Separation of the α- and β-Chains of Globins by Means of Starch-gel Electrophoresis

THE investigation of the molecular weight and the electrophoretic behaviour of globins at *p*H 2–4 has led to a better understanding of the structure of the hæmoglobin molecule. At a *p*H less than 6 there is a progressive decrease of the molecular weight of human hæmoglobin[1]. At *p*H 11 the value of the sedimentation coefficient is 2·55, which points to a molecular weight of about 33,000 instead of 67,000, a value normally found[2].

In an indirect way, Singer and Itano[3] demonstrated that the dissociation at a low *p*H is asymmetrical. Moving-boundary electrophoresis at *p*H 11[2] leads to the assumption that the molecule is split into two identical halves[3].

Reichmann and Colvin[4] estimated a molecular weight of 16,000 at *p*H 2 for horse globin. From the electrophoretic behaviour of globin in acid medium, these authors were able to show that more than one component was present.

Haug, Smith and Wilson[5,6] were able to isolate two different components from horse globin, by fractionated precipitation with acid acetone, by moving boundary electrophoresis and by a chromatographic procedure at low *p*H. Hunt[7], using the same chromatographic method, separated the α- and β-chains and the α- and γ-chains, respectively, of human adult and fœtal globin. Hill and Craig[8] prepared the α- and β-chains by a counter-current distribution procedure.

This communication is a description of the separation of human adult and fœtal globin in chains by means of starch-gel electrophoresis according to Smithies[9], in formate-buffer *p*H 1·9; $\mu = 0{\cdot}02$. A potential of 120 V. was applied during 16 hr. Globins were prepared[10] from the following hæmoglobins: (*a*) normal adult; (*b*) chromatographically pure A_1[11]; (*c*) fœtal purified by the alkali-denaturation method of Chernoff[12].

The electrophoretic pattern of adult globin (Fig. 1*d* and *e*) demonstrates two fast-moving bands of almost equal intensity, and a third band with a smaller mobility. The fast-moving bands are the α- and β-chains of the globin; this was proved in the following way. Globin was separated into two fractions, the α- and β-chains, using the chromatographic method of Wilson and Smith[6]. After concentration the two fractions were separately applied to the gel (Fig. 1*b*, *c*). Fraction I (α-chain) contains the slower of the two fast bands while fraction II contains the faster band.

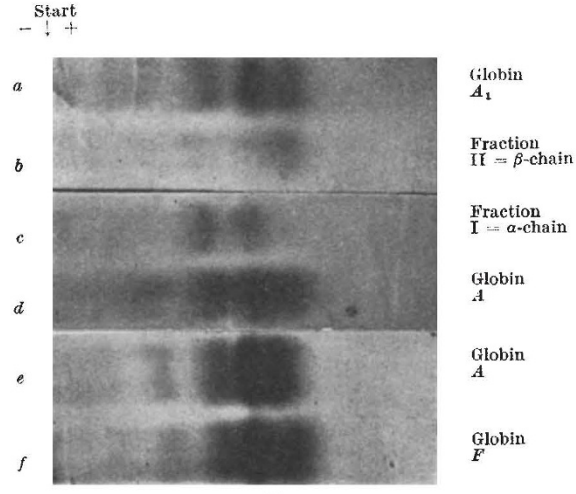

Fig. 1

The electrophoretic pattern of fœtal globin (Fig. 1*f*) compared with the pattern of adult globin (Fig. 1*e*) demonstrates that the α-chains have the same mobility. The mobilities of the β-chains, however, are different. This result is in agreement with the recent findings of Hunt[7].

The nature of the slow band that is present in both separated fractions and in the unfractionated globins is not quite clear. It is possible that they are the result of agglomeration of single chains. Interesting in this respect is the presence of the same band in the electrophoretic pattern of globin of pure hæmoglobin A_1. This excludes the possibility that it is due to the minor components present in the hæmoglobin of an adult human individual. Further research on this third fraction is in progress.

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[1] Field, E. O., and O'Brien, J. R. P., *Biochem. J.*, **60**, 656 (1955).
[2] Hasserodt, U., and Vinograd, J., *Proc. U.S. Nat. Acad. Sci.*, **45**, 12 (1959).
[3] Singer, S. J., and Itano, H. A., *Proc. U.S. Nat. Acad. Sci.*, **45**, 174 (1959).
[4] Reichmann, M. E., and Colvin, J. R., *Canad. J. Chem.*, **34**, 411 (1956).
[5] Haug, A., and Smith, D. B., *Canad. J. Chem.*, **35**, 945 (1957).
[6] Wilson, S., and Smith, D. B., *Canad. J. Biochem. Physiol.*, **37**, 405 (1959).
[7] Hunt, J. A., *Nature*, **183**, 1373 (1959).
[8] Hill, R. J., and Craig, L. C., *J. Amer. Chem. Soc.*, **81**, 2272 (1959).
[9] Smithies, O., *Biochem. J.*, **61**, 629 (1955).
[10] Anson, M. L., and Mirsky, A. E., *J. Gen. Physiol.*, **13**, 469 (1930).
[11] Huisman, T. H. J., Martis, E. A., and Dozy, A., *J. Lab. Clin. Med.*, **52**, 315 (1958).
[12] Chernoff, A. I., *Blood*, **8**, 413 (1953).

Haptoglobin Types in *Macaca irus*

WE have examined the haptoglobin types[1] of ten Cynomolgus monkeys (*Macaca irus*, F. Cuv.). The serum samples have been obtained from the Department of Virus Research, Karolinska Sjukhuset, Stockholm. The monkeys were not born in captivity, but were all captured from wild populations.

The sera were examined by starch-gel electrophoresis, using the discontinuous buffer system described by Poulik[2]. Hæmoglobin from the monkeys was added to the sera prior to electrophoresis, and the resultant gels were stained with benzidine and amido-black.

The resulting protein patterns were, as expected, similar to human serum protein patterns. Two different hæmoglobin-binding serum protein patterns were observed. Double runs revealed that the two patterns were identical with the *Hp* 1–1 and *Hp* 2–1 types found in man. Three of the ten individuals had the *Hp* 2–1 type. It was noted that the *Hp* 2–1 types had a much heavier concentration of haptoglobin in the fastest zone compared with the ordinary human *Hp* 2–1; in other words, it resembled the *Hp* 2–1 (mod), cf. Giblett[3].

Arends and Rodriguez[4] have examined the sera from 27 monkeys (23 *Macaca mulatta*, 1 *Macaca irus*