Haemoglobinopathies, Glucose-6-phosphate Dehydrogenase Deficiency and Allied Problems in the Indian Subcontinent

J. B. CHATTERJEA

The present world-wide interest in haemoglobinopathies and allied disorders has given rise to a very considerable literature over the past two decades. This communication reviews this literature in so far as it refers to the Indian subcontinent. The most common abnormality is thalassaemia, which has been discovered in all regions under consideration: India, Pakistan, Nepal, Bhutan and Ceylon. Haemoglobins S, D and E are also quite common: Hb S has been found mostly in the aboriginal tribes, Hb D in Gujaratis and Punjabis and Hb E in Bengalis, Assamese and Nepalese. A few instances of haemoglobins F, H, J, K, L and M have also been reported. However, there remain many population groups to be investigated.

Studies of the distribution of glucose-6-phosphate dehydrogenase deficiency are also reviewed, and the correlation between the various haemoglobin disorders and various environmental factors is discussed, but it is pointed out that the relevant data are still insufficient to allow any definite conclusions to be drawn.

I. INDIA

THALASSAEMIA SYNDROMES

The first instance of thalassaemia in India was recorded by Mukherji (1938) in a Bengali boy. More precise identification of the disorder was not possible then and it is not known whether this boy was suffering from homozygous thalassaemia or Hb E thalassaemia. However, there is no doubt that he was carrying the thalassaemia gene. Later, instances of thalassaemia were recorded from all parts of India, in both Hindus and Muslims. Among the Hindus, it was reported in different races and communities. Increasing awareness of the condition and better diagnostic facilities have brought to light a large number of subjects with thalassaemia in various forms: heterozygous, homozygous, and in combination with various abnormal haemoglobins (S, D, E, H, J and K). In most of these cases, the investigations started with a patient who was suffering from a certain degree of haemolytic anaemia with jaundice and hepatosplenomegaly. The patient proved to be either homozygous for thalassaemia or a double heterozygote for the thalassaemia gene and one or other of the abnormal haemoglobins referred to above (Chatterjea, Saha, Ray & Ghosh, 1956; Chatterjea, 1959, 1965b; Sarkar et al., 1959; Sharma et al., 1963; Parekh, and Sanghvi et al., personal communications).

Up to 1965, 796 cases of thalassaemia syndromes were investigated in the School of Tropical Medicine, Calcutta. The vast majority were suffering from Hb E thalassaemia or homozygous thalassaemia, with a few other types as shown in Table 1. Most (508) were Bengalis. There were 55 Muslims in the series (Swarup, Ghosh & Chatterjea, unpublished observations).

In a series of 508 cases referred to the Indian Cancer Research Centre, Bombay, for haemoglobinopathic disorders, Sanghvi et al. (personal communication, 1965) found 128 cases of thalassaemia major. In
addition, there were 32 cases in which the thalassaemia trait was found in association with other abnormal haemoglobins, giving the following types of double heterozygous states: Hb S thalassaemia (16 cases), Hb D thalassaemia (7), Hb E thalassaemia (5), Hb F thalassaemia (3), Hb J thalassaemia (1). In the Haematology Clinic of the J.J. Group of Hospitals, Bombay, a total of 164 cases of haemoglobinopathic disorders were investigated during the years 1961-65. As many as 157 were suffering from homozgyous thalassaemia; 6 persons had Hb S thalassaemia and one had Hb E thalassaemia (Parekh, personal communication, 1965). Thalassaemia major appeared to be the most frequent haemoglobinopathic disorder in the Indian patients admitted to the hospitals in Singapore and Malaya (Vella, 1962).

Dr S. J. Baker (personal communication, 1965) of Christian Medical College, Vellore, South India, writes, “We do not find as many abnormal haemoglobins here as in the North. We have about 6 cases of thalassaemia per year; about 1 case of sickle-cell anaemia, but this is invariably in somebody from Orissa or other parts of North India; and we have had 1 case of Hb E which came from the Calcutta region.”

An attempt to differentiate between the various forms of thalassaemia was made by Swarup, Ghosh & Chatterjea on the basis of the relative proportions of Hb A, Hb A₂ and Hb F. Blood samples from 204 parents of thalassaemic patients were analysed. They were classified thus: 157 presumably β-thalassaemia; 46 β-δ trait or α-trait; one α or α-δ thalassaemia. A series of 51 subjects homozygous for thalassaemia gave the following possible groupings: 32 β/β; 10 β/δβ or β/α; 6 βδ/βδ or α/βδ; 3 α/β. No α/α subjects were found.

Data on the relative frequency of different types of thalassaemia have not been published from any other part of the country. Analysis of the details of various cases reported and personal communication with different workers indicate that β-thalassaemia is by far the most common type in India.

