HAEMOGLOBIN Q INDIA (α64(E13) ASPARTIC ACID→HISTIDINE) ASSOCIATED WITH β-THALASSAEMIA OBSERVED IN THREE SINDHI FAMILIES

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P. K. SUKUMARAN, S. M. MERCHANT, MENNA P. DESAI, BARBARA G. WILTSHIRE, and H. LEHMANN

Reprinted from Journal of Medical Genetics
Volume 9, *No.* 4, *pages* 436–442, December, 1972

LONDON
BRITISH MEDICAL ASSOCIATION
TAVISTOCK SQUARE, WC1H 9JR

Journal of Medical Genetics (1972). **9**, 436.

Haemoglobin Q India (α64(E13) Aspartic Acid→Histidine) Associated with β-Thalassaemia Observed in Three Sindhi Families

P. K. SUKUMARAN,* S. M. MERCHANT,† MENNA P. DESAI,† BARBARA G. WILTSHIRE,‡ and H. LEHMANN‡

Haemoglobin Q was first reported in a Chinese man in whom it was associated with Hb H disease, and therefore with α-thalassaemia (Vella *et al*, 1958). It has been discovered in other people, mostly in south-east Asia, and these reports have been summarized by Sagnet *et al* (1968). Although it was known that Hb Q was an α-chain abnormal haemoglobin with a mutation in the tryptic peptide No. 9 of the α-chain (αTpIX) which comprises residues 62–90 of the 141 residues of the α-chain, the exact amino-acid substitution had remained undetermined. However, 2 haemoglobins Q were characterized by Lorkin *et al* in 1970. One type came from both a Chinese and a Thai family (in which it was associated with α-thalassaemia) and had the substitution α74 Asp→His, and the other, α75 Asp→His, came from Iran. Independently, the first variant, described as Hb G Taichung, was found in a Chinese by Blackwell and Liu (1970), and in a Thai, where it was again associated with α-thalassaemia, as Hb Mahidol, by Pootrakul and Dixon (1970). In India only a few cases of Hb H disease have been found, indicating that α-thalassaemia is probably rare; β-thalassaemia is however quite common (Chatterjea, 1966; Chouhan, Sharma, and Parekh, 1970).

We have recently observed 3 Sindhi-speaking Hindu families with children suffering from β-thalassaemia major. Family studies showed some members with β-thalassaemia minor and one with β-thalassaemia major associated with Hb Q. This was shown to arise from an amino-acid substitution α64 Asp→His, and was therefore different both from the Q Thailand (α74 Asp→His) and Q Iran (α75 Asp→His). A preliminary report of this new Hb Q India was presented in 1971 by Sukumaran, Wiltshire, and Lehmann.

Received 23 June 1972.

* Epidemiology Division, Cancer Research Institute, Tata Memorial Centre, Parel, Bombay 12, India.
† Jerbia Wadia Hospital for Children, Parel, Bombay 12, India.
‡ Medical Research Council Abnormal Haemoglobin Unit, University Department of Biochemistry, Cambridge, UK.

Methods

Haematological studies were carried out using standard methods (Dacie and Lewis, 1968). Osmotic fragility tests were performed with Simmel's tyrode (Sanghvi, Sukumaran, and Lehmann, 1958), and Hb F levels estimated according to Singer, Chernoff, and Singer (1951). Paper electrophoresis of the haemoglobins was carried out at *p*H 8·9 using Tris buffer (Cradock-Watson, Fenton, and Lehmann, 1959), and starch gel electrophoresis with discontinous Tris-citrate and borate buffer at *p*H 8·6 (Poulik, 1959). Haemoglobin A_2 levels were determined using cellulose acetate strips (Marengo-Rowe, 1965). The material available was sometimes so small that Hb Q_2 could not be quantitatively measured.

