ABNORMAL HAEMOGLOBINS IN CAPE TOWN*

(OCCURRENCE AND SIGNIFICANCE)

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Abnormal forms of haemoglobin are known to occur in the Coloured population of Cape Town. The carrier rate of Hb. S was estimated by Esrachowitz *et al.*[1] to be 0·58%; and homozygous Hb. S disease (sickle-cell anaemia) was described in two Coloured sisters by Wassermann[2] and three members of a second Coloured family by Anstey.[3] The Hb. C trait was found by Brain[4] in 2 out of 219 Coloured women, giving a carrier rate of about 0·9%; and Brain and Budtz-Olsen[5] reported Hb. E, associated with thalassaemia, in one member of a Cape Coloured family.

There are two reports of abnormal haemoglobin forms in the White South African population. A family with homozygous Hb. C disease in three members and Hb. C trait in a further three members, was studied by Lewis *et al.*[6] Among the four earlier accounts of Hb. S disease in South Africa, is a report[7] of sickle-cell anaemia in a White South African family, although the possibility of Coloured admixture in this instance was admitted on the grounds of physical appearance. In studying this report, it is also noted that there is an unexplained absence from the family pedigree of particulars relating to the mother of the proband. Moreover, in our opinion, there is doubt about the diagnosis in this very early report also. Sickling was not demonstrated in the parent available for study; and in the absence of present-day technical methods, Hb. S was not demonstrated. The fact that the patient had been accepted by the army in the category A1, is hardly in keeping with a diagnosis of sickle-cell anaemia.

In the remaining three South African reports of sickle-cell anaemia, at least one parent was from another country where the Hb. S trait is common, i.e. India and Liberia. Two of these families[8-10] were pure Asiatic (Indian); the other patient[11] was of mixed Xosa and Liberian origin. In addition to this demonstration of the Hb. S trait in a Bantu parent, there are two further accounts of abnormal haemoglobins among South African Bantu. Hb. S carrier rates of 1 in 403[12] and 2 in more than 600,[13] have been reported.

In view of the high incidence of anaemia of pregnancy among certain population groups in Cape Town, an investigation was undertaken to determine the frequency of the carrier state of abnormal haemoglobin forms and of thalassaemia among pregnant women in the area. This report deals with abnormal haemoglobins; the occurrence of various forms of thalassaemia will be reported later.

MATERIAL AND METHODS

Blood is taken by venepuncture from all women attending for the first time during pregnancy at the antenatal clinics of the Cape Town City Health Department. Specimens, with the potassium salt of ethylene diamine acetic acid as anticoagulant, are submitted as a routine for determination of the Hb. value. A proportion of the antenatal specimens received in this laboratory was examined for abnormal haemoglobin. The population sample was not random, since blood specimens with a Hb. value below 10 G/100 ml. were selected in the first instance.

Haemoglobin samples were run on Whatman's No. 3 MM paper in a vertical tank,[14] using barbital buffer at pH 8·6 and ionic strength 0·05, for 17 hours at 5 V/cm. and 0·3 mA/cm. After detection and primary classification, haemolysates containing abnormal haemoglobins were run similarly in TRIS buffer at pH 8·9, as well as in phosphate buffer pH 6·5, of ionic strength 0·1. Specimens containing an abnormal fraction were also separated on an ion exchange column;[15] and specimens of intermediate mobility were examined by Itano's test,[16] by the alkali denaturation test[17] and for sickling.[18]

At the beginning of the study, specimens with different mobilities were submitted to Dr. H. Lehmann† for confirmation of identity. Blood from these individuals served as reference specimens throughout the later investigation.

RESULTS

Table I shows the occurrence of various abnormal haemoglobins among anaemic and non-anaemic antenatal patients in different population groups of Cape Town. Of the Coloured

TABLE I. OCCURRENCE OF VARIOUS ABNORMAL HAEMOGLOBINS AMONG PREGNANT WOMEN IN CAPE TOWN

	Women tested	*Hb. A + C*	*Hb. A + E*	*Hb. A + S*	*Hb. A + J*	*Hb. A + L*
Cape Malay						
Hb. value below 10·0 G/100 ml.	210	1	6	4	–	–
Hb. value above 9·9 G/100 ml.	346	1	2	1	–	–
Non-Malay Coloured						
Hb. value below 10·0 G/100 ml.	510	2	8	3	–	1
Hb. value above 9·9 G/100 ml.	845	2	8	2	1	–
White						
Hb. value below 10·0 G/100 ml.	164	1	–	–	–	–
Hb. value above 9·9 G/100 ml.	1,100	4	2	2	–	–
Bantu						
Hb. value below 10·0 G/100 ml.	40	–	–	–	–	–
Hb. value above 9·9 G/100 ml.	110	–	–	–	–	–

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(taking together Malay and non-Malay) antenatal patients from whom blood samples are received in this laboratory, very nearly 20% have a haemoglobin value of less than 10 G/100 ml. The over-all incidence of abnormal haemoglobins can therefore be calculated approximately from a weighed sample in which the proportion of lower to higher values are restored to normal; the results are shown in Table II. The expected incidence of the homozygous states and of the double heterozygous states (in which 2 different abnormal forms occur together) were calculated and are listed in Table III.