**Frequency of thalassaemia**

Data on frequency are limited. In a survey of 700 normal Bengalis investigated in Calcutta, the frequency of the thalassaemia trait was 3.7%. The population consisted of medical and para-medical personnel of the School of Tropical Medicine, Calcutta, and the donors to the Central Blood Bank, Calcutta (Chatterjea, Swarup et al., 1957). Discovery of the thalassaemia trait in a Sikh girl resident in Vancouver, Canada, led to a search for similar cases in this community: in a study on 80 unrelated individuals there were 5 instances of thalassaemia trait (Siddoo et al., 1956). In the families of these 5 subjects and of the original propositor, there were 38 instances of thalassaemia trait among 79 individuals tested. Using haematological criteria with-

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**Table 1**

DISTRIBUTION OF 796 CASES OF THALASSAEMIA SYNDROMES IN INDIANS ACCORDING TO RACIAL CHARACTER AND RELIGION

<table>
<thead>
<tr>
<th>Racial group</th>
<th>Hb E thalassaemia</th>
<th>Homozygous thalassaemia</th>
<th>Hb S thalassaemia</th>
<th>Hb D thalassaemia</th>
<th>Hb J thalassaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hindu</td>
<td>Muslim</td>
<td>Hindu</td>
<td>Muslim</td>
<td>Hindu</td>
</tr>
<tr>
<td>Bengali</td>
<td>508</td>
<td>48</td>
<td>175</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Bihari</td>
<td>4</td>
<td>1</td>
<td>5</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Oriya</td>
<td>10</td>
<td>-</td>
<td>2</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Sindhi</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Punjabi</td>
<td>1</td>
<td>-</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>South Indian</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Asamese</td>
<td>2</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>526</td>
<td>49</td>
<td>190</td>
<td>3</td>
<td>12</td>
</tr>
</tbody>
</table>

*Investigated at School of Tropical Medicine, Calcutta.*

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HAEMOGLOBINOPATHIES, G-6-PD DEFICIENCY AND ALLIED PROBLEMS IN INDIA

out any reference to the amount of Hb A₂, Vella (1962) recorded a very high frequency (32%) in a small group of 31 Indian subjects in Khartoum.

As part of a comprehensive WHO research project on the genetic structure of some tribal communities in Andhra Pradesh, Siniscalco and his colleagues (personal communication, 1965) surveyed in January-February 1964 the population from 25 Koya Dora and Konda Reddis villages scattered along and around the Godavari river in the Taluk of Polavaram. A total of 1100 individuals were examined. The findings have not yet been published, but preliminary results indicate an appreciable frequency of thalassaemia trait in both tribal communities.

Mathur and co-workers (1962) carried out a study in Uttar Pradesh. During the one-year period from 1 September 1960 to 31 August 1961, 512 infants and children under 12 years with anaemia (less than 10.0 g haemoglobin per 100 ml blood) were studied at the Sarojini Naidu Hospital, Agra. Of the 512 cases, 410 were from Agra, and the remaining 102 came from the neighbouring districts of Western Uttar Pradesh. The large majority of these children were drawn from families of permanent inhabitants of Uttar Pradesh, but 43 infants and children belonged to Sindhi and 11 to Punjabi families who had migrated from West Pakistan and had settled down in Uttar Pradesh after the country’s partition in 1947; 85.7% were Hindus, 13.1% Muslims, 0.6% Christians and 0.6% Sikhs; 60.0% were male and 40.0% female. Standard techniques were employed for the identification and estimation of Hb F, Hb A₂ and other abnormal haemoglobins. (It appears that Hb A₂ was not estimated quantitatively in all cases.) Abnormalities were found in 8 subjects from 7 families: homozygous thalassaemia (6), heterozygous thalassaemia (1) and heterozygous Hb E (1). Of the six families in which thalassaemia was found, four were Sindhi and two Punjabi. The workers concluded that the frequency of the thalassaemia gene in the native population of Uttar Pradesh is very low or zero. In a later study in Uttar Pradesh, Atal & Mital (personal communication, 1965) screened 97 individuals. They found one case of Hb AE and two of thalassaemia major.

Regarding the distribution of thalassaemia among various castes of central and eastern India, it may be mentioned that of the 13 cases reported by Khandelwal & Solanki (1959) from Nagpur, 7 were in Sindhis and 3 in Marwaris. In the series of 26 cases reported by Udani et al. (1961) from Bombay, 14 belonged to the Lohana community.

In the J.J. Hospital, Bombay, some 80 cases were investigated in the period 1956-63 (Sharma et al., 1963). The regional distribution in these cases was as follows: Saurashtra 26, Maharashtra 21, Sindh 12, Gujarat excluding Saurashtra 9, Goa and adjacent region 9, Bengal 1, Uttar Pradesh 1. Among the 26 cases from Saurashtra, 16 were in Memons and Khojas and 10 in Hindus (mostly Lohanas). One of the cases from Sindh was by an intermarriage between a Sindhi and a Marashtrian. Several cases from Sindhi refugees were from persons who had migrated to Sindh from the Punjab several generations ago and intermarried with Sindhis.