The abnormal haemoglobin was purified by repeated paper electrophoresis and by column chromatography using DEAE Sephadex (Huisman and Dozy, 1965). Preliminary studies for the identification of the mutant tryptic peptide were made using the methods summarized by Sick *et al* (1967). For thermolysin digestion the mutant peptide isolated from 55 mg of globin was eluted with 0·5 N NH_4OH and dissolved in 0·8 ml of 0·25 M ammonium acetate buffer, *p*H 8·6. Thermolysin (0·2 mg in 0·1 ml of 50 mM barium acetate) was added and the peptide incubated for 5 hr at 37° C. To stop the reaction, barium was precipitated with 0·1 ml of 0·1 N H_2SO_4. For pepsin digestion the mutant peptide from 20 mg globin was eluted with 0·05 N NH_4OH and dissolved in 0·9 ml of 0·05 N HCl. Pepsin (0·2 mg in 0·1 ml of water) was added and the peptide incubated for 5 hr at 37° C. The reaction was stopped by immersion in boiling water. After both thermolysin and pepsin digestions the supernatant was divided into 2 portions of 0·2 and 0·8 ml for diagnostic and preparative fingerprints, respectively.

Case Histories

Family A. A 1½-year-old boy (II.4) who had developed normally for one year, was admitted to the Jerbai Wadia Hospital for Children, with fever, increasing pallor, and distension of the abdomen. The child

TABLE I

HAEMATOLOGICAL DATA (FAMILY A)

Case	Age (yr)	Hb (g/100 ml)	RBC ($\times 10^6$/μl)	MVC (fl)	MCH (pg)	MCHC (%)	Reticulocytes (%)	Red Cell Morphology: Vacuolation	Red Cell Morphology: Aniso Poikilo	Red Cell Morphology: Target Cells	Osmotic Fragility (% lysis)*	Hb F (%)	Electrophoretic Pattern (when quantitated % Q, A_2, and Q_2 in parentheses),
I.1	40	11·0	6·09	74·0	24·4	28·9	1·0	+ +	+	+	48·5	1·6	$A + Q(8·6) + A(4·3) + Q_2$
I.2	33	11·0	5·28	72·0	20·8	29·0	0·6	+ +	+	Few	62·0	1·5	$A + A_2(5·2)$
II.1	12	10·2	5·68	63·5	18·1	28·3	0·8	+ +	+	+	51·3	1·4	$A + Q(8·0) + A_2(3·3) + Q_2$
II.2	9	12·5	4·55	83·5	27·5	32·8	1·4	−	−	−	96·5	0·5	$A + A_2(2·6)$
II.3	5	12·2	4·13	88·5	29·6	33·5	0·2	−	−	−	88·0	0·7	$A + Q(19·6) + A_2(1·1) + Q_2$ (0·7)
II.4	1½	4·8	—	—	—	—	3·2	+ + +	+ +	+†		32	A + F before transfusion
		7·6	3·66	68·5	20·6	30·2	1·8	+ +	+ +	+‡	71·5	24	A + F + A_2(2·4) after transfusion

* Percentage lysis in 0·4 tyrode solution (normal range 80–100%).
† Large number of nucleated red cells.
‡ One week after blood transfusion, occasional nucleated red cells.

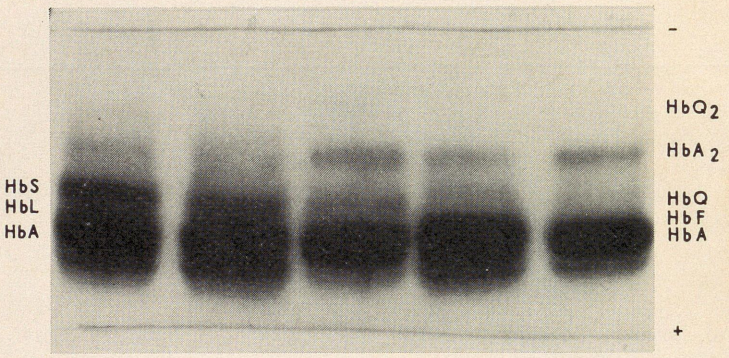

FIG. 1. Electrophoretic pattern of haemoglobins from family A (starch gel). Right to left: Mother (I.2); child (II.4) post transfusion sample; father (I.1); Hb AL and Hb AS.

was dyspnoeic, weighed 7·5 kg and was 70 cm tall. His fontanelle was open and frontal and parietal bossing were evident. The spleen was enlarged to 7 cm and the liver to 5 cm below the costal margin. Haematological data of the boy and his family are given in Table I and are consistent with the diagnosis of the boy having β-thalassaemia major. The percentage of alkali resistant haemoglobin was 31·7% after a packed cell blood transfusion.

