The first observation of Hb D Punjab β^0 thalassaemia in an English family with 22 cases of unsuspected β^0 thalassaemia minor among its members

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SUMMARY A 36 year old local Englishman from Nuneaton was referred to hospital with suspected glandular fever. Relevant tests were negative and the symptoms subsided in due course. The finding of a hypochromic microcytic blood picture without iron deficiency led to the discovery that he was heterozygous for Hb D and β thalassaemia. Hb D trait was established in the father of the proband and β thalassaemia in his mother and a brother. The father's ancestors were miners who came to Nuneaton from Monmouthshire in the 19th century. The mother's ancestors have belonged to the indigenous population of Nuneaton and neighbouring Leicestershire since the 18th century. Twenty local members of her wider family also had thalassaemia. All thalassaemias had a low MCH and raised level of Hb A₂. The Hb F level, however, was normal in five, demonstrating the independent segregation of genetic factors influencing the Hb F level in β thalassaemia trait.

A generation ago thalassaemia was considered rare or absent in Britons. Early descriptions were based on the finding of red cell abnormalities and a raised level of Hb F. In one such case necropsy showed leukaemia.¹ A description in 1955 of 'target cell anaemia' in two English women² is well compatible with thalassaemia. Once it had been shown that raised Hb A₂ levels could be a diagnostic feature, it was possible to report an indisputable case from East Anglia,³ and this was followed by many more observations.^{4 5}

Hb D was originally described as a variant of Hb A which on electrophoresis behaved like Hb S but did not cause the sickling phenomenon.⁶ It was soon found that this description covered a number of variants differing by other investigations,⁷ and denotes a haemoglobin differing from Hb A by an additional positive charge or loss of a negative charge on either both the two α or the two β chains of the Hb $\alpha_2\beta_2$ tetramer. The most widely distributed Hb D which has a frequency of 2 to 3% in the Punjab⁸ was found to have a glutamine instead of a glutamic acid at position 121 of the 146 residues β chain.⁹ When it was found that the original Hb D

described in Los Angeles was identical to Hb D Punjab, the term Hb D Los Angeles was introduced. The association between Hb D and β^0 thalassaemia was first described in a Persian girl¹⁰ and most cases of thalassaemia associated with Hb D Punjab have been β^0 thalassaemias.⁵ The condition is fairly mild and no more severe than β thalassaemia minor on its own. It is more severe, however, than the homozygous state for the Hb D gene.¹¹

Materials and methods

Blood counts were evaluated on a Coulter Counter Model S and other haematological methods, including measurement of serum iron, iron binding capacity,¹² and G6PD, followed established routine.¹³ Preparation of haemolysates, estimation of Hb F, electrophoresis and quantification of Hb fractions, preparation of globin and its tryptic digestion, preparation of two dimensional chromatograms of tryptic peptides (fingerprints), and amino acid analysis followed established routine.¹⁴ Globin chain separation was performed according to Clegg *et al.*¹⁵ Blood groups were determined with monoclonal antisera for ABO groups and immune antisera for Rhesus and other groups.

†Since this paper was submitted, Professor Lehmann has died.

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TABLE Haematological findings in the proband (IV.27) and in 57 members of his family.