Comment

Thalassaemia in some form or other is the commonest haemoglobinopathy in India. It has been reported from almost all regions and is clearly most widespread. Data on its prevalence are limited. The frequency of the different types of thalassaemia in different parts of the country and in various communities is not yet known.

HAEMOGLOBIN S

The first case of sickle-cell anaemia in an Indian was apparently reported from Cape Town by Berk & Bull (1943). The patient was an anaemic married woman aged 22 years. She was born of Indian parents in Durban and there was no suspicion of any admixture of Negro blood in her family. In the stained smear of her capillary blood, about 10% of cells sickled. In the sealed wet preparation, 15% of cells sickled immediately, 60% after 5 hours and 100% after 24 hours. As family members were not studied, it is not possible to state whether this was a case of homozygous Hb S disease (sickle-cell anaemia) or of Hb S thalassaemia, but there is no doubt that she was harbouring the gene for Hb S. It is also not stated from what particular race or community of Indians she originated.

The credit for the first authentic documentation of the occurrence of sickle-cell gene in Indians must, however, go to Lehmann & Cutbush (1952), who demonstrated the presence of sickle-cell trait among the aboriginal tribes (the Pre-Dravidians) of the Nilgiri hills in South India. Their studies, employing the technique of incubation with 2% sodium metabisulfite solution (Daland & Castle, 1948), showed the following sickle-cell trait frequency: 8.4% among 191 unrelated Badagas, 3.3% among 60 Todas and 30% among 80 unrelated Irulas. They did not find any sickle-cell trait among 434 members of
the non-aboriginal or Dravidian populations (Tamils, Malayalis, Canarese and Telugus) of the region. Büchi (1955) carried out similar studies and confirmed the presence of the sickle-cell trait in the Vedoids of South India.

In a later study, Lehmann & Sukumaran (1956) examined the blood of 146 unrelatedaborigines of South India by the sickling test and electrophoresis. The results of their study, shown in Table 2, confirmed their original finding of the presence of Hb S in varying degrees among the Badagas, Irulas and Tudas, and also demonstrated the presence of Hb S in the Kurumbas.

**TABLE 2**

<table>
<thead>
<tr>
<th>Community</th>
<th>No. examined</th>
<th>Sickling test positive</th>
<th>HbS</th>
<th>Normal adult haemoglobin</th>
<th>Mixture of normal adult and sickle-cell haemoglobins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Badaga</td>
<td>30</td>
<td>2</td>
<td>28</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Irula</td>
<td>18</td>
<td>4</td>
<td>14</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Kotha</td>
<td>22</td>
<td>0</td>
<td>22</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Kurumba</td>
<td>28</td>
<td>7</td>
<td>19</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Toda</td>
<td>50</td>
<td>1</td>
<td>49</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

*Reproduced, by permission, from Lehmann & Sukumaran (1956).*

Dunlop & Mozumder (1952) reported 5 cases of sickle-cell trait and 3 presumptive cases of sickle-cell anaemia (either Hb S disease or Hb S thalassaemia) among the tea-garden labourers of Upper Assam, originating from the tribal population of Orissa and Bihar. For the demonstration of sickling, Dunlop & Mozumder examined sealed specimens of finger blood without additives. These findings were confirmed by subsequent studies (Batabyal & Wilson, 1958; Chatterjea, 1959) in which the sodium metabisulfite method for sickling and electrophoretic analysis of haemoglobin were employed. Batabyal & Wilson (1958) recorded a 15% frequency of sickle-cell trait among 100 Oriahs examined in Assam. Dunlop (personal communication, 1957, cited by Batabyal & Wilson) in a tea-estate practice found a frequency of 29% in a random sample of 100 Griza Oriahs of Assam. Vella & Hart (1959) reported sickle-cell anaemia in an Indian family in Malay. The family originally came from Orissa. Nanda et al. (1965) reported 17 patients with haemolytic anaemia, jaundice and splenomegaly. All of them had high concentrations of Hb S and a small amount of Hb F. They came from the Sambalpur-Bolangir region of Orissa and belonged to the Agharia community.