The father (I.1) was healthy, but blood examination revealed a raised Hb A_2 and other red cell stigmata consistent with β-thalassaemia minor. He also had a slow moving haemoglobin, and starch gel electrophoresis showed the presence of 2 haemoglobins A_2, which suggests that there was an α-chain variant present (Fig. 1). Its mobility on paper and on starch gel electrophoresis indicated that it was a Hb Q. The father was therefore a double heterozygote for β-thalassaemia and Hb Q.

The mother (I.2) was shown to have β-thalassaemia minor. Among the sibs, one sister (II.1) had both Hb Q and β-thalassaemia minor, while another sister (II.3) had 20% Hb Q and no β-thalassaemia (Fig. 2). The latter

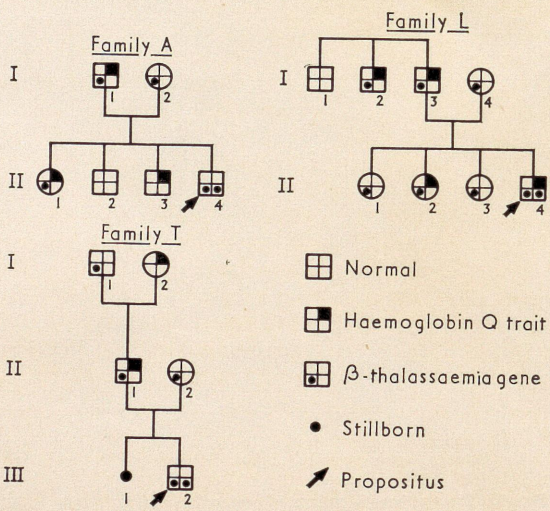

FIG. 2. Pedigrees of families A, L, and T.

sister shows no abnormality at all indicating that this level of Hb Q causes no clinical symptoms.

Family L. A male child (II.4) aged 6 months was admitted to hospital looking pale and weak after having grown well up to the age of 4 months. He weighed 6 kg, was 60 cm tall, and both liver and spleen were enlarged 6 cm below the costal margin. He was assumed to have β-thalassaemia major although x-rays of skull and long bones did not yet show any abnormality.

The haematological data of this child and his family are shown in Table II and can be seen to be in agreement

TABLE II

HAEMATOLOGICAL DATA (FAMILY L)

Case	Age (yr)	Hb (g/100 ml)	RBC ($\times 10^6$/μl)	MCV (fl)	MCH (pg)	MCHC (%)	Reticulocytes (%)	Red Cell Morphology			Osmotic Fragility (% lysis)*	Hb F (%)	Electrophoretic Pattern (when quantitated % Q, A_2, and Q_2 in parentheses)
								Vacuolation	Aniso Poikilo	Target Cells			
I.1	40	13·0	4·47	85·0	29·1	34·2	0·6	–	–	–	100	1·2	$A + A_2(2·2)$
I.2	36	12·2	6·16	58·8	19·9	33·2	1·8	+	–	+	76·5	1·5	$A + Q(14·2) + A_2(3·5) + Q_2$ (1·6)
I.3	38	11·6	5·45	72·5	21·3	29·4	1·6	+	+	+	31·7	1·4	$A + Q(9·6) + A_2(3·4) + Q_2(0·7)$
I.4	30	9·8	5·65	63·8	18·4	27·2	0·6	++	+	+	21·1	1·1	$A + A_2(4·5)$
II.1	8	9·9	5·25	66·5	18·8	28·3	0·6	+	+	Few	21·0	0·9	$A + A_2(4·5)$
II.2	6	10·4	5·20	68·2	20·0	29·3	0·6	++	+	+	15·4	1·7	$A + Q(18·4) + A_2(3·8) + Q_2$ (1·0)
II.3	4	9·6	5·53	62·2	17·3	27·8	1·2	+	–	+	18·0	1·2	$A + A_2(4·1)$
II.4	$\frac{6}{12}$	5·5	2·92	61·6	18·9	30·6	4·6	+++	+++	++†	48·5	56	$F + Q_F$

* Percentage haemolysis in 0·4 tyrode solution (normal range 80–100%).
† Large number of nucleated red cells.

