HAEMOGLOBIN O IN AN ARAB FAMILY

SICKLE-CELL HAEMOGLOBIN O TRAIT

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An 8-year-old Arab boy from the village of Gaser A'zarka, in Israel, was admitted to the paediatric service of Chedera General Hospital, and found to be suffering from a left-lower-lobe pneumonia. The patient was said to have had attacks of abdominal pain about once every year, mainly in the right hypochondrium. These attacks were accompanied by jaundice and dark urine. Several days before admission such an attack had occurred.

On physical examination the boy was pale and jaundiced. In addition to the finding of pneumonia, an enlarged spleen was palpable 4 cm. below the costal margin.

Blood Examination

The peripheral blood smear showed anisocytosis, poikilocytosis, target cells, and occasional sickle-cells. The haematological findings are summarized in the Table. The haemoglobin was examined by the method of Singer, Chernoff, and Singer (1951), and 10% was found to be alkali-resistant by this method.

On paper electrophoresis at pH 9, using TRIS buffer, two fractions were seen, one in the position of haemoglobin A and one moving more slowly. The second fraction amounted to about 70% of the total, and the picture was very much like that seen in sickle-cell thalassaemia. It was, however, noteworthy that the "haemoglobin S fraction" seemed to be moving slightly more slowly than haemoglobin S controls. It was, in fact, only a little in advance of haemoglobin C (Fig. 1).

On paper electrophoresis at pH 8.6, using barbiturate buffer, three abnormal fractions were noted: one in the position of haemoglobin A+F, one in the position of haemoglobin S, and a third which did not separate well

from the haemoglobin S fraction and which moved more slowly than S and faster than haemoglobin C (Fig. 2). Comparison with haemoglobin S+E samples showed that the slowest fraction was faster than E and therefore in the position of haemoglobin O (Lie-Injo Luan Eng and Sadono, 1958). Direct comparison with

FIG. 1.—Paper electrophoresis TRIS buffer, pH 8.9, of propositus's haemoglobin and A+C control. The propositus's haemoglobin divides into two fractions, one corresponding to A+F and one moving more slowly.

FIG. 2.—Paper electrophoresis barbiturate buffer, pH 8.6, of propositus's haemoglobin and A+C control. The propositus's haemoglobin divides into three fractions, one corresponding to A+F, one in the position of S, and one moving between C and S in the position of haemoglobin O.

Findings in a Family in which Haemoglobins A, S, and O are Found as Adult Variants

	Age in Years	Sickle-cell Test	Adult Haemoglobins	Alkali-resistant Haemoglobin (%)	Red Cells (Million/c.mm.)	Haemoglobin (g./100 ml.)	Cell Volume (%)	M.C.H.C. (%)	M.C.V. (cu.μ)	M.C.H. (μg.)	Reticulocytes (%)
Father	28	0	AO	1.9	4.6	14.9	46	31	100	32	1.2
Mother	?	+	AS	1.7	3.5	10.5	35	33	100	30	0.8
Mother's mother ..	67	0	A	3.7	4.1	11.4	40	35	98	28	0.9
Propositus	8	+	SOA	10	2.5	7.0	25	36	100	28	32
Sister 1	4	+	SOA	28	2.8	8.6	31	36	110	31	8
" 2	3	0	AO	—	—	—	—	—	—	—	—
" 3	2 1/2	0	AS	59	3.4	10.6	34	32	100	31	7

a haemoglobin A+O sample, which had kindly been supplied by Dr. Lie-Injo Luan Eng, also confirmed that the third haemoglobin had the mobility of haemoglobin O. Haemoglobin Alexandra has the same mobility under these conditions, but it can be differentiated from O by electrophoresis on agar at pH 6, when Alexandra moves with F. It could also be demonstrated that on the addition of haemoglobin S to a haemoglobin A+O mixture a similar picture could be obtained on paper electrophoresis using barbiturate buffer, and, furthermore, that haemoglobins S and O formed a single band on paper electrophoresis using TRIS buffer. On ion-exchange chromatography, using IRC 50 at pH 6, haemoglobins F, A, S, and O could be demonstrated. Haemoglobins S and O amounted to about one-third each. There was also a small amount of haemoglobin A₂ moving between haemoglobins A and S. These findings suggest that the patient suffered from a modified sickle-cell anaemia of a type hitherto not described—a combination of the traits for haemoglobins S and O.