In Central India, several cases of sickle-cell anaemia were reported in quick succession from Nagpur and surrounding areas (Shukla & Parande, 1956). These reports led to investigations into the local frequency of the sickle-cell trait. A survey was conducted among adult labourers of the Model Mills, Nagpur (Shukla & Solanki, 1958). The people examined were selected at random, except that an effort was made to ensure that no two of them belonged to the same family. The labouring population hailed from different districts within 100 miles (160 km) of Nagpur. In all, 1010 persons were examined. Sickling was demonstrated by the method of Daland & Castle (1948) and the presence of haemoglobin S confirmed by electrophoretic analysis of haemolysates. Most of the 1010 persons examined belonged to the local Marathi-speaking population. Sickling was observed in Mahars, Kunbis and Telis. The other groups—Gond, Koshi, Brahmins, Muslims and a miscellaneous group including various other castes—did not show sickling. In Mahars, the sickling frequency was 22.2% and in Kunbis and Telis 9.4% and 11.3% respectively. Paper-electrophoretic studies in positive sickling cases showed the AS pattern, confirming the presence of sickle-cell haemoglobin in the heterozygous form. The results of this survey are given in Table 3.

Khandelwal & Paitthanking (1961) described 20 cases of sickle-cell anaemia in children. All the cases were Mahar by caste except one, who was a Muslim and whose paternity was doubtful. Twelve cases of sickle-cell anaemia, also in a Mahar community, were reported by Subhedar et al. (1961).

In a recent survey by Roy & Sen (personal communication, 1966), 10 600 tribal people in the Bastar district of Madhya Pradesh and the Koraput district of Orissa were examined for sickling by the metabisulfite method; about 10% appeared to have the sickling trait.

Lele et al. (1962) in their studies on haemoglobinopathies in the Aurangabad region recorded one instance of Hb S thalassaemia in a 9-year-old Muslim boy and two instances of sickle-cell anaemia in one Hindu family of the Mahar community. Their survey of 700 students in a hostel for scheduled castes in Aurangabad revealed the presence of sickle-cell trait in 36. The techniques employed were the meta-
bisulphite method for sickling and a standard procedure for the electrophoretic analysis of haemoglobin.

Sukumaran and co-workers (1956) studied the frequency of sickle-cell trait among some tribes and other groups of Western India. The sickling test was done on the spot, using freshly prepared 2% sodium metabisulphite. Care was taken to read the results within half an hour to avoid false positive reactions. The samples were preserved in ice and were again tested in the laboratory. As an independent check, some of the samples were examined by paper electrophoresis. The results of the sickling test are given in Table 4.

Of 94 samples that showed sickling, 78 were suitable for electrophoresis. All these samples showed mobilities similar to the mixture of Hb A and S used as a control, thus confirming the presence of sickle-cell haemoglobin in heterozygous form. Electrophoresis was also carried out on 191 samples from Dublas that did not show sickling. All showed a pattern similar to Hb A, ruling out the presence of any other abnormal haemoglobin in this sample. The results thus showed a remarkable variation in the prevalence of sickle-cell trait among these tribal groups, varying from practically zero among the Kolis to more than 20% among the Dhodias and Naikas. There was a low prevalence of the R_0 (cDe) genotype among all these tribes.

While investigating the relatives of two cases of Hb S thalassaemia, Parekh (1957) noted that they belonged to a small endogamous community which appeared to have migrated from Saurashtra (Western India) to Palghar on the west coast about 90 km north of Bombay. This community was investigated for the presence of Hb S, using the metabisulphite method for sickling and electrophoretic analysis of haemoglobin (Mital et al., 1962). Initially, a survey of the whole area was carried out. This area consisted of two parts: in the north, Satpatti Aggar, and in the south, Shirgaon Aggar. The total population of Satpatti Aggar was 339 (consisting of 58 families), of whom 325 were investigated. Hb S was detected in 99 persons, i.e., 30.6% of the total examined. Of these persons, 6 had sickle-cell anaemia, 12 sickle-cell thalassaemia and 81 sickle-cell trait. From the above-mentioned 58 families, 125 parents were examined for the presence of Hb S; 38 (30.2%) showed Hb S. It should be noted that in this small endogamous community the possibility of consanguinity could not be excluded. Besides this community, instances of sickle-cell thalassaemia were found in a Mayavanshi Harijan, a Pardeshi from Nasik, a Muslim from Saurashtra, a Maheswari Bhatia and a Vesawa Bhal (Sharma et al. 1963).

In Uttar Pradesh, Bhatia and co-workers (1955) reported 4 cases of sickle-cell trait in a family of 9 members of the Danukh caste in Manipuri District.
Salgia and co-workers (1965) from Indore reported 2 instances of Hb S disorder of which one showed the diagnostic signs of sickle-cell anaemia. A survey of 235 people in Lucknow did not, however, show any instance of sickle-cell trait or of any other haemoglobinopathic trait (Dube et al., 1959). The population was drawn from all parts of Uttar Pradesh; of these 235 persons, 35 were anaemic patients with splenomegaly in whom no cause for splenic enlargement was found. The remaining 200 subjects consisted of doctors, medical students and technicians apparently in good health. Standard electrophoretic techniques were used for the haemoglobin analysis.