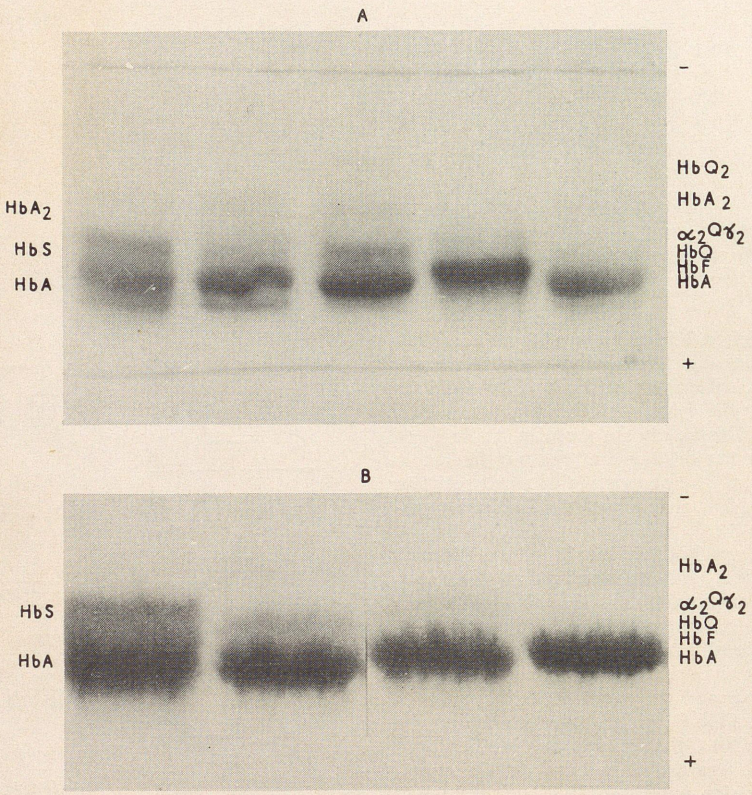

FIG. 3. Electrophoretic pattern of haemoglobins from family L. **A** (starch gel): right to left, mother (I.4); child (II.4); father (I.3); father I.1 of family A; Hb AS. **B** (paper): right to left, thalassaemia major showing Hb F only; child II.4; father I.3; Hb AS.

with the child having β-thalassaemia major. The boy was transfused and given 2·5 mg folic acid by mouth and when discharged had a haemoglobin level of 11·5 g %. Electrophoresis of his haemoglobin on both starch gel and paper (Fig. 3) showed that a slow moving component was present as well as Hb F. It appears from the family studies that this haemoglobin consists of a fetal haemoglobin containing α^Q chains instead of α^A chains, that is $\alpha_2^Q\gamma_2$; it forms 6·9% of the total haemoglobin.

Both father (I.3) and mother (I.2) had β-thalassaemia minor, and the father's haemoglobin also had a slow moving component. The slow moving haemoglobins of both the father and the son can be seen in Fig. 3. Starch gel electrophoresis distinguishes both between normal Hb A and Hb F, and also between the 2 slow components $\alpha_2^Q\beta_2$ and $\alpha_2^Q\gamma_2$. The haemoglobin patterns of I.3 in family L and I.1 in family A can be seen to be identical, a double Hb A_2 being visible in both cases. The inheritance of α^Q chains and β-thalassaemia in the son can be seen from the family tree (Fig. 2).

