SICKLE-CELL TRAIT: A FAMILY STUDY AND A REPORT OF THREE FURTHER CASES OF SICKLE-CELL DISEASE

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The demonstration of the presence of an abnormal haemoglobin in sickle-cell disease by Pauling *et al.* in 1949[13] not only provided the explanation for the clinical manifestations of the disease, but also confirmed the genetic theories[12] postulated to account for its familial occurrence. In addition, it led to the concept, evolved by Pauling, of molecular disease,[14] which holds that some diseases are caused by faulty synthesis of protein, the fault being genetically determined. In sickle-cell haemoglobin the molecular abnormality was recently elucidated by Ingram,[8] who showed that HbA and HbS (as the normal adult and sickle-cell haemoglobins are termed) differ by the replacement of 2 glutamic acid residues in HbA by 2 molecules of valine in HbS.

The disease is rare in South Africa, only 6 case reports being encountered in the local medical literature—Berk and Bull[3] (1943), Altman[1] (1945), Grek and Findlay[7] (1952), Segal and Grusin[15] (1956), and Wasserman[18] (2 cases, 1958). The validity of the diagnosis in the case recorded by Segal *et al.* was questioned by Vandepitte,[17] who suggested that it may well have been a case of sickle-cell/thalassaemia disease. Wasserman's 2 cases were the first to be recorded in the Cape Coloured.

As regards the sickle-cell trait, population surveys have been conducted by several workers amongst the various racial groups in South Africa and Rhodesia—English[4] (1945), Altman[2] (1945), Esrachowitz *et al.*[5] (1952)—and all have stressed the rarity of the trait in the South African Bantu as compared with its frequency in Africans living north of the Limpopo. Esrachowitz and his colleagues surveyed the Cape Coloured population and found the trait present in 0·58%. When, therefore, a case of sickle-cell anaemia was encountered at Groote Schuur Hospital and the mother and father were both found to be members of large Cape Coloured families, it was thought that the results of a family study might prove interesting.

CASE REPORTS

Case 1

The propositus, J.H., Coloured female, presented at the age of 15 months with an acute attack of frequency and dysuria, associated with fever. Her past history included an attack of mild gastro-enteritis, mild upper-respiratory-tract infection, chicken pox, larva migrans and adenoiditis. The illness was diagnosed as acute pyelitis and treated with sulphonamides and streptomycin. There was some improvement after 3 days, but a week later she was reported to be still off-colour, listless and anorexic. On examination the child was found to be well nourished but with marked pallor of the mucous membranes, a firm non-tender 4-finger splenomegaly, and soft non-tender hepatomegaly; there was no lymphadenopathy, jaundice or rash. The haemoglobin was 9 g.% and smear showed anisocytosis, poikilocytosis, some polychromasia, and several normoblasts.

A diagnosis of a haemolytic anaemia of unknown aetiology was made and the patient referred to Groote Schuur Hospital for investigation, where she was admitted on 18 March 1959. The following additional pertinent findings were then made: Reticulocyte count 11%; serum bilirubin 2·5 mg.%; a wet preparation of peripheral blood showed sickling of the red cells, and electrophoresis revealed a pattern of sickle-cell anaemia.

Examination of the mother's and father's blood showed sickling of the father's cells only, but electrophoresis demonstrated that both were heterozygous for HbS, i.e. were cases of sickle-cell trait.

J.H's subsequent course included 2 further haemolytic attacks associated with upper-respiratory-tract infections. After the second of these attacks, the haemoglobin level dropped to 4 g.% and she was readmitted to Groote Schuur Hospital for transfusion. Since then she has remained relatively well. The spleen has decreased in size and is now just palpable. The haemoglobin concentration fluctuates between 7 and 9 g.%.

