

there is difficulty in differentiating between these two conditions clinically, radiologically, and at operation.

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A New Haemoglobin in a Thai Family. A Case of Haemoglobin Siriraj- β Thalassaemia

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Haemoglobin Siriraj

In 1962 an 8-year-old Thai child was admitted to Siriraj Hospital, Bangkok, with severe anaemia and fever. The blood smear showed many nucleated red cells and target cells, and the osmotic fragility of the erythrocytes was reduced. The spleen was enlarged two fingerbreadths and the liver one fingerbreadth below the costal margin. There was no "thalassaemia-mongoloid" facies, and the x-ray picture of the bones was normal. Treatment of infection resulted in improvement, and the haemoglobin level rose gradually from 6 g. to 11.9 g./100 ml. (Table I). Two years later the spleen and liver were only just palpable. The blood picture, however, still showed numerous target cells, and the osmotic fragility was still reduced.

TABLE I.—Haematological Findings in the Propositus

	Hb g./100 ml.	R.B.C. 10 ⁹ /c.mm.	Reticulo-cytes %	Nucleated Red Cells per 100 W.B.C.	M.C.V. c. μ	Target Cells	Osmotic Fragility %NaCl
On first admission	6	2.5	6	12	66	++	0.10-0.35
Two years later	11.9	4.9	3	None	66	++	0.15-0.40

When the patient's haemoglobin was examined by paper electrophoresis (Tris buffer pH 8.9) it showed only one major fraction. It was not in the position of haemoglobin A, but was moving more slowly than A towards the anode. On comparison with known abnormal haemoglobins, this haemoglobin was found to differ from them all, and was therefore thought to be a new variant, which was named haemoglobin Siriraj. In addition to the major fraction, haemoglobin A₂ at a level above that normally seen and 4% haemoglobin F (Betke, Marti, and Schlicht, 1959) were also noted.

Family Investigation

The patient's family were examined. The father possessed 60% haemoglobin A and 40% haemoglobin Siriraj. The mother showed only haemoglobin A as the major fraction, but there was an increased haemoglobin A₂ and a trace of haemoglobin F (2.1%) which was just measurable (Table II). It was concluded that the father had the haemoglobin Siriraj trait and the mother β -thalassaemia minor, and that the haemato-

logical condition of the propositus was the outcome of interaction between the heterozygous states for haemoglobin Siriraj and for β -thalassaemia respectively, resulting in haemoglobin Siriraj- β -thalassaemia. Of three siblings, two also had the haemoglobin pattern of β -thalassaemia minor, and one had the haemoglobin Siriraj trait.

TABLE II.—Haematological Findings in the Thai Family Carrying the Genes for β -Thalassaemia and Haemoglobin Siriraj

	Hb g./100 ml.	Red Cells 10 ⁹ /c.mm.	P.C.V. %	M.C.V. c. μ	M.C.H.C. %	Target Cells	Osmotic Fragility	Serum Iron μ g./100 ml.	Hb F %	Hb Electrophoresis
Father	15.6	5.5	45.5	83	35	None	0.32-0.45		None	A + Siriraj
Mother	10.8	4.8	33	69	32	Some	0.20-0.50	230	2.1	Hb A ₂ raised
Sibling I	12	5.8	37	64	33	Some	0.20-0.45	277	2.9	Hb A ₂ raised
Sibling II	13.4	5.2	39	75	34	None	0.30-0.50		None	Hb A + Siriraj
Sibling III	11.3	4.2	35	60	32	Some	0.28-0.50		3.3	Hb A ₂ raised
Propositus	11.9	4.9	34	69	34	++	0.15-0.40		4.0	Hb Siriraj + F Hb A ₂ raised

Haemoglobin Siriraj

On electrophoresis and chromatography with varying buffers and media the relative mobility of the new haemoglobin differed. On paper electrophoresis at pH 8.9 using Tris buffer

