Technical methods

Quantitative determination of A2 haemoglobin by paper electrophoresis using tris buffer

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Quantitative determination of A2 haemoglobin by paper electrophoresis using TRIS buffer is feasible. There is sufficient separation of haemoglobin A2 from haemoglobins A1 and A3 to allow quantitative estimation by this method. The technique is simple and sufficiently quantitative to be of clinical value.

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MATERIALS AND METHODS

Haemoglobin electrophoresis is performed between siliconized glass plates, on Whatman No. 3 mm. filter paper, using TRIS buffer at pH 9.2 (trishydroxymethylaminomethane 50.4 g., ethylenediaminetetraacetic acid 5.0 g., boric acid 3.8 g., distilled water 1,000 ml.) (Cradock-Watson, Fenton, and Lehmann, 1959), for four hours at 300 volts, 10 milliamperes. Haemoglobin is prepared as described by Chernoff (1955) and 0.02 ml. applied as a 2 cm. band on a single broad filter paper sheet. Several specimens may be run simultaneously. Upon completion of the run the paper is dried, the bands cut into strips and subjected to densitometric scanning (unstained) in a Spinco Analytrol scanner, model RB (B5 cam, slit width 1 mm.) (Figs. 1, 2, and 3). The amount of A2 haemoglobin is determined as the relative percentage of the total haemoglobin. A2 haemoglobin is selected as the area approximately half-way between A1 and A2.

FIG. 1. Illustrative examples of normal haemoglobin patterns after densitometric scanning.

FIG. 2. Haemoglobin pattern on paper electrophoresis in TRIS buffer, pH 9.2. Note prominent A2 haemoglobin in thalassaemia minor.

FIG. 3. Illustrative examples of haemoglobin patterns in thalassaemia minor after densitometric scanning. Note prominent A2 haemoglobin.

RESULTS

NORMALS In 52 normal adults the A2 haemoglobin ranged from 1.5 to 3.1%. The average value was 2.2% (Fig. 4).

THALASSAEMIA MINOR In 24 thalassaemia minor patients, the A2 haemoglobin ranged from 2.9 to 10.0% (Fig. 4). With one exception, in which a normal A2 value was found, the A2 haemoglobin level was raised in all

FIG. 4. Values (%) in normal individuals, in patients with thalassaemia minor, and in patients with anaemias of non-thalassaemia aetiology.

patients with thalassaemia minor. No thalassaemia major was encountered in this study.

NON-THALASSAEMIC ANAEMIAS In 52 patients with anaemias not due to thalassaemia, normal or subnormal A2 haemoglobin levels were found (Fig. 4). These anaemias were due to iron deficiency, infection, acquired haemolytic disease, uremia, marrow failure, abnormal haemoglobinopathies, etc. Low A2 levels were seen in two infants and in iron-deficiency anaemia. No patients with pernicious anaemia were seen in this study.

DISCUSSION

Quantitative determination of A2 haemoglobin by paper electrophoresis in TRIS buffer is feasible and simple. The values observed in most patients with thalassaemia minor are raised and sufficiently distinct from normal levels or those in non-thalassaemic anaemias to merit its use in clinical practice.

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