TWO FAMILIES SHOWING INTERACTION OF HAEMOGLOBIN C OR THALASSAEMIA WITH HIGH FOETAL HAEMOGLOBIN IN ADULTS*

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The difference between the haemoglobin composition in heterozygous sickle-cell trait and in homozygous sickle-cell anaemia is that, in the first, both normal adult haemoglobin (A) and sickle-cell haemoglobin (S) are found, whereas in the latter only haemoglobin S together with smaller amounts of foetal haemoglobin (F) is present. In the double heterozygous condition when the person is a sickle-cell-trait carrier (A+S) and possesses, in addition, a gene for thalassaemia, which causes suppression of haemoglobin-A production, the proportion of haemoglobin A is lowered and may in some instances be so low as to escape detection by electrophoretic methods. In this condition, which is known as sickle-cell thalassaemia, the patient suffers from a modified sickle-cell anaemia, and the red cells show morphological evidence of thalassaemia.

Edington and Lehmann (1955), Jacob and Raper (1958), Went and MacIver (1958), and Herman and Conley (1960) have reported yet a third type of disorder which causes a haemoglobin S+F pattern. In each case family studies excluded the possibility that the persons concerned were sickle-cell homozygotes. It was not possible to decide whether haemoglobin F was inherited in place of haemoglobin A or whether it had been increased as a consequence of suppressed production of haemoglobin A, as is thought to occur in thalassaemia. No other features of thalassaemia, such as microcythaemia, increased level of haemoglobin A_2, and anaemia, were present in these persons. Hence Lehmann (1959) has described this phenomenon as "non-microcythaemic thalassaemia" (N.M.T.). Family studies showed that the inheritance of sickle-cell haemoglobin and the increased level of haemoglobin F were segregated. In some instances the haemoglobin composition in the parents was A+F in one and A+S in the other; in others the offspring inherited either the increased level of haemoglobin F without haemoglobin S or haemoglobin S without haemoglobin F. Only once was it reported (Edington and Lehmann, 1955) that an offspring had inherited a gene for haemoglobin A from such a haemoglobin S+F father; however, mistaken paternity had not been excluded. Other reports on isolated cases of adults with haemoglobin S+F without anaemia have not been supported by family studies (Neel *et al.*, 1956; Griggs and Harris, 1956).

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We have observed two families of American negro origin in whom N.M.T. was inherited in combination with haemoglobin C in one and with classical thalassaemia in the other.

Material and Methods

Red and white blood-cell counts, haematocrit, haemoglobin concentration, reticulocyte count, red-cell osmotic fragility, blood typing, and serum bilirubin estimations were performed, employing standard techniques (Miller, 1960). The percentage of target cells was determined by counting 500 consecutive red cells in well-stained peripheral blood smears. Haemoglobin was fractionated by paper electrophoresis at a *p*H of 8.6 in veronal buffer of ionic strength 0.05, using the Spinco set (Motulsky *et al.*, 1954). Quantitative analysis of the paper strips was carried out with an automatic scanning instrument ("analytrol"). Alkali-resistant haemoglobin was determined as described by Singer *et al.* (1951). Ultraviolet spectra of foetal haemoglobin were obtained on an automatic scanning spectrophotometer. Starch block electrophoresis was performed according to Kunkel and Wallenius (1955).† Red-cell survival was determined by chromium-51 technique. Serum iron was determined and the total iron-binding capacity was calculated as the sum of bound iron and unsaturated iron binding capacity, employing the method of Schade *et al.* (1954).

Family I, Containing Haemoglobin C and F (Fig. 1)

The propositus, Mary H. (II 6), was born in 1910. She was first seen by us at the City of Memphis Hospitals at the age of 48 complaining of pain and swelling in the left leg, particularly in the knee, for approximately two weeks. In 1930 she had been treated for syphilis. Splenomegaly was first discovered in 1945. During the last four years she experienced episodes of mild "arthritis." She had never been jaundiced. The only physical abnormalities consisted of enlargement of both liver and spleen to 4 cm. below the costal margin and painful swelling of the left knee without redness or heat. Aspiration of the left knee produced serosanguineous sterile fluid. Immobilization of the left knee resulted in complete disappearance of her complaints.