Family T. The 3rd child (III.2) was 4 months old and was admitted to hospital because he had been pale for 1 month. He weighed 5 kg, was 62 cm long, and had a spleen enlarged to 7·5 cm below the costal margin. The haematological data of the child and his family are shown in Table III. These confirmed the diagnosis of β-thalassaemia major in the child, who was transfused, given 5 mg folic acid and discharged after 2 weeks with a haemoglobin level of 11·8 g%.

As Table III shows, both parents had β-thalassaemia minor. The father also had the slow moving haemoglobin Hb Q, which he appeared to have inherited from his mother (I.2). Both of them have a double Hb A_2, but the abnormal band is clearer in the case of the father, since β-thalassaemia has led to a raised Hb A_2 level. The grandmother had no sign of β-thalassaemia minor, but the grandfather (I.1) had β-thalassaemia minor but no slow moving haemoglobin (Fig. 4).

Examination of the Abnormality of the Globin

The haemoglobin Q was purified by paper electrophoresis at *p*H 8·9, eluted and concentrated. The globin was precipitated using acidic acetone and digested with trypsin. The fingerprints (Fig. 5) of the haemoglobin from I·3 (family L) and II.1 (family A) showed a new peptide containing both histidine and methionine as well as a reduced amount of peptides α^ATpXI and α^ATpVIII–IX. This suggested that the new peptide was a mutant αTpIX which was confirmed by amino-acid analysis. This showed that an aspartic acid or asparagine

TABLE III

HAEMATOLOGICAL DATA (FAMILY T)

Case	Age (yr)	Hb (g/μl)	RBC (×10⁶/μl)	MCV (fl)	MCH (pg)	MCHC (%)	Reticulocytes (%)	Red Cell Morphology			Osmotic Fragility (% lysis)*	Hb F (%)	Electrophoretic Pattern (when quantitated % Q, A_2, Q_2 in parentheses)
								Vacuolation	Aniso Poikilo	Target Cells			
I.1	55	9·6	4·28	77·0	22·4	29·0	0·1	+	+	+	56·3	1·6	$A+A_2(5·4)$
I.2	45	13·9	4·61	94·2	30·2	32·0	0·3	−	−	−	93·5	0·7	$A+Q+A_2(2·7)+Q_2$
II.1	30	13·0	6·26	71·4	20·8	29·3	0·6	+	−	+	69·4	1·3	$A+Q+A_2(4·4)+Q_2$
II.2	23	8·7	5·31	65·0	16·4	25·2	0·5	+	+	Few	72·8	1·6	$A+A_2(5·1)$
III.2	$\frac{4}{12}$	5·5	2·64	68·1	20·8	30·6	3·6	+++	++	+†	83·3	78	F

* Percentage haemolysis in 0·4 tyrode solution (normal range 80–100%).

† Large number of nucleated red cells.

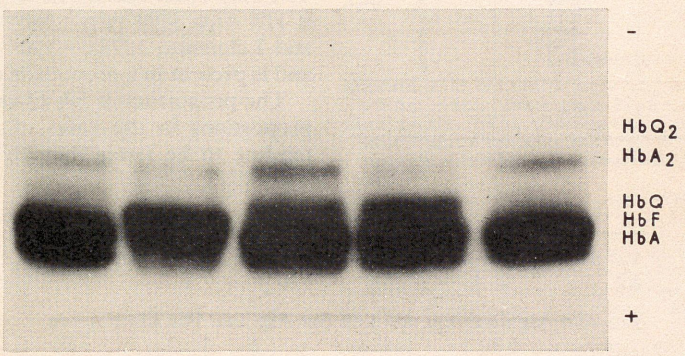

FIG. 4. Electrophoretic pattern of haemoglobins from family T (starch gel). Right to left: paternal grandfather (I.1); grandmother (I.2); father (II.1); child (III.2), mother (II.2).

had been replaced by histidine in both samples (Table IV). From Table V it can be seen that if there were a single charge change in αTpIX the electrophoretic RF would alter by about 0·1 unit. However, since the electrophoretic RF is increased from 0·0 to 0·2 units, it appears that there is a double charge change; indicating a mutation of aspartic acid→histidine as in Hb Q Iran and Hb Q Thailand, because histidine is positively charged under these conditions. The electrophoretic RF was determined taking lysine as unity and the neutral peptides as zero; for the chromatographic RF the solvent front is defined as unity and the origin as zero.