Pedigree	Age	Hb (g/dl)	RBC ($\times 10^{12}/l$)	Hct (l/l)	MCH (fl)	MCH (pg)	MCHC (g/dl)	RBC morphology	Fe ($\mu\text{mol/l}$)	TIBC ($\mu\text{mol/l}$)	Hb A ₂ (%)	Hb F (%)	Hb D (%)	G6PDH
II.10	80	13.5	4.88	421	86	27.5	31.9	N	—	—	—	—	—	—
27	86	11.5	5.41	374	69	21.2	30.7	+	14	45	6.0	1.4	—	N
28	82	12.8	4.72	398	84	27.0	32.0	N	5	96	3.1	0.1	—	N
III.1	78	11.6	5.47	387	71	21.6	30.1	+	11	—	6.7	2.5	—	N
3	75	10.9	5.39	362	68	20.4	30.0	+	14	—	7.0	1.8	—	N
5	73	14.6	4.71	453	97	31.4	32.3	N	8	51	3.2	0.7	—	N
7	65	13.4	4.74	404	86	28.3	33.1	N	7	70	3.4	0.8	—	N
8	63	14.9	4.87	437	90	33.1	34.3	N	12	69	3.7	0.2	—	N
17	69	10.7	5.31	354	67	20.4	30.3	+	22	—	6.9	0.7	—	—
18	74	14.9	4.54	449	99	33.1	33.2	N	13	—	3.3	0.8	—	—
19	71	13.9	6.64	443	66	20.9	21.3	+	25	48	5.9	2.0	—	N
20	73	14.0	4.77	421	88	29.3	33.2	N	11	45	3.2	1.9	39	N
21	62	13.2	6.34	428	67	20.8	30.8	+	24	57	5.7	1.0	—	N
22	61	14.0	4.72	414	87	29.4	33.5	N	32	—	3.5	0.4	—	N
23	67	11.9	5.94	378	63	20.1	32.0	+	20	—	6.0	1.5	—	N
24	69	13.0	4.49	391	87	28.8	32.5	N	18	—	3.6	0.8	35	N
25	75	13.3	4.11	402	85	28.6	32.8	N	18	—	2.2	0.4	—	N
26	70	13.5	4.71	404	85	28.6	33.3	N	18	—	3.1	0.3	—	N
28	70+	15.0	4.91	442	89	30.3	34.1	N	19	48	2.9	0.4	—	N
34	60	13.2	4.37	392	90	30.5	33.7	N	17	60	2.8	0.4	—	N
35	60	12.9	4.40	380	87	29.8	34.6	N	10	54	3.7	0.6	—	N
37	70	12.7	6.59	419	64	19.6	30.4	+	10	60	7.5	1.0	—	N
41	69	12.1	5.40	373	69	22.3	31.8	+	16	—	8.1	0.9	—	N
42	61	14.3	4.75	426	89	29.8	32.9	N	15	—	3.9	0.2	—	N
IV.1	45	11.7	5.56	379	69	21.3	30.9	+	16	69	6.2	0.5	—	N
3	51	13.6	4.52	406	91	30.4	33.5	N	16	66	3.5	0.7	—	N
4	51	11.8	3.74	354	95	11.9	33.4	N	17	78	3.7	0.1	—	N
15	47	13.1	5.52	396	88	29.8	34.0	N	17	54	3.6	0.3	—	N
16	49	13.9	4.34	405	93	11.8	34.1	N	24	60	3.4	1.0	—	N
17	49	13.5	4.57	410	90	29.3	32.7	N	18	—	3.5	0.6	—	N
18	49	15.4	4.83	463	96	31.6	32.9	N	20	—	2.8	0.6	—	N
19	44	11.1	5.14	377	74	21.9	29.5	+	23	66	7.5	2.5	—	N
20	46	12.9	4.19	398	95	31.3	32.6	N	18	70	3.3	0.2	—	N
22	46	12.4	5.80	402	69	21.2	30.6	+	13	—	7.6	4.3	—	N
24	34	10.9	4.97	350	70	21.9	31.0	N	28	78	5.5	1.4	—	N
25	34	14.9	4.91	435	88	30.1	33.9	N	15	48	3.8	0.4	—	N
26	28	11.3	5.62	364	64	19.9	30.7	+	23	48	6.1	1.0	—	N
27	36	12.7	6.0	395	66	21.1	31.5	+	26	—	7.2	1.4	91	N
28	38	12.3	6.01	400	66	20.4	30.6	+	28	—	5.8	1.0	—	N
43	45	5.0	50.4	440	92	11.5	34.1	N	11	69	2.9	0.5	—	N

FIG 1 Family pedigree.