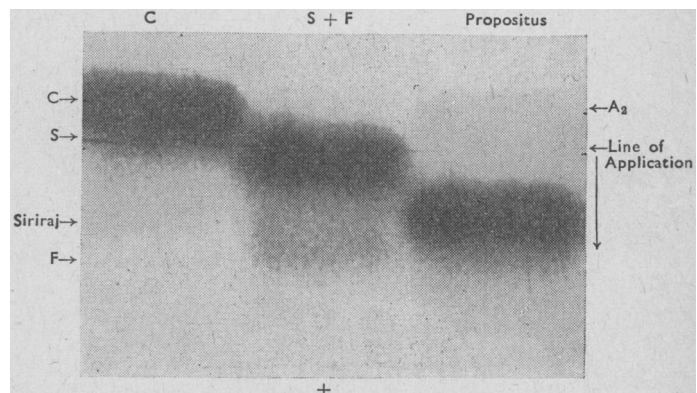


FIG. 1.—Paper electrophoresis—Tris buffer, pH 8.9. From left to right: haemoglobin C, haemoglobin S+F, the haemoglobin of the propositus: haemoglobin Siriraj. It moves faster than haemoglobin C and S, just behind haemoglobin F, in the position which would be occupied by haemoglobin Q.

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(Cradock-Watson, Fenton, and Lehmann, 1959) it moved just behind haemoglobin A in the position of haemoglobin Q in front of haemoglobin G (Fig. 1). On barbiturate buffer at pH 8.6 (see Lehmann and Ager, 1960) it moved more slowly than haemoglobin G, and indeed slightly more slowly than haemoglobin S or D, just in front of haemoglobin E. The same mobility was found (Fig. 2) on discontinuous starch-gel electrophoresis, pH 8.2, according to Poulik (1957). On agar electrophoresis at pH 6 (Robinson, Robson, Harrison, and Zuelzer, 1957) haemoglobin Siriraj moved like haemoglobin C (Fig. 3). On chromatography at pH 6, using resin IRC 50 (Huisman and Prins, 1955), it did not move from the area of application, again resembling haemoglobin C.

We particularly examined these mobilities to compare haemoglobin Siriraj with haemoglobin C-Georgetown (Pierce, Rath, and McCoy, 1963). Haemoglobin C-Georgetown behaves opposite to haemoglobin Siriraj. It is reported to move like haemoglobin C on paper and starch-block electrophoresis at pH 8.6, and similar to haemoglobin S on agar electro-

phoresis at pH 6.2. The cells containing haemoglobin C-Georgetown gave a positive sickling test. Neither the cells of our propositus nor those of the father or the brother, who were haemoglobin-Siriraj-trait carriers, gave a positive sickling test. The solubility of the haemoglobin solution in the Itano (1953) test was also not that found with sickle-cell haemoglobin. Both the haemoglobin of the propositus and that of the father were fully soluble in a concentration of 5 g./l. in 2.24 M phosphate buffer according to Itano. In phosphate buffer 2.58 M the solubilities were 1.20 g./l. and 1.35 g./l. respectively (solubility of haemoglobin A: 1.08–1.69).

On hybridization of haemoglobin Siriraj with canine haemoglobin (Itano and Robinson, 1959; Huehns, Shooter, and Beaven, 1962) the mobility of α_2 Siriraj β_2 canine was the same as that of $\alpha_2^A \beta_2$ canine, but the hybrid of α_2 canine and of β_2 Siriraj moved more slowly than α_2 canine β_2 Siriraj, indicating that the abnormality in haemoglobin Siriraj was located in the β -chain.

