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 Grönnwall (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 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) is 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 from 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.

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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: Sickle-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.

REFERENCES