TABLE II. CALCULATED INCIDENCE OF THE HETEROZYGOUS STATE OF VARIOUS ABNORMAL HAEMOGLOBINS IN CAPE TOWN

	Hb. A + C	*Hb. A + E*	*Hb. A + S*
Cape Malay Coloured	0·3	0·9	0·5
Non-Malay Coloured	0·3	1·0	0·3
White	0·4	0·2	0·2
Bantu	—	—	—

TABLE III. EXPECTED INCIDENCE PER 100,000 OF SOME HOMOZYGOUS AND DOUBLE HETEROZYGOUS COMBINATIONS OF CLINICAL SIGNIFICANCE

	Cape Malay	*Non-Malay Coloured*	*White*
Hb. C + C	1·0	0·7	1·6
Hb. E + E	7·7	10·6	0·3
Hb. S + S	2·5	0·8	0·3
Hb. C + E	5·5	5·4	1·3
Hb. C + S	3·1	1·5	1·3
Hb. E + S	8·8	5·8	0·5

DISCUSSION

Incidence

The occurrence of Hb. C is confirmed, and the occurrence of Hb. E and Hb. S established in the White population. The individuals carrying these haemoglobins were from White families which have been in the Cape for at least two generations; and in all probability, from much further back in every instance. Whereas Hb. C is the commoner in the White population, Hb. E and Hb. S were found more often in the Coloured population groups.

Among the Coloured women in this study, Hb. C is so much less common than Hb. E, that the previous report[4] of two examples of Hb. C giving a carrier rate of 0·9% in a Coloured sample apparently free from Hb. E, seems remarkable. This earlier report did appear when Hb. E had only just been discovered, but the designation of the Cape Town variant as Hb. C had been confirmed after comparison with the electrophoretically similar Hb. E.

Among all Coloured women from whom blood specimens were received during the period of this study, the proportion of Malay to non-Malay is 6:1. Calculated on this basis, the carrier rate of Hb. S in the combined Coloured population sample would be 0·30, which is approximately half of that reported previously. The earlier investigation[3] was based on tests for sickling, which may be susceptible to false positives.[19]

Clinical Considerations

The carrier state, in which an abnormal haemoglobin is in combination with normal adult Hb. A, is generally reported to be an asymptomatic, benign condition. In some instances there may be, in addition to the abnormal physico-chemical behaviour of the variant form, some minor morphological changes in the red cells.

Among the pregnant Coloured women of the Cape, abnormal haemoglobins are distinctly more common in those who are anaemic. It has to be considered, therefore, whether there is some degree of causal relationship. Detailed haematological investigation and follow-up study of these patients were undertaken only towards the end of this survey and will be reported later.

The early impression is that, in some instances, the anaemias associated with an abnormal haemoglobin have the same stigmata (hypochromic red cells, low mean corpuscular haemoglobin concentration and low serum iron) as iron-deficiency anaemia, which is common among pregnant women at the Cape. In a small number of patients who were followed up after treatment, the response to iron was variable, but not more so than was observed in anaemic patients without haemoglobin variants. In this group of patients (represented by Table IV, nos. 3, 5, 6 and 8), the possession of an abnormal haemoglobin trait may be no more than incidental. On the other hand, some anaemias accompanied by an abnormal haemoglobin are normochromic, associated with a normal serum iron level (in some instances without treatment), and of an order which is probably not ascribable entirely to blood volume changes of pregnancy (patients 1, 2 and 4). On the whole, haemoglobin values well below 10 G/100 ml. were found only when there was evidence of iron deficiency.