Cases 1 and 3

The families of the parents were also linked by a marriage between an uncle of the mother and a sister of the father (Fig. 1). Two of the 8 children of this marriage had suffered from recurrent bouts of pyrexial illnesses which, in the elder, Coloured male aged 16, were associated with polyarthritis, and, in the younger, Coloured male aged 8, with mono-arthritis. Neither has ever been overtly jaundiced and neither has a palpable spleen; their haemoglobin levels are 10 g.% and 11 g.% respectively. The plasma from specimens of blood (diluted with sodium citrate) gave a positive indirect v.d. Bergh reaction. The elder patient had been hospitalized on several occasions and had been regarded as a case of rheumatic fever, atypical in the response to salicylates and in having a normal ESR, and no cardiac murmurs.

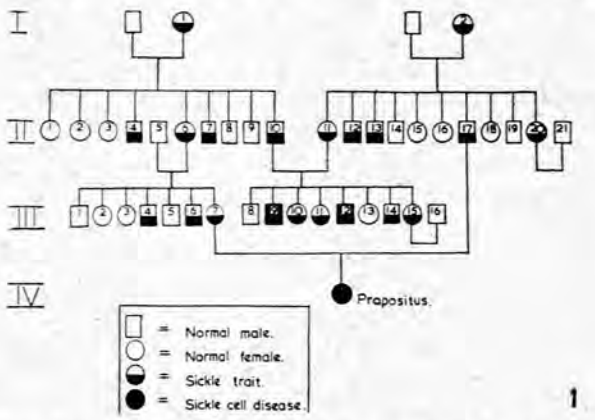

Fig. 1. Family tree, showing the incidence of the HbS gene in the family. II 11 had not had any miscarriages or stillbirths, nor had any of her children died in infancy.

Laboratory Investigations

Electrophoresis. A starch-block supporting medium was chosen in preference to paper for the following reasons: (*a*) Separation of components was better, and trailing was not nearly so marked as on paper; (*b*) elution and determination of components was accomplished with a minimum of trouble; (*c*) relatively large quantities could be applied to the block at one time and, as a consequence, reasonable quantities of purified components could, if required, be further investigated; and (*d*) increases in the proportion of the HbA2 fraction could be readily detected. The method used was basically that of Masri, Josephson and Singer,[11] modified according to the available apparatus.

Alkali denaturation. The one-minute method of Singer, Chernoff and Singer[16] was adopted.

Solubility determination. Assay of the proportion of HbS in the samples was performed according to the method of Itano.[9]

The sickling phenomenon. One drop of whole blood was treated on a slide with one drop of 2% sodium dithionite, covered with a cover-slip and examined microscopically.

RESULTS AND DISCUSSION

The blood of the propositus was examined and the diagnosis of sickle-cell anaemia confirmed. The proportion of foetal haemoglobin was assayed and found to be 10% of the total. Investigation of the family began and work proceeded in routine fashion until the unexpected discovery was made that another link between the paternal and maternal sides of the family also involved 2 heterozygotes (II 10 and II 11). On genetic grounds it was expected that, of the 8 children of this marriage, 2 should be homozygous for HbS, and such indeed was the case (cases 2 and 3). As indicated in the clinical data, one of these two children had, for years, masqueraded as a case of rheumatic fever.

The electrophoretic pattern of cases 2 and 3 calls for comment, however (Fig. 2). It resembles closely the patterns

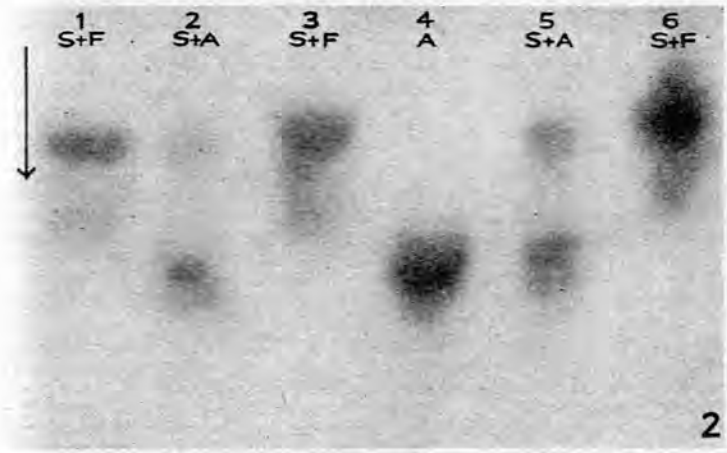

Fig. 2. Electrophoretic patterns. 1 = case 3; 3 = case 2; 6 = case 1; 2 and 5 = parents of cases 2 and 3; 4 = a normal adult. S + F = sickle-cell disease; S + A = sickle-cell trait; A = normal. Note the high proportion of HbF in the 3 cases of the disease.

