Drying

Dye Solution.—To 0.10 g. bromophenol blue and 50.0 g. $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ add about 25 ml. of 95% ethanol. Mix thoroughly and add 5% (v/v) acetic acid to make 1 litre.

Wash Solution A.—Acetic acid, 5% v/v.

Wash Solution B.—Glycerine, 10 ml., 5 ml. acetic acid, 1 g. sodium acetate, water to 100 ml.

After electrophoresis the gel is placed in the dye solution for eight hours, or overnight, and then washed in solution A until the background is clear. It is finally rinsed in water. After this, the gel is placed on a perspex tray, covered with filter paper, and dried overnight at 37° C. The agar film is then placed for 10 minutes in solution B to enhance the colour of the stained proteins and to make the film pliable. If the agar film has dried on to the filter paper, the filter paper can be removed at this stage. The film is finally hung up to dry at room temperature.

Discussion

Agar gel electrophoresis has many applications in the analysis of serum constituents and is a useful method to have available in the laboratory when required. The technique described enables a simple paper electrophoresis box to be used at any time with agar gel, the only addition necessary being a suitable bridge between the buffer solution compartments. By resting the gel on a celluloid support the necessity for a separate connexion between the gel and buffer solution was eliminated. The buffer and staining solutions were those also employed for paper electrophoresis. The separation of serum proteins was satisfactory and would enable further analysis of the fractions to be carried out. One advantage over the use of paper was that larger quantities of serum could be handled and good separation still obtained. Preparing the gel strips in batches greatly reduced the time taken to set up the apparatus for electrophoresis in agar gel. The time taken to complete the separation and staining of the proteins was not found to be a disadvantage, as the various processes could be left unattended. The changing of the staining and washing solutions took very little time.

Summary

An adaptation of a paper electrophoresis box for use with agar gel and of its use in the electrophoresis of serum proteins is described.

REFERENCES

- Gordon, A. H., Keil, B., and Sebesta, K. (1949). *Nature (Lond.)*, **164**, 498.
- Grabar, P., and Williams, C. A. (1955). *Biochim. Biophys. Acta*, **17**, 67.
- Pezold, F. A. (1958). *Clin. chim. Acta*, **3**, 40.
- Uriel, J. (1958a). *Ibid.*, **3**, 17.
- (1958b). *Ibid.*, **3**, 234.