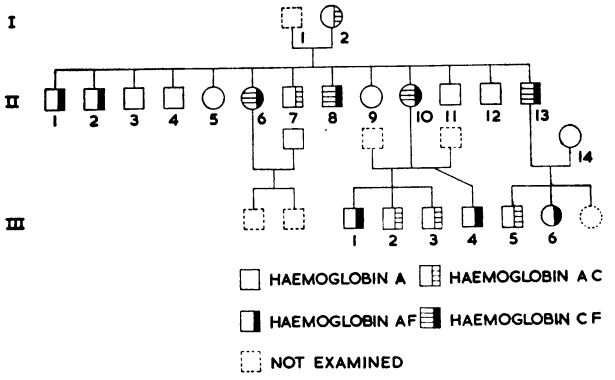

FIG. 1.—Pedigree of Family I.

A skeletal *x*-ray survey showed nothing of note. The results of the laboratory studies performed can be seen in Table I. Electrophoresis of haemoglobin revealed 69% haemoglobin C and 31% haemoglobin F. As the clinical picture and the haematological findings were not fully compatible with homozygous haemoglobin C disease, particularly in

†We are indebted to Miss Virginia Minnich, Washington University, St. Louis, Missouri, for the determination of haemoglobin A_2; to Dr C. Argall, Director of Blood Bank, Baptist Memorial Hospital, Memphis, Tennessee, for the detailed typing of the blood; and to Dr. H. Lehmann, St. Bartholomew's Hospital, London, England, for valuable suggestions.

view of a normal reticulocyte level, we examined the patient's family.

The father had died two years previously at the age of 83, having been in good health all his life.

The mother, Josie R. (I 2), had always been in good health except for arthritis and hypertensive cardiovascular disease in recent years. No history of jaundice could be elicited, and she denied splenomegaly and anaemia.

Nine brothers and three sisters of the propositus were available for study. Since, except for a few individuals, all members of this large family were in good health and, since physical examination revealed no abnormal findings, they are presented in tabular form (Table I). The few exceptions are as follows:

Andrew R. (II 1) had an episode of pain in the head and right shoulder six years before examination. Unfortunately his wife and child could not be studied.

Alfred R. (II 2) had "arthritis" in the right shoulder. His wife, daughter, and grandson were not available.

Johnny R (II 6) was said to have had malaria and typhoid fever in 1937. He was now in good health except for "rheumatism" in the neck, shoulder, elbow, and knees. This indisposition did not prevent him from doing manual labour.

Lennie S. (II 10) had always been in good health with the exception of two episodes of cystitis which responded to antibiotics. She had been married twice and separated from both husbands. The first husband fathered children III 1, 2, and 3; the second husband, child III 4. Neither of these men was available for study.

Ray R. (II 13) had always been in good health. His wife, Daisy Lee, is reported as II 14.

The results of the laboratory studies performed on these individuals are summarized in Table I.

The blood groups (ABO, CDE/ce, Kell, Duffy, Kidd, MNS, and P) of all members of this family (except I 2, II 9, and II 12) were determined. The results obtained do not contradict the assumption, as given in Fig. 1, that the family members are related, except possibly for II 4.

In summary, this family had inherited the haemoglobin C trait from I 2 and the high level of foetal haemoglobin presumably from her husband (I 1). Two members of the second generation (II 1 and 2) are assumed to be similar to their father in that they have a high level of haemoglobin F and a normal A_2 in the absence of haemoglobin C. Six of their siblings are homozygous for haemoglobin A. Another is a haemoglobin C trait (II 7). The remaining four are postulated to have inherited haemoglobin C from their mother and haemoglobin F from their father, and they appear to have the haemoglobin phenotype C+F, although three of them (II 8, 10, and 13) may have a small amount of haemoglobin A in the haemolysates. This conclusion was based upon the observation that, when scanning the paper strips of haemoglobin electrophoresis, the amount of haemoglobin present in the region of A+F was consistently slightly higher than the values for haemoglobin F obtained by alkali denaturation. Agar electrophoresis, kindly carried out by Miss Virginia Minnich, also revealed in these three individuals a slight deviation from the zero line in the region of haemoglobin A. This was not the case in II 6. The amounts of haemoglobin A as demonstrated by these techniques are so small and the methods of determination not sufficiently sensitive, so that it is impossible to state with complete assurance that haemoglobin A was truly present.

