Haemoglobin Ocho Rios (β 52 (D3) Aspartic Acid $\rightarrow$ Alanine): A New β -Chain Variant of Haemoglobin A Found in Combination with Haemoglobin S

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During a routine study of the parents of a 3-month-old Jamaican child with the sickle-cell trait the father was found to have a haemoglobin electrophoretic pattern identical to that of homozygous sickle-cell disease. Because he was perfectly well and had no haematological abnormalities detailed studies of his haemoglobin constitution were undertaken. These indicate that he is heterozygous for both haemoglobin S and a previously undescribed β -chain haemoglobin variant.

Methods

Haemoglobin analysis was carried out by electrophoresis on cellulose acetate (Kohn, 1969), starch gel using a TRIS-EDTA-borate system, pH 8.5 or phosphate system, pH 7.0 (Weatherall, 1965), agar gel (Robinson *et al*, 1957; Marder and Conley, 1959) and starch block using a phosphate buffer system, pH 6.5 (Weatherall, 1965). Haemoglobin A₂ levels were estimated by the modification of the method of Marengo-Rowe (1965) described by Weatherall *et al* (1971). Alkali resistant haemoglobin levels were determined by the methods of Singer, Chernoff, and Singer (1951) and Betke, Marti, and Schlicht (1959). Blood films were also examined for the presence of fetal haemoglobin-containing cells by the method of Kleihauer, Braun, and Betke (1957). Preparation of globin, separation of globin chains on carboxymethyl (CM)-cellulose columns in 8M urea, isolation of purified chains, fingerprinting, and amino-acid analysis followed the methods described by Clegg, Naughton, and Weatherall (1966).

Results

Clinical and Haematological Investigation.

The proband was a 3-month-old Jamaican Negro child with the sickle-cell trait and no haematological abnormalities. The child's mother and father were available for study. The mother's blood picture and haemoglobin electrophoretic pattern were

completely normal. The father was a 35-year old Negro who had been born in Ocho Rios on the North Coast of Jamaica. He had no history of illness and clinical examination was completely normal. His haemoglobin level was 15.4 g/100 ml, packed cell volume 45%, and reticulocyte count 2%. The peripheral blood film showed occasional target cells and, on incubation of his blood with *coliform* organisms, the red cells sickled.

Haemoglobin analysis. Electrophoresis of the father's haemoglobin on cellulose acetate (Kohn, 1969) revealed two components; a major fraction migrating in the position of haemoglobin S and a minor fraction migrating in the position of haemoglobin A₂. The haemoglobin A₂ value obtained by a quantitative cellulose acetate electrophoresis was 3.3%. This is at the upper limit of the normal adult range (Weatherall *et al*, 1971). Electrophoresis on agar gel, pH 6.0 (Robinson *et al*, 1957) or pH 5.9 (Marder and Conley, 1959), revealed two major components; one in the position of haemoglobin S and the other, of almost equal concentration, close to the origin. Starch gel electrophoresis, pH 8.5, of the father's haemolysate gave a pattern identical to that of homozygous sickle-cell anaemia with haemoglobins S and A₂ only. No haemoglobins F or A were present. Starch gel electrophoresis, pH 6.5, and starch block electrophoresis, pH 6.5, gave only a single component and the second fraction seen on agar gel electrophoresis was not resolved. There was no increase in alkali resistant haemoglobin by either the method of Singer *et al* (1951) or Betke *et al* (1959) and none of the red cells showed resistance to acid elution.

Globin was prepared from the father's haemolysate without prior purification and the constituent globin chains examined by CM-cellulose chromatography in 8M urea, pH 6.7. Two globin chain

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fractions were isolated, one eluting in the position of the β -chain of haemoglobin S and the other in the position of a normal α^A -chain. The β^S -chain was aminoethylated, digested with trypsin, and fingerprinted. When compared with a fingerprint of aminoethylated β^A -chain three abnormal peptides were noted. One was in the known position of haemoglobin β^{S1} and had the expected amino-acid composition. The normal β^{A1} peptide was also present at a similar concentration to the β^{S1} peptide. The other two abnormal peptides were slightly more basic than, and had very similar chromatographic mobilities to, β^{A5} and $\beta^{A5_{ox}}$, both of which were also seen on the fingerprint. Amino-acid analysis of the two abnormal peptides gave compositions identical to those of β^{A5} and $\beta^{A5_{ox}}$ except that there was an additional alanine residue and one less aspartic acid residue in each case. Peptide β^5 has one residue of asparagine and two of aspartic acid and since the haemoglobin variant is basic relative to haemoglobin A the alanine substitution must involve one of the two aspartic residues rather than asparagine.

A larger sample of the abnormal β^5 peptide was therefore isolated from a tryptic digest of the abnormal β -chains and digested with chymotrypsin. The resulting digest was fractionated by electrophoresis at pH 6.5 and 3.5 and by chromatography in BAWP (Clegg *et al*, 1966). A peptide was isolated with the following composition: Ser,Thr,Pro,Ala₂, Val, Met, corresponding to residues 49–55 of the abnormal β -chain. In the β^A -chain, residue 52 is normally aspartic acid. The substitution in the abnormal variant must therefore be $\beta^{52} \text{ Asp} \rightarrow \text{Ala}$.

The finding in the father of two haemoglobin components on agar gel electrophoresis and of both β^{A1} and β^{S1} and normal and abnormal β^5 peptides in the β -chain obtained by urea chromatography indicate that he is heterozygous for both haemoglobin S and a β -chain variant, $\beta^{52} \text{ Asp} \rightarrow \text{Ala}$. Since the latter haemoglobin has not been previously reported it has been designated haemoglobin Ocho Rios after the place of origin of the father.

Discussion

The discrepancy between the clinical and laboratory findings in this case led to the identification of a new haemoglobin variant, haemoglobin Ocho Rios. A similar discrepancy led Konotey-Ahulu *et al* (1971) to identify the heterozygous state for both haemoglobins S and Osu-Christiansborg ($\beta^{52} \text{ Asp} \rightarrow \text{Asn}$) which also has the same electrophoretic mobility as haemoglobin S on paper electrophoresis at pH 9 and starch gel electrophoresis at pH 8.6. Thus the two substitutions which have now been

described at this site both result in a haemoglobin variant which has the same mobility as sickle-cell haemoglobin on electrophoresis by routine screening techniques but which is separable by either agar gel electrophoresis or peptide mapping, from sickle haemoglobin. Both these studies underline the importance of a full structural analysis of the 'haemoglobin S' from individuals with 'benign sickle-cell anaemia'. The presence of a second β -chain variant can be deduced by either agar gel electrophoresis or peptide analysis of the ' β^S -chain' isolated by CM-chromatography in 8M urea even in the absence of critical family members, as in the present case.

Haemoglobin Ocho Rios is a further example of a β -chain variant which does not cause a clinical disorder when inherited in the heterozygous state with haemoglobin S. Like haemoglobin Osu-Christiansborg the substitution is at an external position in the D helix; the gradual acquisition of information of this type may provide information regarding the complementary sites involved in the stacking of haemoglobin S molecules.

Summary

A new human haemoglobin variant, haemoglobin Ocho Rios ($\beta^{52}(\text{D3})$ aspartic acid $\rightarrow$ alanine) has been characterized. It was found in an individual also heterozygous for the sickle-cell gene and the presence of the two β -chain variants is not associated with any clinical or haematological disorder.

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