Amino-acid Substitution in Haemoglobin Siriraj

The haemoglobin was purified and a "fingerprint" of its soluble tryptic peptides was prepared according to Ingram (1958), Baglioni (1961), and Ingram and Stretton (1962). (For more details of these and the following procedures and techniques see Watson-Williams, Beale, Irvine, and Lehmann (1965).) Tryptic peptide β TpI, which in haemoglobin A represents the first eight amino-acid residues of the β -chain,

1 2 3 4 5 6 7 8
val-his-leu-thr-pro-glu-glu-lys

was missing and a new peptide had appeared (Fig. 4). Its mobility at pH 6.4 was +0.53, taking that of lysine as +1.0. Colour tests on the new peptide for tryptophan, tyrosine, methionine, and arginine were negative, but the test for histidine was positive. The N-terminal residue of this peptide was determined as the dinitrophenyl derivative (Ambler, 1963), and was found to be valyl. The overall amino-acid composition of the peptide was determined on the amino-acid analyser (Spackman, Stein, and Moore, 1958), and the molar ratio was (± 0.2): lys 2, his 1, thr 1, glu 1, pro 1, val 1, leu 1. The composition of the new peptide differed, therefore, from that of β TpI by containing one fewer glutamic acid residue and one more of lysine. The likely explanation was an amino-acid replacement glu \rightarrow lys at position 6 or 7 of the β -chain. As the mutation $\beta 6$ glu \rightarrow lys is known to be that occurring in haemoglobin C, from which quite different fingerprints are obtained (Hunt and Ingram, 1958), $\beta 7$ glu \rightarrow lys seemed the likely mutation in haemoglobin Siriraj. It is unusual for a tryptic peptide to contain two lysine residues and that there should not have been at least a partial breakdown, because trypsin hydrolyses the carboxy bonds of lysine residues. However, the electrophoretic mobility of the peptide was in full agreement with this finding. This mobility could arise only from the presence of two positive groups in addition to the N-terminal group. In view of the amino-acid composition, these could be only two lysine residues.

The amino-acid sequence was further investigated by partial digestion with papain and with carboxypeptidase. Papain breaks amino-acid chains at leucyl and lysyl bonds. After treatment with papain the digestion products were separated by paper electrophoresis at pH 6.4 and six components could be recognized. These were eluted and hydrolysed and the products of this hydrolysis were identified by paper electrophoresis at pH 2.

Products of Papain Digestion

Mobility (Lysine +1.0)	Composition
1 ... +1.0 ...	lysine
2 ... +0.53 ...	val his leu thr pro glu lys
3 ... +0.48 ...	val his leu
4 ... +0.41 ...	thr pro glu lys
5 ... +0.24 ...	val his leu thr pro glu lys
6 ... 0 ...	thr pro glu lys

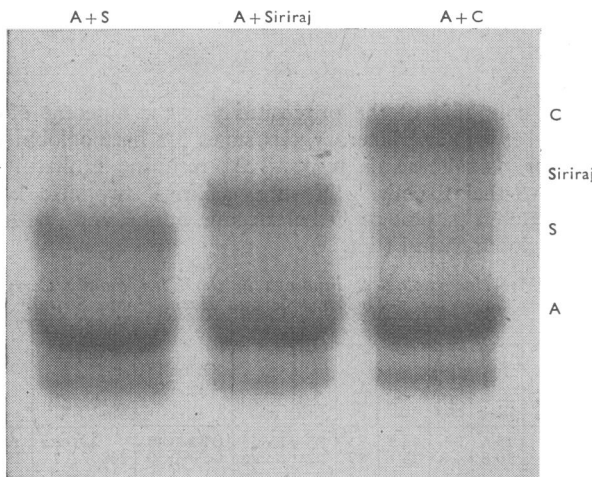


FIG. 2.—Starch-gel electrophoresis—discontinuous buffer, pH 8.2. From left to right: haemoglobin A+S, the haemoglobin of the propositus's father (A+Siriraj), and haemoglobin A+C. Haemoglobin Siriraj moves just behind haemoglobin S and well in front of haemoglobin C.

