DETERMINATION OF THE ELECTROPHORETIC MOBILITY OF HAEMOGLOBIN USING PAPER ELECTROPHORESIS

by

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INTRODUCTION

Paper electrophoresis of haemoglobins has been used extensively for comparative purposes in the last few years (see MOTULSKY *et al.*[1], GOLDBERG[2]).

But, within our knowledge, the electrophoretic mobility of the haemoglobins has not been determined by paper electrophoresis. As a knowledge of the mobilities of the haemoglobins would be useful for the identification of the blood pigments we have studied the possibility of determining them with this simple procedure.

From theoretical considerations (DE WAEL[3, 4]), it can be expected that these determinations will be possible by comparing a paper electrophoresis diagram of haemoglobin with a diagram, obtained under identical conditions, consisting of several bands of known mobility, for instance a diagram of human blood serum. From equation (6) of the paper cited[4] it follows that the ratio of the distances between the bands of the serum diagram depends only on the electrophoretic mobilities. This is true if the evaporation of water from the filter paper strip is uniform over the whole strip, and it is even true if evaporation and electro-osmosis are not constant in time (DE WAEL[4], p. 114). Therefore, if we plot the distances of the bands in the serum diagram from a chosen origin against the known mobilities, we should obtain a straight line, which in fact, we do. By measuring the distance of the haemoglobin band to the same origin, the corresponding mobility can be read from the graph obtained from the serum diagram.

METHOD

Paper electrophoresis was carried out, using the apparatus that has been described by one of us (DE WAEL[3]).

Strips of Whatman No. 3 filter paper (50 × 7 cm) were used. A slit, 20 cm long and 2 mm wide, was cut in the horizontal part (25 cm) of the strip, so as to obtain two parallel strips with a width of *ca.* 3.5 cm, on which paper electrophoresis can be carried out under identical conditions. A barbitone–barbitone sodium buffer (0.01 M = 1.84 g barbitone; 0.05 M = 11.4 g barbitone sodium; water to 1 liter) of pH = 8.6 was used. To this buffer 0.01% commercial human albumin was added to counteract adsorption of haemoglobin on the filter paper, which is considerable in the absence of albumin.

Haemolysates were prepared according to CHERNOFF[5]. These haemolysates were usually diluted ten times with barbitone buffer, corresponding to a final haemoglobin concentration of *ca.* 1.5%. 0.03 ml of the haemoglobin solution was put on one side

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of the double filter paper strip using a perspex wedge-shaped trough (DE WAEL[3, 6]). A similar amount of normal human blood serum was put on the other side of the double strip. After electrophoresis for 16 h at room temperature with a field strength of 4.0–4.5 V/cm, the strips were dried at 105° for 15 min, stained with azocarmine B, and dried again. The strips were made transparent by immersion in a lacquer ("Bril-

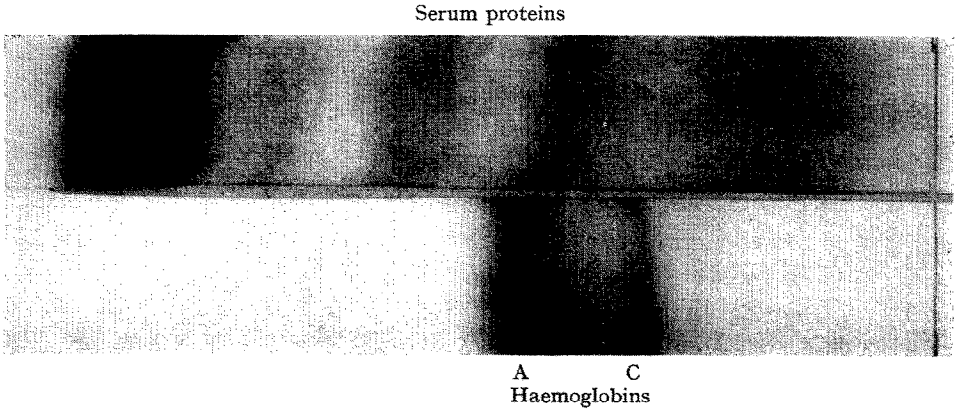

Fig. 1. Double filter paper strip with electrophoresis diagrams of serum proteins and haemoglobins A and C.

lant lak" of Sikkens, Sassenheim, Netherlands). After drying, the positions of the protein bands relative to the line where the haemoglobin and the serum had been put on the paper, were measured with a densitometer using a green interference filter with a maximum transmission at 530 mμ.

The mobility of the haemoglobin was found from a graph as indicated in the introduction.