From this preliminary study it cannot be decided whether the heterozygous state of an abnormal haemoglobin may be responsible for mild pregnancy anaemia, either by itself or by complicating a state of iron deficiency. It is known that where Hb. A and an abnormal haemoglobin occur together the abnormal form is practically always in a lower proportion, usually 25 - 45% of the total haemoglobin. The rate of synthesis of Hb. A is

TABLE IV. HAEMATOLOGICAL FINDINGS IN 8 PREGNANT COLOURED WOMEN WITH A HETEROZYGOUS ABNORMAL HAEMOGLOBIN

	Hb. form	*Stage of pregnancy*	*Previous treatment*	*Hb. value G/100 ml.*	*MCHC percent*	*Serum iron µg./100 ml. (N = 60–120)*
1.	A + C	20 weeks	Oral iron	9·9	31	
		24 weeks	Oral iron	10·0		
		26 weeks	Oral iron	9·9		
		30 weeks	Oral iron	9·1		
		36 weeks	IM iron	8·8	32·5	144
			Blood transfusion			
2.	AE	22 weeks	Nil	10·7	30·0	75
		24 weeks	Oral iron	11·1	30·0	
		28 weeks	Oral iron	12·2	30·0	
		37 weeks	Oral iron	11·6	30·5	
3.	AE	25 weeks	Nil	7·3	23	27
		33 weeks	Oral iron	8·6	28·5	68
		37 weeks	IM iron	11·2	32	
4.	AE	35 weeks	Nil	10·1	31·5	98
		39 weeks	Oral iron	9·7	31·0	
5.	AE	32 weeks	IM iron	7·3	28	
		36 weeks		8·5	30	
			Blood transfusion			
6.	AS	20 weeks	Nil	8·7	30	22
		23 weeks	Oral iron	9·7	30·5	81
		26 weeks	Oral iron	10·4	30·5	
		29 weeks	Oral iron	12·0	30·0	
7.	AS	25 weeks	Oral iron	9·5	31	50
		27 weeks	Oral iron	9·9	30	55
8.	AS	31 weeks	Nil	6·2	26	35
		37 weeks	IV iron	10·3	28·5	97

therefore more rapid than that of abnormal haemoglobins. Since carriers of abnormal haemoglobins commonly have normal levels of total haemoglobin, it has been postulated that the genes for normal Hb. A possess a reserve, which remains hidden when the genes are in company of a second gene for Hb. A. In company of an abnormal haemoglobin, this reserve may enable the single gene for Hb. A to compensate for the important fact that the clinically significant abnormal Hb. forms, such as C, E and S, have an inherently slower rate of synthesis. From the few patients studied to date, there did not seem to be any evidence that the stress of iron deficiency produced a state of preferential utilization of iron by Hb. A, since the ratios of Hb. A to abnormal Hb. were of the same order before and after correction of severe anaemia.

As regards the homozygous state of haemoglobin abnormality, it is well known that pregnancy increases the disability which is associated with one particular condition. Homozygous Hb. S disease (sickle-cell anaemia) is a serious condition in itself; in the small proportion of women who reach puberty and fall pregnant in spite of this condition, the pregnancy is often fatal.[20] Homozygous Hb. C and homozygous Hb. E disease is clinically much less severe than sickle-cell anaemia. The two conditions may present as mild haemolytic anaemias with indefinite and vague symptoms; on the other hand, homozygous individuals may be quite healthy. In these conditions, pregnancy has not been noted as a hazard.

The double heterozygous state, in which there occurs a combination of two differing abnormal haemoglobins, is generally intermediate in clinical severity. Hb. S and C and Hb. S and E, that have been well studied in West Africa and South East Asia respectively, may produce haemolytic anaemias that tend to be less severe than sickle-cell anaemia, but more severe than Hb. C or Hb. E disease. Pregnancy is commonly a serious complication; among pregnant women with Hb. S and C disease, a maternal mortality rate of 10% is reported from West Africa.[21]

From the figures in Table III, it would be expected that among the general population of Cape Town, i.e. children and adults of both sexes, homozygous Hb. E disease would be relatively more common; in practice, however, proportionately more cases of homozygous Hb. S disease have been recognized. The fact that homozygous Hb. E disease has not previously been described from South Africa is taken as an indication that the relatively milder symptoms, that may be associated with homozygous Hb. E, are treated at outpatient level and not referred for specialized laboratory investigation. The same argument may well apply to other homozygous and double heterozygous combinations, since sickle-cell anaemia is the haemoglobinopathy most commonly reported to date.

Genetic Aspects

Because of their clear anthropological association, Hb. C and Hb. E may serve as genetic markers in population studies.

