Tris buffer for differentiation between haemoglobins C and E and separation of haemoglobins S from F and 'lepore' from A

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Trishydroxymethylaminomethane (TRIS) buffer was introduced for the finer separation of serum proteins by paper electrophoresis by Aronsson and Grönwall (1957). Cradock-Watson, Fenton, and Lehmann (1959) found this buffer not suitable for the paper electrophoresis of haemoglobin variants but reported that with a slight, but important, modification it could be used to demonstrate

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haemoglobin A₂. We should now like to report three further uses for this modified buffer in the investigation of haemoglobins.

DIFFERENTIATION BETWEEN HAEMOGLOBINS C AND E

On paper electrophoresis with barbiturate buffer pH 8.6 haemoglobin C and haemoglobin E have a very similar mobility. It is often difficult to differentiate between the two, although it can usually be established that haemoglobin C moves more slowly. The modified TRIS buffer can be used to help in distinguishing C and E in two ways.

(1) Whereas with barbiturate buffer, E (and A₂) are seen to move in front of C, the position is reversed with TRIS buffer (Figs. 1 and 2), where E and A₂ move more slowly than C. Thus the examination of an unknown haemoglobin mixture, together perhaps with a haemoglobin A+C control by paper electrophoresis with both buffers, will give a reliable identification.

(2) If haemoglobin S is added to a sample which contains C or E respectively, paper electrophoresis with barbiturate buffer will show a separation of S in both, but TRIS buffer paper electrophoresis will show S and C moving as a single band, and S and E as separate bands.

FIG. 1A. Unstained strip showing from left to right haemoglobins A+S, A+E, and A+C. Paper electrophoresis using TRIS buffer. E moves more slowly than C.

FIG. 1B. Stained strip showing from left to right haemoglobins A+C and A+E. Paper electrophoresis using barbiturate buffer. C moves more slowly than E.

FIG. 2. Unstained paper strip showing result of TRIS buffer electrophoresis. Left: Sick-cell anaemia—S+10% F; note that A₂ can be recognized. Right: Thalassaemia minor—A, 1.2% F (not separating from A), 3.8% A₂.

FIG. 3. Stained (light green) paper strip showing the result of TRIS buffer electrophoresis. Left: normal—A and A₂. Right: Lepore trait—Lepore haemoglobin between A and A₂.

In other words TRIS buffer paper electrophoresis fails to separate C and S, but can separate E and S.

SEPARATION OF HAEMOGLOBINS F AND S

The second advantage of TRIS buffer is that, unlike barbiturate buffer, it will separate haemoglobin F from haemoglobin S (Fig. 3).

SEPARATION OF HAEMOGLOBIN 'LEPORE' FROM A

One of the characteristics of Lepore is that it can be

separated from A by starch electrophoresis with barbiturate buffer, but either not at all, or only very poorly, by paper electrophoresis (Gerald and Diamond, 1958). Paper electrophoresis with TRIS buffer separates the two haemoglobins.

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