seen in sickle-cell/thalassaemia disease, a condition in which the patient carries the trait for both sickle-cell disease and that for thalassaemia. It conforms also with that of a group of cases discussed by Jacob and Raper,[10] characterized by the presence of the sickle-cell trait and a gene for the persistent production of foetal haemoglobin (the 'F' gene of Went and MacIver[20]).

The latter possibility was ruled out by the absence of any appreciable quantity of HbF in the parents, and the former rendered unlikely (*a*) by the fact that both parents were heterozygous for HbS, (*b*) by the absence of HbA in both children, and (*c*) by the fact that neither parent appears, on clinical grounds at any rate, to be suffering from sickle-cell/thalassaemia disease. Evidence of a biochemical nature was obtained by measurement of the HbA2 fractions in the haemoglobin samples of the parents and the children. Gerald and Diamond[6] showed that in thalassaemia trait this fraction was increased from the normal 3% or less to values ranging from 3·3 to 6·8%. The following were the values obtained: 1·6% and 3% (parents), and 1·5% and 1·8% (children).

An unusual feature of both the cases under discussion is the unduly high proportion of foetal haemoglobin. A similar situation has been reported by Watson[19] where the foetal-haemoglobin fraction amounted to 39%.

Alkali denaturation studies were undertaken to confirm that the fraction of haemoglobin other than HbS was indeed

TABLE I. METHOD OF DETERMINATION

	Elution from Block			*Solubility Measurement*		*Alkali Denaturation*	
	HbA %	*HbS* %	*HbF* %	*HbA or F* %	*HbS* %	*HbA or S* %	*HbF* %
Cord Blood						35	65
Normal Adult						98	2
Normal Adult						98·5	1·5
Sickle Cell:							
Disease IV 1	0	90	10			86	14
Disease III 9	0	67	33			75	25
Disease III 12	0	65	35			72	28
Sickle Trait	66	34		70	30		
Sickle Trait				60	40		
Sickle Trait				56	44		

HbF and not yet another abnormal haemoglobin. While agreement between the results of this technique and that of elution from the block was not very precise, the degree of agreement was such as to confirm the presence of HbF (Table I).

Failure to elicit the sickling phenomenon in the erythrocytes of the mother of the propositus gave rise to the possibility that we were dealing with HbS/HbD disease. HbD has the same mobility as HbS and the only way to distinguish one from the other is to demonstrate the insolubility of HbS (in the reduced form) in 2·24 M phosphate buffer. This was done and the presence of HbS confirmed (Table I).

The gene was traced back to the paternal grandmother and maternal great-grandmother, but no connection with Central or West African antecedents could be established.

SUMMARY

1. The results of a family study of the sickle-cell trait are presented. A consequence of the investigation has been the discovery of 3 more cases of sickle-cell anaemia.

2. The advantage of electrophoresis over the demonstration of the sickling phenomenon in such a survey is shown by the fact that by the latter method some cases of the trait were missed and two cases of the full-blown disease were denoted as sickle-cell trait.

3. The necessity of supplementing electrophoresis with such techniques as alkali denaturation and solubility studies is well demonstrated by this survey.

We gratefully acknowledge the help and encouragement of Prof. J. E. Kench. Thanks are also due to Drs. P. M. Smythe and C. Merskey for valuable advice and criticism, and to Dr. M. C. Berman for assistance with the photography. We also wish to thank the Medical Superintendent of Groote Schuur Hospital for his permission to quote from hospital records.

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