Haemoglobin Osu-Christiansborg: A New β -chain Variant of Haemoglobin A (β 52 (D3) Aspartic Acid $\rightarrow$ Asparagine) in Combination with Haemoglobin S

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A 63-year-old man, a member of the Ga tribe from the Osu-Christiansborg area in Accra was examined for hypertension. On incubation with 2% sodium metabisulphate his red cells readily sickle. Only two haemoglobin bands were demonstrated by paper electrophoresis at pH 8.9 (Cradock-Watson, Fenton, and Lehmann, 1959) and starch gel electrophoresis at pH 8.6 (Poulik, 1957), a minor one corresponding to haemoglobin A₂ in normal concentrations and a major one in the position of haemoglobin S (Fig. 1). The finding of a single haemoglobin band in the position of haemoglobin S would suggest sickle-cell anaemia. The man was not anaemic, his haemoglobin level was 13.8 g/100 ml and the haematocrit was 42.9%, and there was no jaundice. The man did however remember in his twenties having bone pains of sufficient severity to wake him at night and pains in his thighs on running; these pains have not recurred. The only abnormal findings were an arcus senilis and blood pressure of 200/120 mm with an occasional ectopic heart beat and bilateral perception deafness of uncertain cause, as had his father.

Laboratory Investigations

The solubility test of Itano (1953), was compatible with the sickle-cell trait rather than sickle-cell anaemia, suggesting that two haemoglobins were present, and that the proportion of the non-sickling haemoglobin was like that of haemoglobin A in a sickle-cell trait carrier. On agar gel electrophoresis at pH 6.0 (Robinson *et al*, 1957), two haemoglobins separated, one in the position of haemoglobin S and one in the position where haemoglobin A or D are found (Fig. 2). After staining and drying the agar gel, the two fractions were measured on a

densitometer. Haemoglobin A₂ migrates with haemoglobin A by this technique, and after correcting the proportion of the haemoglobin in the position of haemoglobin A, this was 56% and haemoglobin S was 42%. A control sample from a sickle-cell trait carrier showed 57% haemoglobin A and 41% haemoglobin S respectively under the same conditions (see Table III). These proportions are similar to those reported in a Nigerian with haemoglobin S and haemoglobin D Ibadan, (Watson-Williams *et al*, 1965), and haemoglobin Korle-Bu and haemoglobin S (Konotey-Ahulu *et al*, 1968).

On examination of the family, one member was found to have haemoglobin A together with the non-sickling haemoglobin. The haemolysate from this sample was used to isolate the variant haemoglobin. The haemoglobin was isolated by paper electrophoresis at pH 8.9, and also by column chromatography on DEAE-Sephadex (A 50), with a pH gradient of 8.5 to 7.0 by the method of Huisman and Dozy (1965). The abnormal fraction was concentrated by vacuum dialysis and re-run on paper

FIG. 1. Paper electrophoresis (Tris buffer pH 8.9) of the haemoglobin solution of the propositus with haemoglobins A + S as control. A single band is seen in the position of haemoglobin S.

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FIG. 2. Agar gel electrophoresis at pH 6.0. By this method haemoglobin Osu-Christiansborg migrates in the position of haemoglobin A and separates from haemoglobin S.

FIG. 3. Fingerprint (peptide chromatogram) of the new haemoglobin. Electrophoresis at pH 6.4 β^A TpV (residues β 41–59) is missing, and a new peptide is seen instead (see text).

electrophoresis at pH 8.9 to assess the purity. On hybridization with canine haemoglobin (Huehns, Shooter, and Beaven, 1962) the abnormality was found to be in the β -chain. Globin was prepared at pH 6.4 by the methods summarized by Sick *et al* (1967). From the fingerprint (peptide chromatogram) in Fig. 3 it can be seen that the tryptic peptide (Tp) β V is absent, and that a new, more positively charged peptide with a slight increase in chromatographic mobility is found instead. The amino-acid residues comprising β TpV are residues 41–55 of the 146 of the β -chain. Though it can be seen in the fingerprint (Fig. 2) that the variant peptide is more positively charged and has a slightly higher chromatographic mobility than β^A TpV, the result of the amino-acid analysis of the two peptides is the same in both (Table I). This suggests that an asparagine or gluta-

mine residue in the variant peptide has been converted into the free acid during the hydrolysis preceding amino-acid analysis. From the amino-acid sequence (Fig. 4) there are 3 acid residues, aspartic acid at position 47 and 52 and glutamic acid at position 43 in β^A TpV, and one of these must be present as the amide in the variant. To determine the exact position of substitution, β TpV was isolated from 5 preparative prints (30 mg globin) from both haemoglobin A and the variant by elution with 0.1 M ammonium bicarbonate, and taken to dryness *in vacuo*. The eluate was hydrolysed with thermolysin at pH 7.5 using the method of Lorkin *et al* (1970). In addition one diagnostic and one preparative fingerprint were prepared at pH 6.4 for each haemoglobin. The expected position of hydrolysis by thermolysin is given in Figure 4. From the thermolysin fingerprint of β TpV