While studying the histological changes in the spleens from 500 autopsy examinations conducted in Andhra Medical College, Reddy & Baruah (1964) observed 3 cases of sickling. The first was in a 16-year-old boy, a naval recruit; the second was in a 25-year-old Muslim male and the third in a 24-year-old Christian male from East Godavary District. None of these patients apparently belonged to aboriginal tribes. Reddy and co-workers (1966) recorded 2 instances of Hb S in their necropsy material and one case of sickle-cell anaemia in a scheduled tribe of Andhra Pradesh. They wrote: "It should be no surprise that in and around the districts of Visakhapatnam and Srikakulam bordering Orissa there may be a limited number of families possessing sickle-cell trait in whom in-breeding is common".

In a series of 190 Malayalis obtained from the staff and students of the Armed Forces Medical College, Poona, electrophoretic analysis of haemoglobin did not reveal any Hb S or any other abnormal haemoglobin (Bird et al., 1962). The Malayalis inhabit the State of Kerala in south-western India. Siniscalco and his colleagues in the WHO research project already referred to recorded a high frequency of sickle-cell trait in the tribal communities in Polavaram Taluk of Andhra Pradesh. Preliminary reports indicate that the average frequency of sickle-cell trait for all the tribal populations studied was 15.5%. In male Koya Doras it was 19.4%, whereas in male Hill Reddies it was 9.7% (Meera Khan, personal communication, 1964).

Hb S is extremely rare in Bengalis. In a series of 10 000 blood samples from the Bengali population, there was one instance of Hb S trait in a female subject belonging to the Quiri caste in the Jalpaiguri district, and there was one family with Hb S thalassaemia from Calcutta. Other instances of Hb S reported from the School of Tropical Medicine, Calcutta, during the period when these 10 000 Bengali blood samples were examined were in people hailing from Orissa, Bihar, Andhra Pradesh, Madhya Pradesh, Maharashtra, Mysore, Uttar Pradesh and Rajasthan (Chatterjea et al., 1958; Chatterjea, 1959).

Chaudhuri, Ghosh & Mukherjee (1964) reported the results of their survey on the Santhal tribe in the Midnapore district of West Bengal. Blood samples were drawn from 336 individuals from 119 families in 24 villages, covering a total population of 250 000. The metabisulphite method of sickling and the electrophoretic technique of haemoglobin analysis were employed. Instances of sickle-cell β-thalassaemia were found in 2 families. Blood group study did not show any R₅ (cDe) patterns.

Foy and co-workers (1956) examined 5000 samples from the five racial groups of India (Dravidian, Kolarian, Burmo-Mongol, Tibeto-Mongol and Indo-Iranian). In their very brief note, they reported wide variations of sickle-cell trait within subsections of the same tribes; for example, the Parjah Konachs had a sickle-cell trait rate of 55%, while the Jijmor Konchs had only 3%. As far as can be traced in the available literature, they did not publish any further details regarding their large sample.

Comment

Available data on the distribution of Hb S indicate that this abnormal haemoglobin is particularly common among the tribal populations in different parts of the country. It is, however, not unknown in non-tribal populations. A few cases in Muslims are also on record. This haemoglobin has so far been more extensively searched for than any other abnormal haemoglobin. Adequate data from all parts of the country and from various races and tribal populations have yet to be gathered.

HAEMOGLOBIN D

The first Indian in whom Hb D was detected was a 19-year-old Sikh soldier, from Kararchharwar in the Hoshiarpur district of East Punjab (Bird et al., 1955). In view of the above finding, Bird & Lehmann (1956) examined 109 unrelated Sikhs and found that one of them was heterozygous for Hb A and Hb D. They investigated a further series of 62 unrelated Sikhs and found another instance of Hb D in homozygous form. Thus, in their combined series of 171 cases there were 2 instances of Hb D. In a series of 350 normal subjects recently investigated in Punjab,
there were 4 instances of heterozygous Hb D—3 in the Sikh community and 1 in a Punjabi Hindu (Saha & Banerjee, 1965).

In 326 Indians of the Gujarati community living in Uganda, a frequency of about 1% was reported by Jacob and co-workers (1956) and by Lehmann (1959). In a series of 500 Indians, which included the 326 Indians mentioned above, living in and around Kampala, Uganda, there were 4 instances of Hb D; the population of this series was mostly from the Bombay region, with a few Sikhs and Gujaratis (Raper, 1957). Instances of Hb D trait were also reported from Bombay by Sukumaran & Shah (1962) in a 17-year-old pseudohermaphrodite girl suffering from testicular feminization syndrome, and in her mother and one sibling.

In a survey of 2500 blood samples from unrelated Indian subjects residing in the Federation of Malaya and Singapore, Vella (1959) found 13 instances of Hb D, giving a frequency of 0.5%. Of the above samples, 1700 were derived from healthy blood donors attending the Blood Transfusion Service at the General Hospital, Singapore, and 800 from hospital patients suffering from a variety of disorders, not primarily haematological. No attempt was made to classify samples according to the geographical origin of the donor, religion or language spoken.

