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

SHEILA WORTHINGTON* AND HERMANN LEHMANN†
From *the Pathology Laboratory, George Eliot Hospital, Nuneaton; and †the Department of Biochemistry, University of Cambridge, Cambridge.

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 $\beta$ thalassaemia. Hb D trait was established in the father of the proband and $\beta$ 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 $\beta$ 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 $\alpha$ or the two $\beta$ 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 $\beta$ 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 $\beta^0$ thalassaemia was first described in a Persian girl10 and most cases of thalassaemia associated with Hb D Punjab have been $\beta^0$ thalassaemias.5 The condition is fairly mild and no more severe than $\beta$ 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.
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Results

Findings in the proband were WBC 8.7×10⁹/l, RBC 6.0×10¹²/l, Hb 12.7 g/dl, PCV 0.395 1/l, MCV 66 fl, MCH 21.1 pg, MCHC 31.5 g/dl, and reticulocytes 1.8 per 100 RBC. The red cells were microcytic and hypochromic with slight polychromasia, moderate leiptocytosis, and slight basophilic stippling. The differential white cell count was normal and glandular-lyr cells were not seen. Platelets were normal. The slide test for glandular fever was negative. The G6PD was normal. The serum iron level was 26 μmol/l (normal 13 to 32 μmol/l). Hb electrophoresis on cellulose acetate at pH 9.2 showed no Hb A and only a band migrating in the position of Hb D or S amounting to 91%, Hb A₂ 7.2% (normal 2.0 to 4.0%), and Hb F 1.4% (normal less than 0.9%). Hb F was heterogeneously distributed among the red cells. On agar gel electrophoresis at pH 6.2 the abnormal haemoglobin did not migrate like Hb S but like Hb A, which indicated that it was Hb D. Fingerprinting and amino acid analysis showed that the Hb D was Hb D Punjab α₂β₂ 121 Glu→Gln.

The mother of the proband had β thalassaemia trait and the father had Hb D trait. Their blood groups were compatible with the son's first degree relationship. On investigating the parents' families it was found that the mother's maternal aunt also had thalassaemia trait. This led us to examine the mother's family. The aunt was the survivor of nine sibs. Two of them had no descendants. Of the other six it was possible to demonstrate thalassaemia in descendants of four of them. The table shows the haematological findings and fig 1 shows the family pedigree. It will be noted that in addition to the proband, his mother, and brother, there were another 20 cases of unsuspected thalassaemia. Hb D was not demonstrated in any of the father's three sibs but was found in a paternal cousin. It has not been possible to examine more members of this family.

Discussion

The proband is the first case of Hb D Punjab (Los Angeles) β⁺ thalassaemia found in a native Englishman. Schneider et al described a similar case in a person of English, Irish, and Scottish ancestry in the USA. That thalassaemia was, however, of the β⁺ type and the clinical picture was much more severe. Although β thalassaemia trait is found occasionally among the indigenous population of Nuneaton (10 cases from different families have been diagnosed in the last five years), it was surprising to find 22 unsuspected cases among 57 relatives of the proband. The proband, an engineer with the local
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 \( \beta \) 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-
The first observation of Hb D Punjab β⁺ thalassaemia in an English family

![Diagram of Hb D Punjab β⁺ thalassaemia in an English family]

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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Correspondence and requests for reprints to Dr Sheila Worthington, Department of Haematology, George Eliot Hospital, College Street, Nuneaton, Warwickshire CV10 7DJ.
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S Worthington and H Lehmann

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