Haemoglobin A_2 was consistently within normal limits in all individuals examined. Furthermore, the peripheral blood smears failed to show hypochromia, basophilic stippling, or any other abnormalities suggestive of thalassaemia minor except for increased numbers of target cells in all of the individuals carrying haemoglobin C and in some of those with high haemoglobin F. Our observations differ, therefore, from those of the previous authors in that we have not found a complete absence of morphological changes in all persons of the haemoglobin A+F phenotype.

Family II, Containing High Haemoglobin F in Combination with Thalassaemia Minor (Fig. 2)

The family tree is given in Fig. 2. The propositus of this family (II 4) was a 26-year-old negro employee of the V.A.M.T.G. Hospital. His Army records showed that his spleen was palpable 2.5 cm. below the left costal margin in 1954 and 1955. He was in good health and was working regularly except during an occasional episode of aching in the left upper quadrant of the abdomen. These attacks lasted one to one and a half hours and were not related to respiration, food position, or activity. He denied ever having been jaundiced. The spleen was palpable 1 cm. below the left

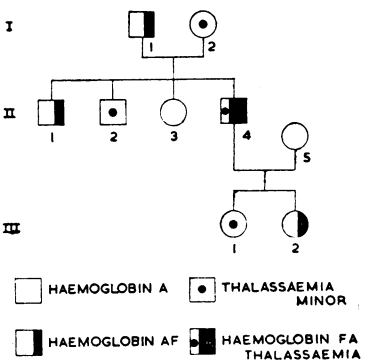

FIG. 2.—Pedigree of Family II.

TABLE I.—*Clinical and Laboratory Data of Family I*

Case No.	Sex	Year of Birth	Liver Palp. (cm.)	Spleen Palp. (cm.)	R.B.C. × 10^6 c.mm.	Hb (g./100 ml.)	Haematocrit (%)	M.C.V. (c.μ)	M.C.H. ($\mu\mu$g.)	M.C.H.C. (%)	Retic. (%)	Target Cells (%)	Serum Bilirubin (mg./100 ml.)	R.B.C. Osmotic Fragility %NaCl	Serum Iron (μg./100 ml.)	Total Serum Iron Binding Capacity (μg./100 ml.)	Hb A (%)	Hb C (%)	Hb F (%)	Hb A_2 (%)
I 2	F	1879	3	0	4·37	13·3	39·0	89	31	34	1·2	16			90		50·0	50·0	<2	*
II 1	M	1900	0	0	4·80	14·9	45·0	94	31	33	0·6	12			127		73·2	0	25·2	1·6
II 2	M	1902	0	0	4·68	17·0	47·0	97	31	32	0·8	11			138		70·8	0	27·6	1·6
II 3	M	1903	0	0	4·25	13·8	43·9	103	33	32	0·9	2			110	238	97·6	0	<2	2·4
II 4	M	1906	0	0	4·26	13·3	43·4	102	31	31	0·7	1			74	329	98·1	0	<2	1·9
II 5	F	1907	0	0	3·99	12·7	42·5	106	32	31	1·0	1			114	342	97·7	0	<2	2·3
II 6	F	1910	4	4	3·82	11·3	31·3	83	28	35	1·3	27	0·5	0·36–0·24	92		0†	69·0	31·0	*
II 7	M	1912	0	0	5·30	15·7	45·0	85	30	35	1·0	27			120	266	50·7	49·3	<2	*
II 8	M	1913	0	0	5·76	15·0	42·5	74	26	35	4·1	33	0·4	0·36–0·20	130		2·6†	63·4	34·0	*
II 9	F	1915	0	0	4·13	13·2	38·0	92	31	35	0·6	0			126		100·0	0	<2	—
II 10	F	1917	2	2	5·00	13·1	35·0	70	26	37	4·2	34	0·8	0·36–0·24	160		0†	61·4	38·6	*
II 11	M	1921	0	0	4·62	13·2	42·5	91	28	31	0·9	0			150	310	97·4	0	<2	2·6
II 12	M	1923	0	0	5·24	16·1	48·5	93	31	34	1·0	0					97·8	0	<2	2·2
II 13	M	1924	0	1·5	5·06	13·3	38·2	77	27	35	0·9	38		0·40–0·28	85	263	4·5†	59·2	36·3	*
II 14	F	1929	0	0	4·30	13·5	38·0	90	31	35	1·1	1					97·6	0	<2	2·4
III 1	M	1941	0	0	4·95	15·3	48·0	96	31	33	0·3	1			165		65 4	0	33·0	1·6
III 2	M	1945	0	0	4·30	13·3	35·5	83	31	38	0·7	2	0·2	0·36–0·28	100	324	57·0	43·0	<2	*
III 3	M	1947	0	0	4·48	13·2	38·0	87	30	35	0·7	2	0·2	0·36–0·28	118	250	61·2	38·8	<2	*
III 4	M	1952	0	0	4·88	13·4	41·0	83	27	32	0·6	1			91		66·4	0	32·3	1·3
III 5	M	1953	0	0	4·20	10·9	33·9	80	26	32	0·3	13			25	243	57·0	43·0	<2	*
III 6	F	1954	0	0	4·94	11·9	40·0	90	27	30	1·3	1					69·6	0	28·9	1·5