In a survey on abnormal haemoglobins in Mysore, 68 random samples of haemoglobin obtained from various parts of the Shimoga district in Mysore State were analysed electrophoretically. An instance of Hb D in heterozygous form was obtained in a 20-year-old Hindu male from Sorab Taluk (Swarup et al., 1959).

In an examination of 10,000 blood samples of Bengalis in Calcutta, Hb D was detected in 16 subjects from 5 unrelated families (Chatterjea, 1965a; Swarup et al.). This group of 16 subjects consisted of Hb D trait (9 cases), Hb D thalassaemia (6) and Hb DE disease (1). The case of Hb DE disease was associated with a hypothyroid condition (Ghose, 1965). In examinations at the School of Tropical Medicine, Calcutta, Hb D had been previously recorded in people from other States—namely, in Punjabis, in a Muslim family from Uttar Pradesh and in an orphan boy possibly of Bihar origin.

Instances of D-thalassaemia were also recorded by Shukla (personal communication, 1957) in a Sikh family; by Ghai et al. (1961) in a Punjabi family; by Sukumaran et al. (1960) in 2 Lohana families, one a Sindhi-Lohana and the other a Gujarati-Lohana; and by Lele et al. (1962) in a Kunbi family in a village 6 miles from Aurangabad.

HAEMOGLOBIN E

The original patient in whom Hb E was first discovered by Itano, Bergren & Sturgeon (1954) was a double heterozygote possessing genes for Hb E and thalassaemia. Hb E in this subject was inherited from the father, who was of Guatemalan origin with Spanish and Hindu ancestry (Sturgeon et al., 1955). Independently, Hb E was reported from Thailand by Chernoff et al. (1954). In 1955, instances of a slow-moving abnormal haemoglobin without any evidence of sickling was discovered for the first time in Bengalis from India; this was identified as Hb E, which had just been discovered (Chatterjea, Saha, Ray & Ghosh, 1956); the relevant results were presented to the VIth International Congress of the International Society of Haematology, held in Boston in 1956. In Bengal, Hb E was found both in Hindus and in Muslims. Subsequent studies indicated the presence of Hb E in Assamese (Chatterjea, 1959), a Jat-Sikh family from Patiala, one Tamil family from Madras, one Punjabi family and a Muslim family from Aurangabad (Swarup et al., 1960a; Lele et al., 1962; Kochhar & Kathpalia, 1963).

Sukumaran and co-workers (1961) reported the first case of Hb E thalassaemia from Bombay, in a Muslim Bohri. It was, however, found that the grandmother came from Bengal. Mathur et al. (1962) in their survey on 512 anaemic children found one instance of Hb E trait in Uttar Pradesh. Sharma and co-workers (1963) from Bombay found two cases of Hb E thalassaemia; one was born of a local Muslim mother and a Pathan father, and the other was a Bhayya from Gondia, Uttar Pradesh.

A large number of cases of Hb E have been described in Bengalis. These include instances of Hb E trait, Hb E disease, Hb E thalassaemia, Hb EK thalassaemia and Hb DE disease (Chatterjea, Saha, Ray & Ghosh, 1957; Swarup et al., 1960a; Chatterjea, 1965a; Chatterjea, 1965b; Swarup et al.). There was one interesting subject who had a combination of Hb E, G-6-PD deficiency and prophyria erythropoietica (Chatterji et al., 1963).

In a survey of 700 normal Bengalis investigated in Calcutta, the Hb E frequency was 3.9% (Chatterjea, Swarup et al., 1957). In Assamese, the Hb E frequency may be similar to or perhaps higher than

that in Bengalis; but available information does not permit of any definitive conclusion on this point. The study of Chaudhuri, Chakravartti et al. (1964) corroborates the presence of Hb E in Bengalis and suggests that a high frequency may exist in certain tribal populations.

The frequency in other parts of the country would appear to be very low. In a series of 2500 blood samples from unrelated Indians (unclassified) residing in the Federation of Malaya and Singapore, already referred to (Vella, 1959), there were 8 instances of Hb E, giving a frequency of 0.3%.

HEREDITARY PERSISTENCE OF FOETAL HAEMOGLOBIN

Sukumaran et al. (1961) reported 13 instances of this variant in heterozygous form. They recorded 3 instances of the doubly heterozygous state showing interaction between the gene for hereditary persistence of Hb F and that for \( \beta \)-thalassaemia (Sanghvi, personal communication, 1965). Two Bengali families with such an interaction were observed in Calcutta as early as 1957 (Swarup & Chatterjea, unpublished observations). A similar interaction was also recorded by Barkhan & Adinolfi (1962) in a family of mixed Indian and Portuguese origin. Parekh and co-workers (1963) described two Indian families, in which this Hb F gene had interacted with \( \beta \)-thalassaemia. Bird and co-workers (1964) reported a further example in a 5½-year-old boy of pure Indian stock.

