

TECHNICAL METHODS

TRIS Buffer for the Demonstration of Haemoglobin A₂ by Paper Electrophoresis

J. E. CRADOCK-WATSON, J. C. B. FENTON,
AND H. LEHMANN

From the Department of Pathology, St. Bartholomew's Hospital, London

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Tris(hydroxymethyl)aminomethane (TRIS) buffer has been recommended for finer separation of serum proteins in paper electrophoresis (Aronsson and Grönwall, 1957). We have employed it for the examination of haemoglobin variants and have found that, with a slight modification, it is suitable for this purpose. When used with a vertical tank the fractions do not move as far from the point of application as they do with barbiturate buffer (pH 8.6), but they are more sharply defined and the bands are narrower. In view of this it is possible to recognize, with the naked eye, small fractions which would escape discovery on paper electrophoresis with barbiturate buffer. This is particularly useful for the examination of specimens for haemoglobin A₂. Even normal proportions of haemoglobin A₂ are easily visible, and abnormal proportions of haemoglobin A₂ are recognized at once. By this technique the non-haemoglobin protein X of Derrien (1959), which is present in some haemolysates, separates clearly from haemoglobin A₂, and on

staining the strips and eluting the protein dye complex the risk of inaccuracy due to the admixture of protein X to haemoglobin A₂ is avoided.

In Fig. 1 a haemoglobin specimen from a patient with haemoglobin H disease is compared with that from a case of thalassaemia minor. Fig. 1a is a photograph of the unstained strip and shows the absence of haemoglobin A₂ in the specimen of haemoglobin H disease. Fig. 1b shows the strip after staining with "light-green," and it will be noted that the X fraction is now visible, and that it is well separated from haemoglobin A₂.

Fig. 2 shows a photograph of an unstained strip of the electrophoretic pattern of a haemoglobin from a normal person and of a haemoglobin from a case

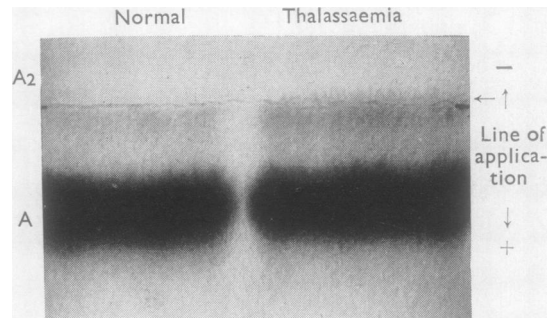


FIG. 2.—Unstained strip showing after electrophoresis haemoglobin A₂ just visible in the normal specimen and increased in thalassaemia.

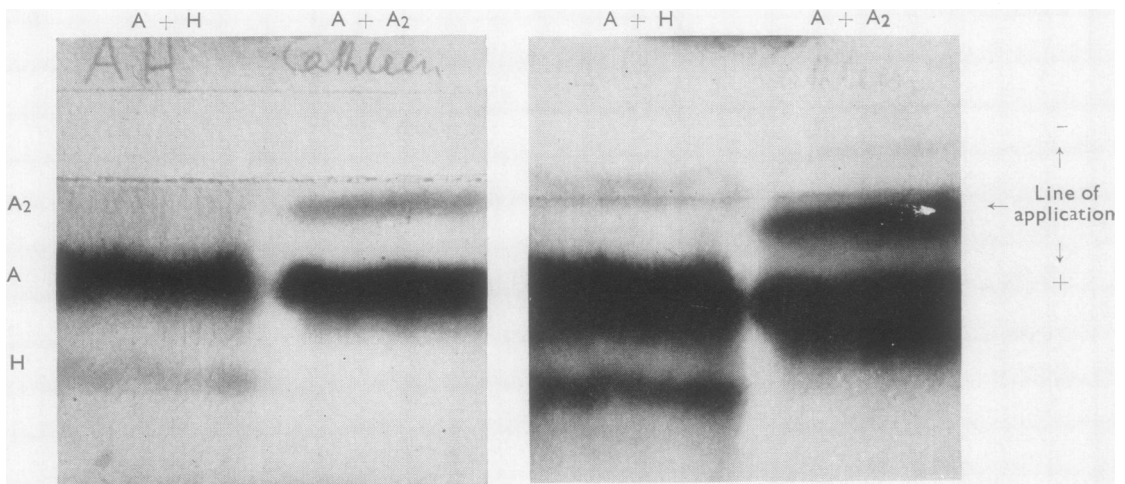


FIG. 1a

FIG. 1b

FIG. 1a.—Unstained strip showing haemoglobins A and H on the left and haemoglobins A and A₂ on the right.

FIG. 1b.—Strip stained for protein with "light green." Protein X is now visible in both specimens. On the right it is well separated from A₂.

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

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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