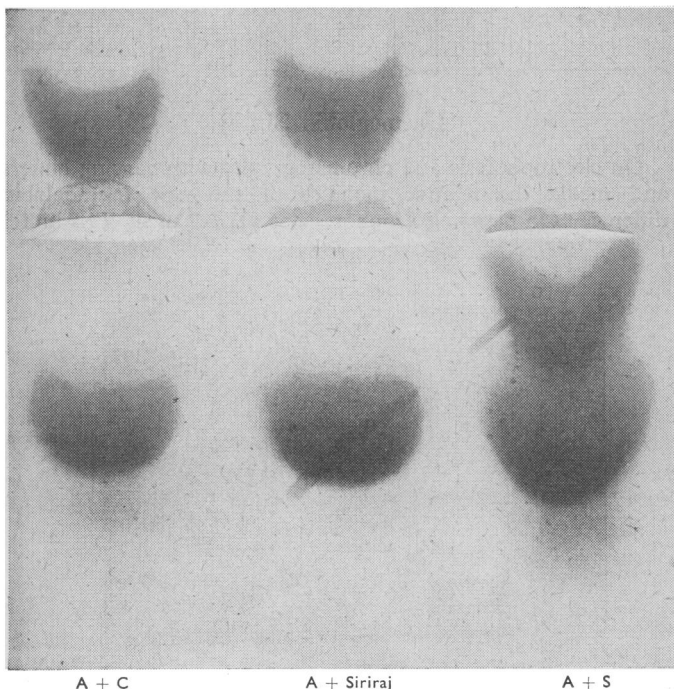


FIG. 3.—Agar-gel electrophoresis—pH 6. From left to right: haemoglobin A+C, haemoglobin A+Siriraj, haemoglobin A+S. Haemoglobin Siriraj moves like haemoglobin C and very differently from haemoglobin S.

The sequence in peptide 3 must be val-his-leu, because val was the N-terminal residue, and leu must be the C-terminal of a peptide arising from papain digestion. For the same reason peptides 2, 4, 5, and 6 must have at least one C-terminal lysine residue.

The mobility of +0.53 for peptide 2 suggests two lysine residues, and this peptide undoubtedly was the original peptide unchanged. The mobility of +0.24 for peptide 5 suggests only one lysine residue, and peptide 5 must have been the original peptide minus one C-terminal lysine residue. This is supported by the fact that component 1 was free lysine. In the same manner the observed mobilities for peptides 4 and 6 respectively suggest that 4 had one lysine residue more than 6. The action of papain can thus be summarized tentatively.

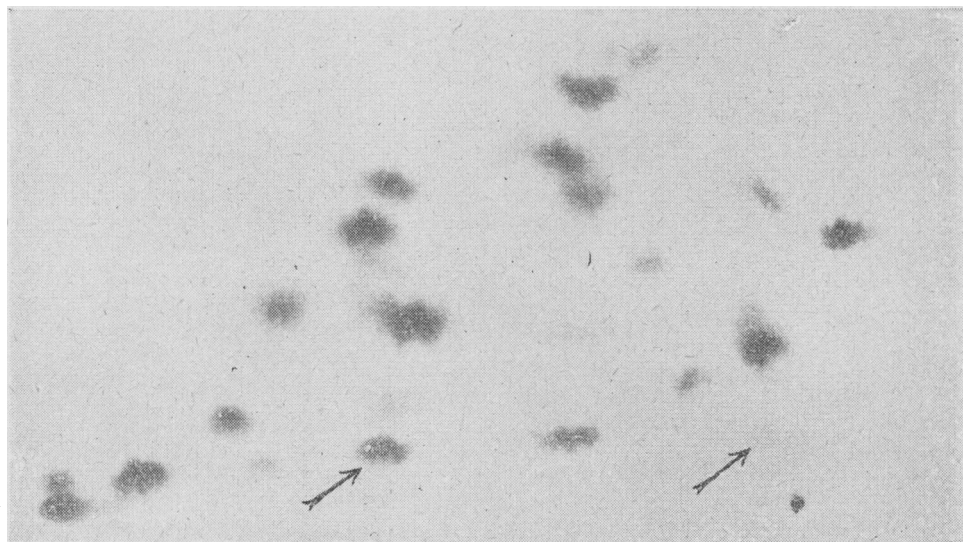


FIG. 4.—“Fingerprint” of haemoglobin Siriraj. Right arrow: β^A TpI is missing. Left arrow: A new peptide can be seen— β^S SirirajTpI.