HAEMOGLOBIN BART'S AND FESSAS & PAPASPYROU

Haemoglobin Bart's (Ager & Lehmann, 1958), a tetramer of apparently normal \( \gamma \) chains, is perhaps identical with the variant described by Fessas & Papaspyrou (1957). Vella (1959) reported 2 instances of Hb Fessas & Papaspyrou in 222 samples of cord blood obtained from Indian women confined in Malaya. In a survey carried out among 278 newborn infants in the General Hospital, Kuala Lumpur, there were 3 instances of Hb Bart's (Lie-Injo Luan Eng & Ti, 1961). In the above two series, there is no information to indicate the part or community of India from which the persons with abnormal haemoglobins had originated. Out of 100 samples of cord blood obtained from a neonatal population born of Bengali parents, there were 4 instances of Hb Bart's (Swarup, Banerji et al., 1965).

HAEMOGLOBIN H

Only one case of Hb H has so far been reported in Indians (Chatterjea, 1961). The propositus, a 19-year-old Bengali Hindu, was admitted for an episode of fever with anaemia. Red cells showed characteristic inclusion bodies of Hb H which according to densitometric estimation of the electrophoretogram accounted for about 15% of the total haemoglobin. One of the parents and one of the 3 brothers examined had thalassaemia trait. Hb H was not demonstrable in the parents and siblings. The subject conformed to the diagnostic criteria for Hb H thalassaemia.

HAEMOGLOBIN J

The first instance of Hb J in an Indian was found (Raper, 1957) in a Gujarati woman during a survey of 326 Indian immigrants residing in Uganda. Sanghvi and co-workers (1958) recorded cases of Hb J in 2 unrelated women belonging to the Gujarati-speaking Lohana community in Bombay. One was an instance of Hb J thalassaemia without any detrimental genetic interaction between genes for Hb J and thalassaemia. It was possibly an example of Hb J (a) with an anomaly in the \( \alpha \)-chain. Subbedar and co-workers (1961) from Nagpur found Hb J in a Harijan family. Vella (1959) in his survey of 2500 Indians in Malaya found Hb J in a 33-year-old male Sikh who originated from the Ludhiana district of Punjab. Swarup and co-workers (1963) reported Hb J in a Bengali family in which there were 2 instances of Hb J trait and 4 instances of Hb J thalassaemia. In the Hb J thalassaemia cases in this Bengali family, formation of Hb A was completely suppressed and the Hb J content varied from 75% to 93%. The composite clinical and haematological features indicated interaction between genes for Hb J and thalassaemia, and indirect evidence suggested that this was possibly an instance of Hb J (\( \beta \)) with an anomaly in the \( \beta \) chain.

HAEMOGLOBIN K

Ager & Lehmann (1957a) were the first to find this haemoglobin variant in two unrelated East Indians in London. In his survey of 2500 Indians in Malaya, Vella (1959) found a mixture of Hb A and Hb K in several members of 4 unrelated families. In the first family, it was found in a mother and her 2 children; the mother, who was adopted when young, had no definite
information on the geographical origin of her family. In the second family, Hb K was found in a Tamil-Muslim father originating from Nagapattinam and his son. In the third family, originating from Mayavaram, Hb K was found in a mother and 4 of her children (Vella & Wells, 1959). The fourth subject was a Tamil-Hindu male blood donor aged 20 years. In August 1959, a Bengali family with Hb K was detected in Calcutta. The family was briefly mentioned in the annual report of the School of Tropical Medicine, Calcutta (Chatterjea, 1961-62). In the same family, there were also instances of Hb E and thalassaemia. In the propositus, there were three abnormalities, namely, Hb E, Hb K and thalassaemia (Swarup et al., 1963).

**HAEMOGLOBIN L**

This abnormal haemoglobin was first found in an Indian (Ager & Lehmann, 1957b). The subject was a Hindu Punjabi, of Khawsthi caste, born in the Maniwal district, Pakistan, an Indian citizen temporarily resident in London. Vella (1959), in his survey on 2500 Indians in Malaya, recorded two instances of Hb L. One was a male blood donor born in Karachi of Sindhi parents. The other was a male Punjabi Sikh. The haemoglobin was identified by paper electrophoresis and ion-exchange resin chromatography. Sukumaran and co-workers (1959) found 8 instances from 3 families of Gujarati-speaking Lohanas in Bombay. No other instance of Hb L appears to have been recorded so far.