Hb. C is characteristic of certain Negro races of West Africa, particularly Ghana, where it occurs with a frequency of 30% in some areas.[22] It occurs also, with a lower incidence, in negroid populations which originated from there, such as the negroid Berbers in Algeria;[23] and in the New World, among American Negroes[24] and the negroid population of the West Indian islands.[25]

Hb. C or Hb. C-like haemoglobin in a White family has been reported in 4 instances. A French family,[26] without known Algerian association, was exceptional in that the abnormal haemoglobin was not clearly recognized as being Hb. C; in any event, the complete absence of an abnormal haemoglobin from both parents largely discounts the suggestion that this may have been classic Hb. C in a White family. The remaining three families were in geographic association with a negroid population with Hb. C. A White Dutch family[27] had lived for several generations in Curacao, and the authors suggest the possibility of a negroid ancestor; an Italian family was studied[28] in the United States; and in a report,[6] to which earlier reference was made, a White South African family of Dutch descent was said to have been at the Cape since 1691.

The association between Negroes direct from West Africa and the population of the Cape occurred early. West European immigrants settled in 1652; the earliest significant importation of involuntary immigrants were two shiploads of West African Negroes from Angola and Guinea in 1658.[29] The majority of these people were soon taken to the Dutch East Indies, but some remained. Importation of Negroes from the west coast of Africa to the Cape was not repeated, but substantial numbers were later brought from the east coast and particularly from Madagascar.

Hb. E is characteristic of South East Asia, where its distribution has a distinct pattern.[30] The highest general frequencies (up to 13%) occur among Burmese, Thais and the people of North Eastern Malaya. Lower frequencies are reported in Western and Southern Malaya, among the Bengali of India to the West and the Filipinos in the East, and the Vedda people of Ceylon. While the Indonesian people generally have a low frequency,[31] it is of note that Indonesian communities with a higher frequency are found on the islands to the east of Java. Eng *et al.* have reported[32] figures of 16·4% for Sumba, Sumbawa and Timor.

The names of these islands, that had formed part of the Dutch East Indian possessions, are recorded[33] relatively frequently as the place of origin of South East Asian people who were brought to the Cape during the first 150 years after the White settlement. Among others similarly mentioned, such as the Balinese, Buginese (from Sulawa, previously Celebes) and the people of Makassar, Hb. E occurs with the more general Indonesian frequency of 1 - 4%.

Outside the South East Asian population, Hb. E has been recorded, in isolated instances, among the Eti-Turk,[34] in Greece,[35] in an Indonesian-Dutch individual[32] and in a Cape Coloured family.[5]

Hb. S is the most widespread of the abnormal haemoglobins,[36] and is therefore of lesser value in a genetic study of newer populations. It is common, but with variable frequency, in tropical Africa, south of the Sahara and north of the Limpopo, reaching a carrier rate of 40% in parts of East Africa; it is also found in Madagascar. The low frequency in South African Bantu and its absence from the Hottentot[37] and Bushmen,[38] is noteworthy. Foci of Hb. S occur along the Mediterranean, in Greece, Italy, Sardinia and Turkey, as well as in North Africa and it is

irregularly distributed in South Arabia and the Indian peninsula. It occurs sporadically elsewhere along the Mediterranean and in South East Asia, but is distinctly uncommon in Indonesia.

Hb. J is rare and is irregular in distribution ; most instances have been found in Asians, including Indonesians. Hb. L is even more rare, and was first described from India. These two haemoglobins have not been found to be clinically significant. The haemoglobins that have been designated Hb. J, on the grounds of physico-chemical behaviour, are not necessarily identical in their structure. The single example of Hb. J from Cape Town is a new form, not previously identified by amino-acid analysis.[39]

It may be concluded that the occasional occurrence of Hb. C and Hb. E indicates a West African and South East Asian genetic contribution respectively to the population of the Cape ; the isolated finding of Hb. J and Hb. L* may well confirm the Asian contribution, while the presence of Hb. S is not as clearly of anthropological significance.

The possibility that two abnormal haemoglobins may combine to cause disease in individuals of these population groups, must be remembered. There are additional clinical considerations. The same anthropological association may be responsible for other genetically-determined illnesses. Deficiency of glucose-6-phosphate dehydrogenase, of either Negro or non-Negro type, is an example. Knowledge of the genetic constitution of people is clearly of medical importance.

CONCLUSION

It is not clear whether the heterozygous state of some of the abnormal haemoglobins, that occur among pregnant women of Cape Town, is of clinical significance as a cause of anaemia. This possibility should be examined further. Their occurrence is medically significant in wider respects.

SUMMARY

A variety of abnormal haemoglobins has been found in certain population groups of the Cape. The possible significance of these observations are considered.

*The haemoglobin J identified in this series has now been designated Hb J Cape Town, while the haemoglobin L has been designated Hb L Bonteheuwel (personal communication, Dr. H. Lehman, Cambridge, England).

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