Scheme of Papain Digestion of Tryptic Peptide (Original Peptide—Component 2)

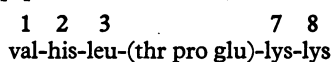
2	val-(his leu thr pro glu lys)-lys
5+1	val-(his leu thr pro glu)-lys + lysine
2	val-(his leu thr pro glu lys)-lys
3+4	val-his-leu + (thr pro gly lys)-lys
4	(thr pro gly lys)-lys
6+1	(thr pro glu)-lys + lysine

To prove conclusively that the new peptide had lys-lys as its C-terminal sequence, it was digested with carboxypeptidase B. This enzyme removes the C-terminal lysine or arginine residues of tryptic peptides, and, following this, can remove the new C-terminal, but only if this again is a residue of lysine or of arginine. The products of digestion with carboxypeptidase B were again separated by paper electrophoresis at pH 6.4, eluted, hydrolysed, and the resulting hydrolysates were examined for their composition as before.

Products of Carboxypeptidase Digestion

Mobility (lysine = +1.0)	...	Amino-acids found
B	... +0.24	val-his-leu (thr pro glu lys)
C	... negative	val-his-leu (thr pro glu)
A	... +1.0	lysine

B was presumably the same peptide as the papain peptide 5—namely, the original peptide minus the C-terminal lysine residue. C represents the original peptide after both its lysyl residues have been removed by carboxypeptidase B. The formula of the peptide can therefore be written



and that of haemoglobin Siriraj as $\alpha_2\beta_2^{7\text{glu}\rightarrow\text{lys}}$.

It was surprising that on tryptic digestion of the new haemoglobin no peptide val-his-leu-(thr pro glu)-lys was found. The sequence $\text{gen}^+-\text{lys}^--\text{lys}^-$ does not seem to be a substrate for tryptic digestion. Even by varying the pH (usually 8.2) from 9 to 7.5 and by doubling the time of digestion, only the peptide val-his-leu-(thr glu)-lys-lys could be obtained.

There was supporting evidence for the complete absence of hydrolysis of the lys-lys bond in β^S SirirajTpI. If there had been some hydrolysis, one of the tryptic peptides would have been β^S TpII plus a lysyl at its N-terminal. As β^S TpII contains tryptophan, a new tryptophan-positive peptide would have appeared, but none was noted.

The formula $\alpha_2\beta_2^{7\text{glu}\rightarrow\text{lys}}$ has been claimed for haemoglobin C-Georgetown. As stated earlier the electrophoretic and solubility properties of haemoglobin Siriraj and C-Georgetown are quite different. The haemoglobin C-Georgetown erythrocytes sickle, but the haemoglobin Siriraj erythrocytes do not. The fingerprints also differ; those of haemoglobin Siriraj show a new peptide with about twice the electrophoretic mobility of the abnormal peptide from haemoglobin S (β^S TpI), whereas the fingerprint of haemoglobin C-Georgetown was indistinguishable from that of haemoglobin S.

Summary

A Thai family is described in which the father carries the trait for a new haemoglobin—haemoglobin Siriraj—and the mother has β -thalassaemia minor. Two of their children had thalassaemia minor, one had the trait for the new haemoglobin, and one, the propositus, had haemoglobin Siriraj-thalassaemia.

The new haemoglobin was analysed and its formula found to be $\alpha_2\beta_2^{7\text{glu}\rightarrow\text{lys}}$. This formula has also been claimed for haemoglobin C-Georgetown, but Siriraj and Georgetown differ in their electrophoretic properties, and in their “fingerprints”; also, whereas C-Georgetown cells sickle, those containing haemoglobin Siriraj do not.

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