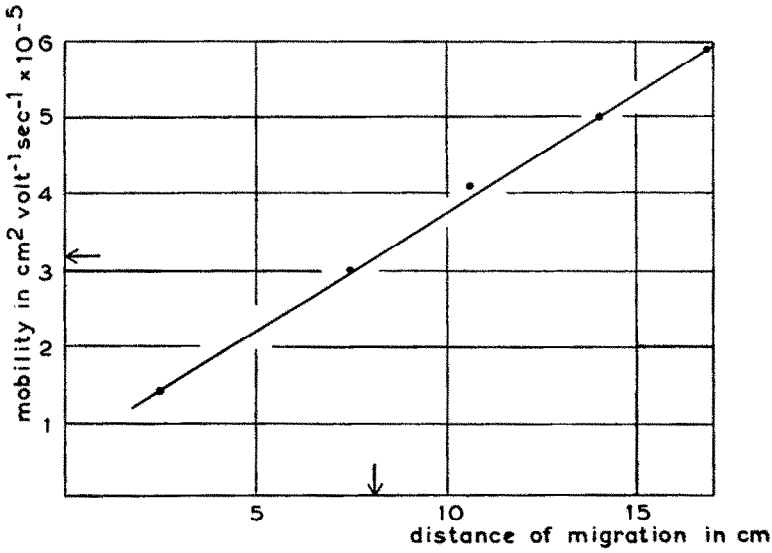

Fig. 2. Distance of migration of serum proteins plotted against corresponding mobilities.

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RESULTS

Fig. 1 represents an electrophoresis diagram of a mixture of haemoglobin A and C, together with a diagram of blood serum.

It can be seen that the separation of the haemoglobins is rather good, and that the haemoglobins are only slightly adsorbed on the filter paper.

Fig. 2 is an example of a graph obtained by plotting the distance of migration of the serum protein bands against the corresponding mobilities.

The mobilities computed by MEULEMANS[7] from paper electrophoresis experiments on 12 normal serums were used (Table I)*.

TABLE I

MOBILITIES OF SERUM PROTEINS IN $cm^2 V^{-1} sec^{-1} \times 10^{-5}$

Albumin	*Globulins*			
	α_1	α_2	β	γ
5.9	5.0	4.1	3.0	1.4

The points obtained lie on or near a straight line; this is practically always found. The distance of migration of haemoglobin A and the corresponding mobility are indicated by arrows.

In Table II the mobilities of haemoglobin A, F and C, determined by the method described, the standard deviations, and the standard deviations of the means are

TABLE II

	Haemoglobins		
	A	*F*	*C*
Mobility	3.15	2.85	2.25
,,	3.15	2.95	2.30
,,	3.05	2.85	2.10
,,	3.15	2.85	2.25
,,	3.05	2.85	2.10
,,	3.20	2.87	—
Mean mobility	3.12	2.87	2.20
Standard deviation	0.075 = 2.4%	0.040 = 1.4%	0 094 = 4.3%
Standard deviation of mean	0.034 = 1.1%	0.016 = 0.6%	0.042 = 1.9%

given. From Table II it is clear that the accuracy of the measurement of the mobility is of the order of a few per cent. Therefore, it is felt that the determination of the mobility will be helpful in identifying haemoglobins, especially when solutions of known haemoglobins for comparative purposes are not available.

ACKNOWLEDGEMENT

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* These mobilities are very similar to those computed by S. H. ARMSTRONG *et al.*[8] from free electrophoresis experiments: 5.92, 4.85, 3.87, 2.88 and 1.15. We prefer, however, to use the figures obtained by paper electrophoresis, the results of which are undoubtedly more comparable with our own results.

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SUMMARY

The authors have determined the mobilities of haemoglobins by comparing the electrophoretic migration of haemoglobin and serum proteins on paper strips. The accuracy of the measurements is of the order of a few per cent. Therefore, these measurements can be helpful in identifying haemoglobins.

RÉSUMÉ

Les auteurs ont déterminé les mobilités des hémoglobines, en comparant par électrophorèse sur des bandes de papier la migration de l'hémoglobine et des sérum-protéïnes. L'exactitude des mesures est dans l'ordre de quelques pourcents. Ces mesures peuvent donc être utiles pour l'identification des hémoglobines.

ZUSAMMENFASSUNG

Die Verfasser haben die Beweglichkeit von Hämoglobinen bestimmt indem sie die elektrophoretische Wanderung von Hämoglobin und Serumproteinen auf Papierstreifen verglichen haben. Die Genauigkeit der Messungen ist von einer Grössenordnung von einigen wenigen Prozenten. Daher können diese Messungen bei der Identifizierung von Hämoglobinen behilflich sein.

РЕЗЮМЕ

Авторы определяли подвижности гемоглобинов путем сравнения электрофоретических миграций гемоглобина и сывороточного белка на бумаге. Точность измерения порядка нескольких процентов, что позволяет применять эти измерения для опознавания гемоглобинов.

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