FIG. 3.—Paper electrophoresis TRIS buffer, pH 8.9, of the father's haemoglobin and A+E control. The father's haemoglobin divides into two fractions, one in the position of A and one moving faster than E. Other electrophoretic comparisons had shown that the non-A haemoglobin of the father moved under these conditions more slowly than haemoglobin S.

FIG. 4.—Chromatography IRC 50, pH 6, of sister's haemoglobin. Starting from below, haemoglobins F, A, S, and O can be seen.

There were no other striking findings in this patient's blood. Total serum protein was 8.3 g./100 ml. (albumin 5.2 g., globulin 3.1 g.). Serum thymol turbidity and cephalin cholesterol turbidity were 4 units and 0 respectively.

Patient's Family.—The parents, the maternal grandmother, and three siblings were investigated. Two siblings had died in infancy. The haematological findings, so far as could be obtained, are included in the Table. Examination of the haemoglobin showed that the father was a haemoglobin O trait carrier (Fig. 3), and the mother a sickle-cell trait carrier. One sibling was a sickle-cell trait carrier, one a haemoglobin O trait carrier, and one possessed haemoglobins F, A, A₂, S, and O (Fig. 4). One notable fact was that the patient's grandmother had a more than normal amount of foetal haemoglobin (3.7%), otherwise no abnormalities were found in her. The older sister, who also suffered from sickle-cell haemoglobin O "disease," had a higher percentage of haemoglobin F (28%) and a small amount of haemoglobin A (8%). The remainder consisted of about equal parts of haemoglobin S and O (Fig. 5).

FIG. 5.—Family tree. Haemoglobins A, S, and O.

Discussion

Haemoglobin O was discovered by Lie-Injo Luan Eng and Sadono (1958) in Indonesia, and has hitherto not been found in any other part of the world. The exact ethnic origin of the Arab family described in this report could not be established, but it is noteworthy that another East Asian haemoglobin, haemoglobin E, has been observed sporadically in the Eti-Turks (Aksoy, Ikin, Mourant, and Lehmann, 1958). Until now it had not been possible to study the mode of inheritance of haemoglobin O, and the present investigation demonstrates that it is inherited in a Mendelian fashion, and that when it is inherited with haemoglobin S haemoglobin A is formed as well.

The presence of haemoglobin A together with S and one other abnormal haemoglobin has been noted before with Hopkins II (Smith and Torbert, 1958) and P (Dherte, Lehmann, and Vandepitte, 1959). Ingram (1959) suggested that there was one gene determining the formation of the α -chain and one determining the formation of the β -chain. Thus if an individual inherited an abnormal haemoglobin in which the α -chain was different from the normal he would still possess a normal β -chain, and if he also inherited another abnormal haemoglobin in which the β -chain was abnormal he would still possess a normal α -chain. This would enable him to form three haemoglobins—the abnormal α -chain haemoglobin, the abnormal β -chain haemoglobin, and a haemoglobin formed by the normal α - and β -chains: haemoglobin A.

Itano and Robinson (1959) dissociated a mixture of haemoglobin Hopkins II, which carries its abnormality

in the α -chain, and of haemoglobin S, which carries its abnormality in the β -chain into α - and β -chains. On recombination they obtained haemoglobin A as well as a hybrid composed of abnormal α - and β -chains. One might therefore expect that when haemoglobin S and another abnormal haemoglobin are found together with haemoglobin A, S being a β -chain mutant, the other abnormal haemoglobin would be an α -chain mutant. We have reason to believe, however, that haemoglobin O is not an α -chain mutant, and indeed the same applies to haemoglobin P. A full account of this will be given by two of us (B.R. and H.L.) in collaboration with Drs. E. M. Shooter and E. R. Huehns, of University College, London, and a tentative explanation for this apparent paradox will then be put forward.