* Hb A_2 determination is not possible in presence of Hb C.
† This figure is calculated from the difference between alkali denaturation and paper electrophoresis (see discussion).

TABLE II.—*Clinical and Laboratory Data of Family II*

Case No.	Sex	Year of Birth	Spleen Palp. (cm.)	R.B.C. × 10^6/c.mm.	Hb (g./100 ml.)	Haematocrit (%)	M.C.V. (c.μ)	M.C.H. ($\mu\mu$g.)	M.C.H.C. (%)	Retic. (%)	Target Cells (%)	Serum Bilirubin (mg. 100 ml.) Total/1. min.	Serum Iron (μg./100 ml.)	Blood Type			Hb A (%)	Hb F (%)	Hb A_2 (%)	Hb A_2'(%)
														ABO	MN	Rh				
I 1*	M	1900		4·00	12·6	42	105	32	30	0·5	15·8	0·45/0·15		A	MN	cDe/c–e	79·5	19·5†	0·9	1·1
I 2*	F	1903		3·50	10·4	35	100	30	30	0·5	2·5	0·35/0·15	80	B	MN	cDe/c–E	95·9	<2·0†	4·1	
II 1*	M	1942		3·60	11·6	35	97	32	33	0·5	0·8	0·35/0·10		A	MN	cDe/c–E	95·9	<2·0†	2·4	1·7
II 2*	M	1940		3·95	12·6	40	101	32	32	1·1	0·6	0·35/0·10		AB	MN	cDe/c–E	78·1	20·0†	1·9	
II 3*	F	1945		3·85	11·4	36	94	30	32	0·4	1·6	0·35/0·20		AB	MN	cDe c–e	97·4	<2·0†	1·6	1·0
II 4	M	1933	1	4·75	11·5	40	84	24	29	1·2	28·3	1·27/0·19	110	B	MN	cDe/c–e	29·0	67·6	3·4	
II 5	F	1935	0	3·70	9·9	34	94	27	29	2·2	0·3	0·60/0·11		A	MN	cDe/c–e	97·2	<2·0	2·8	
III 1	F	1956	0	5·00	9·3	30	60	19	31	0·2	0·5	0·54/—		A	MN	cDe/c–e	94·3	3·2	5·7	
III 2	F	1958	0	4·60	10·0	33	72	22	30	1·8	0·2	0·67/0·07		AB	N	cDe/c–e	66·2	32·0	1·8	

