Haemoglobin E Saskatoon $\beta^{22 \text{ glu} \rightarrow \text{lys}}$ in the Shetland Islands

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Summary. A survey of blood samples from the inhabitants of the Shetland Islands has revealed the presence of a variant haemoglobin in 1 individual out of the 345 tested.

The variant haemoglobin accounted for 46% of the total haemoglobin. Tryptic digestion and amino acid analysis indicated that the variant haemoglobin was caused by the substitution of a glutamic acid residue at position 22 in the β chain by a lysine residue. This is the same amino acid substitution as found in haemoglobin E Saskatoon.

Hb E Saskatoon ($\beta^{22 \text{ glu} \rightarrow \text{lys}}$) was first discovered by Vella *et al* (1967) in a woman of mixed Dutch-Scottish parentage from Saskatoon, Canada. The variant haemoglobin was electrophoretically indistinguishable from Hb E. In view of the known connexion between the Dutch and South-East Asia, it was at first thought that the variant was another example of Hb E. However a family study revealed that the woman had inherited the variant haemoglobin from her Scottish father. It was subsequently shown that the variant haemoglobin was the result of the replacement of the glutamic acid residue at position 22 in the β chain by a lysine residue. This is the same type of amino acid substitution as in Hb E, only in the case of Hb E it is a replacement of the glutamic acid at position 26 in the β chain. The new haemoglobin variant was called Hb E Saskatoon.

This paper reports the discovery of haemoglobin E Saskatoon in a woman from the Shetland Islands.

Materials and methods

Characterization of the abnormal haemoglobin was by the standard techniques as described by Jonxis and Huisman (1968).

Results

During the course of a survey of 345 inhabitants of the Shetland Islands for red cell enzyme polymorphisms by starch gel electrophoresis, an individual was discovered with two major haemoglobin

components (Fig. 1). One of the haemoglobin migrated to the position of HbA, while the second haemoglobin migrated further towards the cathode at pH 6.8.

The abnormal haemoglobin was separated from HbA by ion-exchange chromatography on DEAE-Sephadex, and was found to comprise 46% of the total haemoglobin. Canine hybridization showed that the abnormality resided in the β chain. The

Hb Shetland Starch Gel phosphate pH 6.8

FIG. 1. Starch gel electrophoresis of haemoglobin in phosphate buffer pH 6.8.

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FIG. 2. Peptide maps of the β chains from Hb A and Hb Shetland.

β chain was isolated on a CM-cellulose column in 8M urea and digested with trypsin. A peptide map of the soluble peptides is shown in Fig. 2. In the β chain of Hb Shetland the peptide corresponding to β Tp III in the normal β chain is missing and has been replaced by two new peptides labelled S I and S II. Peptides β Tp III, S I, and S II were eluted from the cellulose plates and their amino acid compositions were determined. The results are given in Table I and have been calculated as the minimum residue number. The results for the amino acid composition of peptide β Tp III are in agreement with the published amino acid composition for this peptide (Lehman and Huntsman, 1966).

The replacement of one peptide by two new peptides is usually found to be the result of the substitution of an amino acid by a lysine or arginine residue, thereby creating an additional site for the enzyme trypsin to attack. Peptide S I has an amino acid composition identical to the terminal 8

TABLE I
AMINO ACID COMPOSITION OF
PEPTIDES β Tp III, S I, S II
EXPRESSED AS MINIMUM RESIDUE
NUMBER

Amino Acid Residue	Peptides		
	β Tp III	S I	S II
Aspartic/asparagine	2	—	2
Glutamic/glutamine	2	1	—
Glycine	3	3	—
Alanine	1	1	—
Valine	3	1	2
Leucine	1	1	—
Lysine	—	—	1
Arginine	1	1	—

residues of peptide Tp III (β 23–30). The first 5 residues of β Tp III (β 18–22) have a glutamic acid residue at the C-terminal end (β 22). If this glutamic acid were to be replaced by lysine, thereby creating an additional site for cleavage by trypsin, the resulting peptide (β 18–22) would have an amino acid composition identical to that found for the peptide S II in Hb Shetland. These results are summarized in Table II.

Conclusions

The variant haemoglobin found in the Shetland Islands has been shown to be the result of a replacement of the glutamic acid residue at position 22 in the β chain by a lysine residue. This is the same substitution as in Hb E Saskatoon. The connexion between the two examples of the same rare variant is most likely explained by the Scottish parentage of the woman with Hb E Saskatoon described by Vella *et al* (1967), and the Scottish ancestry of the present-day inhabitants of the Shetland Islands.

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TABLE II
AMINO ACID SEQUENCE OF PEPTIDES β Tp III, S I, S II

β Tp III											
18	19	20	21	22	23	24	25	26	27	28	29
Val	Asn	Val	Asp	Glu	Val	Gly	Gly	Glu	Ala	Leu	Gly
18	19	20	21	22	23	24	25	26	27	28	29
Val	Asn	Val	Asp	LYS	Val	Gly	Gly	Glu	Ala	Leu	Gly
18	19	20	21	22	23	24	25	26	27	28	29
S II				S I							