Summary

A new sickling condition—sickle-cell haemoglobin O trait—has been demonstrated in two children of an Arab family. This is the first occasion on which haemoglobin O has been seen outside Indonesia and that its mode of inheritance could be examined.

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A CASE OF SUBACUTE RICKETTSIAL ENDOCARDITIS; WITH A SURVEY OF CARDIAC PATIENTS FOR THIS INFECTION

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A few reports during the last 10 years suggest that Q fever in man sometimes pursues a chronic course with infection of the heart valves. Marmion *et al.* (1953) reviewed three possible cases of the condition—two Californian patients and one English.

More recently it was possible to observe the course of the disease during a two-year period in a patient of Dr. F. H. Scadding's at the Middlesex Hospital, London. A brief description of the findings was given at the Sixth International Congress of Tropical Medicine and Malaria (see Marmion, 1958), and a detailed account has been published together with a review of the literature on persistent Q fever infections (Robson and Shimmin, 1959; Andrews and Marmion, 1959). The patient was

a man aged 47, who had pneumonia clinically resembling Q fever, recovered, and was apparently healthy for six months, then became chronically ill with a swinging fever. Aortic valve incompetence developed and gradually increased until his death some 18 months later. *Rickettsia burneti* was isolated from his bloodstream at the beginning of the chronic febrile illness and again just before death; it was also shown to be present in large numbers in aortic valve vegetations collected after death.

Evans, Powell, and Burrell (1959) have also reported the isolation of *R. burneti* from the aortic valve vegetations and from the spleen of a patient dying five months after an influenza-like illness.

Although subacute rickettsial endocarditis is probably a rare sequel to an acute infection with *R. burneti*, it seemed desirable to examine a number of cardiac patients for rickettsial antibodies so as to obtain an estimate of its frequency.

This paper describes the results of these examinations so far, together with the clinical state, history, and laboratory diagnosis of another patient with subacute Q fever endocarditis who was detected as a result.

Selection of Patients and Methods

Sera were obtained from suspected cases of subacute endocarditis in Leeds and certain other centres. The sera were tested for complement-fixing antibodies to the phase 1 and 2 antigens of *R. burneti* (see Andrews and Marmion, 1959), and also for those against murine typhus and rickettsial-pox group-specific antigens. The complement-fixation test used was either that described by Stoker, Page, and Marmion (1955) or a locally developed micro-method using essentially the same reagent concentrations and incubation times.

Results

Fifty-six patients were tested in the period between April and December, 1959. A diagnosis of subacute bacterial endocarditis, with isolation of *Streptococcus viridans*, enterococci, or other bacteria, was made in 13 of the patients. In 35 of the remainder there was evidence of valvular damage and a strong suspicion of an infective process, but blood cultures were sterile. The remaining eight patients had valvular damage but no evidence of infection.

No patient had C.F. antibody to the group-specific typhus antigen. One patient had Q fever C.F. antibodies and a case report is given below.

Another patient had a C.F. antibody titre of 1/128 to rickettsial-pox group-specific antigen (Lederle). Dr. J. Colquhoun (Department of Bacteriology, Royal Infirmary, Sheffield) kindly informed us about his investigations, which indicate that this patient's endocarditis was of bacteriological origin. No antigenic relationship was shown between the causative bacterium and the rickettsial-pox antigen, and it seems probable that the rickettsial-pox antibody resulted from some past experience. Dr. C. C. Shepard (Montgomery, Ala.) kindly confirmed the specificity of the serological findings by C.F. tests with their rickettsial-pox antigen and by staining smears of *Rickettsia akari* with 1/10 and 1/100 dilutions of the patient's serum, using the indirect fluorescent antibody technique. The patient has not been out of the country, and local investigations into the possible presence of a member of the spotted-fever