TABLE V

ELECTROPHORETIC AND CHROMATOGRAPHIC MOBILITIES (RF VALUES)

Peptide	Electrophoretic RF	Chromatographic RF	Charge Change from α^{A}IX
α^{A}IX	0·00	0·14	0
α^{A}VIII–IX	0·09	0·11	+1
$\alpha^{Q\ Iran}$IX	0·19	0·13	+2
$\alpha^{Q\ India}$IX (I.3, family L)	0·21	0·13	+2
$\alpha^{Q\ India}$IX (II.1, family A)	0·22	0·13	+2

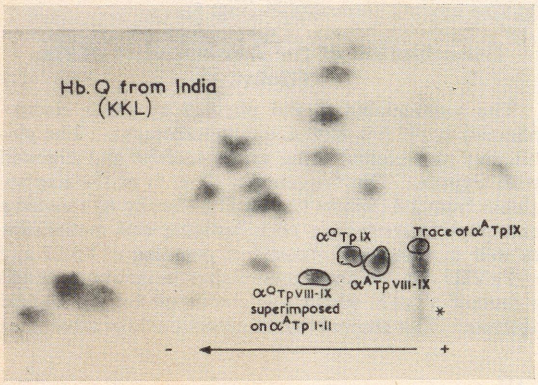

FIG. 5. Fingerprint (*p*H 6·4) of the soluble tryptic peptides of Hb Q India from father (I.3) of family L. (For details of interpretation see text.) * = Point of application.

TABLE IV

AMINO-ACID COMPOSITION OF α^{Q}TpIX (62–90)

Amino Acid	Residues Found		Expected in Hb A
	Sample (II.1, family A)	Sample (I.3, family L)	
Asp	5·01	5·07	6
Thr	1·04	0·90	1
Ser	1·94	1·77	2
Pro	0·94	1·00	1
Ala	7·35	6·70	7
Val	3·02	2·94	3
Met	0·73	0·51	1*
Leu	4·18	4·02	4
His	3·77	4·30	3
Lys	1·11	1·33	1
Yield (n-moles)	20·3	37·0	

* Methionine is partially destroyed on acid hydrolysis.

The new peptide from 1.3 (family L) was digested with thermolysin which hydrolyses αTpIX in the positions shown in Fig. 6. The thermolysin fingerprint (Fig. 7) had a new positively charged histidine-containing peptide which was α62–65 with a histidine replacing the aspartic acid at α64. In digests of α^{A}TpIX the peptide α62–65 runs on top of α83–85 but in the abnormal α^{Q}TpIX only the peptide α83–85 is present in this position on the fingerprint. The only other peptide containing aspartic acid, α73–79, was present in its normal position (Table VI). The mutation in this haemoglobin must therefore be α64 aspartic acid→histidine.

In the case of I.1 (family A) a peptic digest of α^{Q}TpIX was attempted. The results were unsatisfactory but it appeared that the mutation Asp→His was probably at position α64 (Table VII). The fingerprint (Fig. 8) showed a new histidine-containing spot which is not present in peptic digests of α^{A}TpIX. This spot was found to contain the peptide α62–70 without aspartic acid.

Discussion

Hb Q India does not cause any haematological disorders, perhaps because the residue involved, α64 (E13), is on the surface of the haemoglobin tetramer. Charge changes at such positions do not usually affect the properties of the haemoglobin. The only other mutant so far found at this position is Hb Aida (α64 Asp→Asn) (Ramot, Kinderlerer, and Lehmann, 1972). This is also asymptomatic, and is present in a proportion of 27%.

The proportion of Hb Q varies from 8–20%, the proportions in the cases of β-thalassaemia minor tending to be lower than that in the normal case

Val Ala Asp Ala Leu Thr Asn Ala Val Ala His Val Asp Asp Met
62 ↑ ↑70↥ ↑

Pro Asn Ala Leu Ser Ala Leu Ser Asp Leu His Ala His Lys
↑80↥ ↑ ↥ ↑ ↥ ↑ 90

FIG. 6. Amino-acid sequence of α^{A}TpIX. ↥ = hydrolysis by pepsin; ↑ = hydrolysis by thermolysin.