* Blood counts, reticulocyte counts, and serum bilirubin determinations were performed at VA Hospital, Columbia, S.C.
† Oxalated refrigerated blood was sent from S.C. Haemolysates were prepared three days after blood was obtained from patients.

costal margin. No other abnormalities were noted. Peripheral blood and other laboratory findings of this patient are summarized in Table II. The apparent half-survival of Cr-51 labelled red cells was 20 days (normal value in this laboratory is 27–32 days). These data, together with the microcytosis, target cells, hypochromia with normal serum iron level, and the presence of an enlarged spleen suggested to us that he was suffering from a well-compensated haemolytic anaemia, probably thalassaemia minor. The remarkably high foetal haemoglobin level of 67% is, however, usually associated with the more severe thalassaemia major, although exceptions to this rule have been recorded by Josephson *et al.* (1958) and Aksoy (1959). Haemoglobin A_2 was 3.4%, a value which in reference to total haemoglobin is within the upper level of normal, but which is much higher than normal if referred to haemoglobin A alone. This again is a feature of thalassaemia major rather than of thalassaemia minor.

Examination of the family (see Table II) revealed members who were examples of typical thalassaemia minor and others who were examples of N.M.T. All were clinically well, including the relatives with thalassaemia minor.

Our propositus apparently inherited both thalassaemia minor and N.M.T., one from each of his parents, and these seemed to have interacted to produce a thalassaemia of intermediate severity. The possibility of such an interaction has been suggested by Olesen *et al.* (1959) in their study of atypical thalassaemia patients in Liberia. They were unable to produce direct proof but presented impressive circumstantial evidence. In the present family the A+F phenotype was free from the morphological features which suggested thalassaemia, and it corresponded, therefore, much more closely with the observations of previous authors who had reported N.M.T. On the other hand, it differed from some of the A+F phenotypes of our first family.

Discussion

The human haemoglobin molecule contains four polypeptide chains. Two α and two β chains are found in haemoglobin A, and two α and two γ chains are present in haemoglobin F (Schroeder, 1959). The transition from foetal to adult haemoglobin, from $\alpha_2\gamma_2$ to $\alpha_2\beta_2$, consists, therefore, in a switchover from α chains combining with γ chains to α chains combining with β chains. It is this switchover which seems greatly suppressed in N.M.T. Ingram and Stretton (1959) surveyed the current ideas which suggest that there are two types of thalassaemia—one in which the α chain production is reduced and one in which the β chain production is lowered. It is obvious that the β chain thalassaemia is not a uniform condition. Thus Ingram and Stretton suggest that it might include β chain deficiency due to the production of abnormal variants of the β chain, in which there is no alteration of the electrical charge, and which, therefore, cannot be differentiated from the normal β chain by electrophoresis. Whatever the precise nature of N.M.T., it seems to fall within the group of β chain thalassaemias.

There are obvious differences between N.M.T. and classical β chain thalassaemia. The compensatory increase of haemoglobin F is much higher and there is no rise in haemoglobin A_2. There is no microcythaemia or anaemia. Yet the target cells present in some, though not all, of the A+F individuals in the first of the above families suggest that these differences may be quantitative rather than qualitative. There certainly is no indication that the suppression of the β chain production in N.M.T. is anything but quantitative. The deficiency of β chain in N.M.T. would constitute a corollary to the deficiency of α chains in which the surplus β and/or γ chains form respectively β_4 and γ_4 (haemoglobin H and haemoglobin "Barts") (Ramot *et al.*, 1959; Sturgeon *et al.*, 1960).

Summary

Two families are reported in whom a hereditary persistence of foetal haemoglobin production into adult life (non-microcythaemic thalassaemia) is found together with haemoglobin C in one and with classical microcythaemic thalassaemia in the other. Double heterozygosity for the genes responsible for non-microcythaemic thalassaemia and haemoglobin C, and for non-microcythaemic thalassaemia and classical thalassaemia, has been investigated.

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