Water Board, had been well apart from the brief illness mentioned and showed no abnormality on physical examination later. The relatives with thalassaemia trait were mostly well. Two of them, however, had been treated previously for hypochromic anaemia. The findings were consistent: Hb A₂ was always raised and the MCH was near 20 pg. Hb F level, however, was not raised in all thalassaemics. Fig 2 shows the independent segregation of the genetic factors which influence the Hb F levels in β thalassaemia trait. It is noteworthy that in the two

Hb D trait carriers the Hb F is raised in one (III.20) but normal in the other (III.24). Though thalassaemia is rare in Britons its significance is important as there are genetic implications for those in whom it is diagnosed. It is essential that in the differential diagnosis of hypochromia the clinician is aware that thalassaemia is a possibility and should be considered (among others with sideroblastic anaemia and aleukaemic leukaemia) so that mistreatment with iron is avoided. The Coulter Counter indices produced by the majority of haematology depart-

FIG 2 Haemoglobin F levels associated with the β thalassaemia trait through four generations of the family.

ments these days give a clear indication that thalassaemia may be present, and when a low MCV, low MCH, and normal MCHC are reported patients should be investigated thoroughly to establish the true diagnosis. Heterozygotes for thalassaemia can be recognised easily, and family studies of thalassaemia adumbrate what will become possible, when other heterozygous states which at present cannot be diagnosed as easily as thalassaemia will become recognised by restriction enzyme analysis of the DNA.

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References

- Bywaters EGL. The Cooley syndrome in an English child. *Arch Dis Child* 1938;13:173-87.
- Israels MCG, Turner RL. A British target cell anaemia. *Lancet* 1955;ii:1356-66.
- Havard CW, Lehmann H, Bodley-Scott R. Thalassaemia minor in an English woman. *Br Med J* 1958;i:304-5.
- Callender ST, Mallett BJ, Lehmann H. Thalassaemia in Britain. *Br J Haematol* 1961;7:1-3.
- Weatherall DJ, Clegg JB. *The thalassaemia syndromes*. 3rd ed. Oxford: Blackwell Scientific Publications, 1981.
- Itano HA. Third abnormal hemoglobin associated with hereditary hemolytic anemia. *Proc Natl Acad Sci USA* 1951;37:775-84.
- Benzer A, Ingram M, Lehmann H. Three varieties of human haemoglobin D. *Nature* 1958;182:852-3.
- Bird GWG, Lehmann H. Haemoglobin D in India. *Br Med J* 1956;i:514.
- Baglioni C. Abnormal human haemoglobins. VIII. Chemical studies on haemoglobin D. *Biochim Biophys Acta* 1962;59:437-49.
- Hynes M, Lehmann H. Haemoglobin D in a Persian girl: presumably the first case of haemoglobin D thalassaemia. *Br Med J* 1950;ii:923.
- Politis-Tsegos C, Kynoch PAM, Lang A, et al. Homozygous haemoglobin D Punjab. *J Med Genet* 1975;12:269-74.
- Ramsey WMN. The determination of iron binding capacity of serum. *Clin Chim Acta* 1957;2:221.
- Dacie JV, Lewis SM. *Practical haematology*. 5th ed. Edinburgh: Churchill Livingstone, 1975.
- Lehmann H, Huntsman RG. *Man's haemoglobins*. Revised ed. Amsterdam: North-Holland, 1974.
- Clegg JB, Naughton MA, Weatherall DJ. Abnormal human haemoglobin. Separation and characterization of the α and β chains by chromatography. *J Mol Biol* 1966;19:91-108.
- Schneider RG, Ueda S, Alperin JB, Levin WC, Jones RT, Brimhall B. Hemoglobin D Los Angeles in two Caucasian families: hemoglobin SD disease and hemoglobin D thalassaemia. *Blood* 1968;32:250-9.

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