TABLE VI

AMINO-ACID ANALYSIS OF THERMOLYSIN DIGEST IN I.3 (FAMILY L)

Amino Acid	62–65		73–79		83–85	
	Ratio	Expected	Ratio	Expected	Ratio	Expected
Asp	—	1	2·94	3	1·07	1
Ser	0·33	—	—	—	0·88	1
Pro	—	—	0·91	1	—	—
Gly	0·31	—	—	—	—	—
Ala	1·96	2	1·08	1	—	—
Val	1·00	1	1·06	1	—	—
Met	—	—	0·70	1	—	—
Leu	—	—	—	—	1·05	1
His	1·05	—	—	—	—	—
Yield (n moles)	35		29		79	

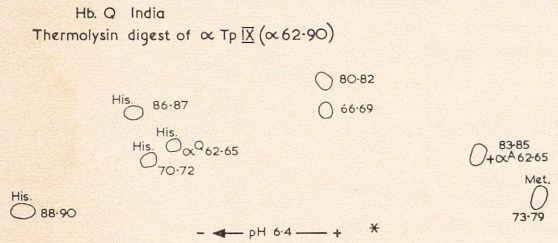

FIG. 7. Comparison of the fingerprints of thermolysin peptides of α^{A}TpIX and α^{Q}TpIX (α62–90). (For details of interpretation see text.) * = Point of application.

TABLE VII

AMINO-ACID ANALYSIS OF PEPTIC DIGEST IN I.1 (FAMILY A)

Amino Acid	62–69		84–86	
	Ratio	Expected	Ratio	Expected
Asp	1·07	2	0·93	1
Thr	0·97	1	—	—
Ser	—	—	1·28	1
Ala	3·08	3	—	—
Val	0·97	1	—	—
Leu	0·91	1	0·85	1
His	0·30	—	—	—
Yield (n moles)	9·1		2·2	

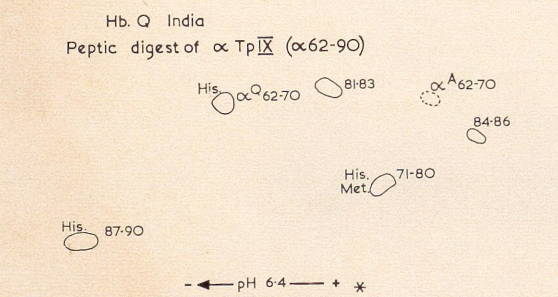

FIG. 8. Comparison of the fingerprints of peptic peptides of α^{A}TpIX and α^{Q}TpIX (α62–90). (For details of interpretation see text.) * = Point of application.

(Tables 1 and II). The proportion of Hb $\alpha_2^Q\gamma_2$ compared to $\alpha_2^A\gamma_2$ in II.4 (family L) is 6·9%, even lower than in the trait. It is possible that when there is a shortage of non α-chains the α^A chains are preferentially incorporated into haemoglobin while the precipitated α-chains contain a greater proportion of α^Q. However, without more data on the proportions of Hb Q India in non-thalassaemic people, it is difficult to judge whether the lowering of the proportion of Hb Q in cases of β-thalassaemia minor is significant.

Summary

A new haemoglobin, Hb Q India, α64 Asp→His has been discovered. It was found in association both with β-thalassaemia major and β-thalassaemia minor. It seems that the proportion of this haemoglobin is lower when β-thalassaemia is present.

Our thanks are due to Dr L. D. Sanghvi MSc, PhD (Columbia), Head, Epidemiology Division and Dean, Cancer Research Institute, Tata Memorial Centre, Parel, Bombay 12, for his continued encouragement in this work and to the Superintendent, Jerbai Wadia Hospital for Children, for permission to publish these data.

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PRINTED IN GREAT BRITAIN
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