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Note

Comparison between electrophoresis and DEAE-microchromatography for the quantitation of haemoglobin A_2

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Electrophoresis followed by scanning or elution[1–11] and DEAE-cellulose chromatography[12,13] are usually applied for the separation and quantitation of haemoglobin A_2 (Hb A_2). A microchromatographic separation was introduced by Bernini[14], who used small columns of DEAE-cellulose to separate Hb A_2, subsequently estimated photometrically. The purpose of this note is to compare the DEAE-microchromatographic method of Bernini with the electrophoretic technique used for several years in our laboratory.

EXPERIMENTAL

Electrophoresis has been performed on 10×20 cm Cellogel* strips by applying a constant voltage of 250 V for 2 h, with Tris–EDTA–borate buffer at pH 8.5. The haemoglobin fractions were eluted separately in Drabkin's solution. The optical density at 540 nm was then determined using a Beckman spectrophotometer.

Microchromatography has been carried out according to Bernini, using DEAE-cellulose (Selectacel Type 40; Brown) in 20×0.8 cm columns and a 0.01 *M* Tris–sodium phosphate+0.0015 *M* KCN buffer at pH 8.5. The optical density of the eluates was determined at 418 nm (Model D. U. spectrophotometer; Beckman).

RESULTS

The reproducibility of the two methods is shown in Table I. Hb A_2 levels were determined by the two methods in sixty normal subjects and in sixty-nine carriers of β-thalassaemia (Table II). Slightly but significantly higher values were obtained in each group by chromatography ($P < 0.001$).

In Fig. 1 are shown the Hb A_2 levels obtained by chromatography in six cases with α-thalassaemia, ten with iron-deficiency anaemia and thirteen with anaemias of various origin (four haemolytic, three autoimmune haemolytic and three aplastic anaemias, one spherocytosis, one chronic myeloid leukaemia and one erythroleukaemia).

Five of the six patients with α-thalassaemia and three out of ten with iron-deficiency anaemia had values lower than normal. The four values found to be lower

* Supplied by Chemetron, Milan, Italy.

TABLE I

REPRODUCIBILITY OF ELECTROPHORESIS AND MICROCHROMATOGRAPHY

	Number of determinations	*Mean Hb A_2 (%)*	*S.D.*
Electrophoresis	22	2.02	0.15
Chromatography	20	2.30	0.09

TABLE II

MEAN VALUES OF Hb A_2 OBTAINED ON ELECTROPHORESIS AND MICROCHROMATOGRAPHY OF SAMPLES FROM NORMAL SUBJECTS AND β-THALASSAEMIA CARRIERS

	Normal subjects			*β-Thalassaemia carriers*		
	Number of cases	*Mean Hb A_2 (%)*	*S.D.*	*Number of cases*	*Mean Hb A_2 (%)*	*S.D.*
Electrophoresis	60	1.96	0.46	69	4.22	0.86
Chromatography	60	2.49	0.25	69	5.06	0.77

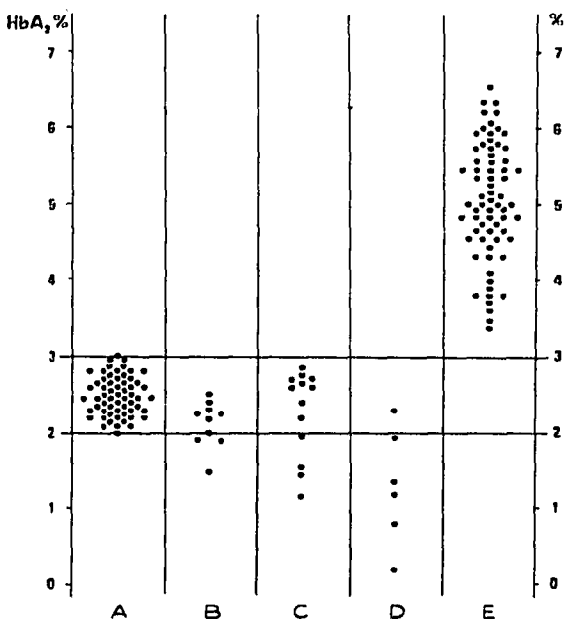

Fig. 1. Hb A_2 values obtained on microchromatography of samples from normal subjects and patients with haematologic diseases. A = Normal subjects; B = iron deficiency; C = miscellaneous anaemias; D = α-thalassaemia; E = β-thalassaemia.

than normal in the anaemias of various origin belonged to two patients with aplastic anaemia, one with haemolytic anaemia and one with erythroleukaemia.

DISCUSSION

The main advantages of the microchromatographic technique compared to electrophoresis are easier and more rapid execution, higher reproducibility and, mainly, the possibility of detecting minor changes in Hb A_2 levels. Cellogel electrophoresis with elution was a very suitable method for the separation and quantitation of the haemoglobin fractions when Hb A, Hb A_2 and abnormal haemoglobins are present. These two methods, having their respective characteristics, proved to be of great value in screening for different forms of thalassaemia and other haemoglobinopathies.

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