AMINO-ACID COMPOSITION OF β TpV IN HAEMOGLOBIN OSU-CHRISTIANSBORG AND HAEMOGLOBIN A

* Methionine value corrected for loss on acid hydrolysis.
† Phenylalanine value low due to loss on acid hydrolysis.
‡ Lysine analysis lost, but taken as one residue as this is a tryptic peptide.

At the time of the investigation (1968) the propositus and two sisters were the only survivors of 11 children (Fig. 6). The oldest sib, aged 87 and still living in 1970 completely asymptomatic, has haemoglobins Osu-Christiansborg and S, whereas her sister (aged 74) now deceased, had the sickle-cell trait. The 45-year-old son (now 47) of the oldest sib was heterozygous and had haemoglobins Osu-Christiansborg and A. The only daughter of 5 children of the propositus available for

AMINO-ACID COMPOSITION OF THERMOLYSIN PEPTIDES FROM β TpV
IN HAEMOGLOBIN OSU-CHRISTIANSBORG AND HAEMOGLOBIN A

Sequence	Amino Acid	Haemoglobin Osu-Christiansborg			Haemoglobin A		
		n Mole	Molar Ratio	Charge (pH 6·4)	n Mole	Molar Ratio	Charge (pH 6·4)
41-44	Ser	35·4	1·08	- 1	27·4	1·07	- 1
	Glu	35·7	1·09		31·2	1·23	
	Phe	60·2	1·83		43·3	1·70	
45-47	Asp	86·5	1·08	- 1	91·5	0·70	- 1
	Gly	88·0	1·10		182·0	1·39	
	Phe	65·0	0·82		120·0	0·92	
48-53	Asp	75·5	1·07	0	85·4	0·78	- 1
	Thr	71·7	1·02		122·0	1·12	
	Ser	71·4	1·01		125·0	1·15	
	Pro	64·0	0·91		116·0	1·05	
	Ala	72·0	1·02		126·0	1·16	
	Leu	67·5	0·96		79·5	0·73

FIG. 6. Family pedigree.

identification showed haemoglobin A only. It was considered inappropriate to investigate this seeming contradiction too closely. Haemoglobin Osu-Christiansborg, when present with haemoglobin S, was found in the same proportion as haemoglobin A in a sickle-cell trait carrier. When haemoglobin Osu-Christiansborg is found with haemoglobin A the proportions are not equal as they are for example, with haemoglobin Korle-Bu (Konotey-Ahulu *et al*, 1968), and there was more haemoglobin A present than haemoglobin Osu-Christiansborg. For percentages of each haemoglobin see Table III.

TABLE III
PROPORTIONS OF HAEMOGLOBINS A, S, AND OSU-CHRISTIANSBORG IN HETEROZYGOTES*

Genotype	Haemoglobin (%)		
	A	Osu-Christiansborg	S
A + Osu-Chr	62	36	
S + Osu-Chr		56	42
A + S	57		41

* Haemoglobins A and Osu-Christiansborg were separated by cellulose acetate electrophoresis, the others by agar gel electrophoresis (see text).

Discussion

The identification of haemoglobin Osu-Christiansborg during investigation of a patient with the electrophoretic findings of sickle-cell anaemia, underlines the importance of characterizing the haemoglobin where the clinical pattern deviates from that of the disease. The substitution of aspartic acid $\beta 52$ (D3) by asparagine occurs at an external position in the D helix. This helix is absent from the α -chain (Perutz *et al*, 1968) and may account for the fact that isolated α -chains do not form tetramers whereas the β -chains do, but the new haemoglobin may well provide information on the complementary site involved in the stacking of deoxygenated haemoglobin S.

4—J.M.G.

Summary

A new variant of haemoglobin A, haemoglobin Osu-Christiansborg $\beta 52$ aspartic acid to asparagine has been found in a Ghanaian family. In combination with haemoglobin S, this haemoglobin did not cause overt sickle-cell disease.

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