**HAEMOGLOBIN M**

Only one family with haemoglobin M has so far been detected at the haematological centre of the School of Tropical Medicine, Calcutta. This was a Punjabi family living in Amritsar. Three samples of blood from three different members of the same family were found to have Hb M levels of 7%, 33% and 50%. As detailed amino-acid analysis was not carried out, it is not possible to specify the type of Hb M. The samples were kindly supplied by Dr Baldev Raj, who was at the time working in the Pathology Department, Amritsar Medical College (Chatterjea, 1961-62).

**GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G-6-PD) DEFICIENCY**

In a retrospective analysis of the literature, evidence for the presence of G-6-PD deficiency can be found in records relating to the use of pamaquine or primaquine as antimalarial agents. British troops were found to be less susceptible to the haemolytic action of pamaquine than were Indian troops (Manifold, 1931; Amy, 1934; Hockwald et al., 1952; Dimson & McMartin, 1946; Smith, 1943; Beutler, 1959). It was reported in the proceedings of the Conference of Medical Specialists (1944) that all cases of haemolysis occurred in Indians and Burmese (Beutler, 1959).

The observation that a high proportion of subjects with Hb E thalassaemia have an unstable erythrocytic reduced glutathione (GSH) level as determined by the glutathione stability test (Swarup et al., 1960b) led to the decision to investigate the frequency of GSH instability and glucose-6-phosphate dehydrogenase (G-6-PD) deficiency in a normal population. Of a series of 22 normal Bengalis examined as controls in the above study, two had a grossly unstable erythrocytic GSH pattern. In an enlarged series of 60 normal Bengalis, 3 had an unstable pattern, as determined by the method of Beutler (1957) (Swarup et al., 1961a, 1961b; Chatterjea & Swarup, 1963).

A series of 82 normal Bengalis, consisting of 56 males and 26 females, were examined for the GSH stability and G-6-PD activity of their red cells by the method of Kornberg & Horekar (1955), as modified by Marks (1958). Marked G-6-PD deficiency was demonstrated in 2 male subjects. An intermediate pattern was observed in 2 female subjects. An interesting picture was found in 5 subjects who had an unstable GSH but normal G-6-PD activity, thus showing that this combination is possible. A similar association of GSH instability with normal G-6-PD activity was also observed in Hb E thalassaemia and other haemolytic syndromes. The GSH instability in such subjects is presumably due to deficiency either of glutathione reductase or of nicotinamide-adenine dinucleotide phosphate (NADP) (Swarup et al., 1961b; Chatterjea et al., 1964).

At the School of Tropical Medicine, Calcutta, 173 normal subjects have so far been examined for G-6-PD deficiency by the assay technique of Marks (1958). The frequency of deficiency in different populations is shown in Table 5. During this period, 15 cases of drug-induced haemolytic anaemia with haemoglobinuria were also investigated. G-6-PD deficiency was found in all of them. These 15 subjects consisted of 10 Bengalis, 2 Punjabis, 2 Muslims and 1 Bihari. A case of drug-induced haemoglobinuria in a G-6-PD-deficient subject (a
25-year-old Bengali boy) was reported by Chatterjee & Dawn (1962).

Baxi and co-workers (1961) examined an unselected sample of 110 individuals (81 males and 29 females, between the ages of 20 and 40) from Bombay. They employed the brilliant cresyl blue coloration test of Motulsky & Campbell-Kraut (1961). G-6-PD deficiency was found in 6 males and 9 females. Their data on the distribution of normal and deficient individuals by community are shown in Table 6.

In a later study, Baxi and co-workers (1963) tested a larger sample from the Parsi community in Bombay. The sample consisted of 100 men and 116 women.

A deficiency was found in 19 (19%) of the men and 15 (12.9%) of the women. There was no association between the enzyme deficiency and ABO or Rhesus blood groups. In this context, it would be pertinent to refer to the origin of the Parsis of Bombay. After the fall of the Zoroastrian Empire of Persia in AD 651, religious persecution forced some Persians to quit their country. After some wanderings, they reached India and were allowed to settle down at and near Surat, Gujarat. Later, Bombay city became one of their main centres. In the early stages, some intermarriage with the local populations might have taken place, but afterwards marriages were contracted exclusively within the community. The Parsis speak Gujarati. Their level of education and standard of living are generally high, and members of the community occupy high positions in the social, economic and intellectual life of the city.

Examination of 132 Indians in Singapore by the Motulsky & Campbell-Kraut technique revealed 4 instances of deficient subjects (3.3%) (Vella, 1961). Details of the origin or racial stock of these subjects were not stated.

A frequency of 6% was recorded by Motulsky and co-workers (1960).

Baxi and co-workers (1964) investigated the role of G-6-PD in neonatal jaundice and haemolytic syndromes. The first group consisted of 20 neonates developing jaundice within from 12 hours to 7 days; G-6-PD deficiency was detected in 6 of these. The ethnic background of these cases was as follows: Hindu Punjabi Kshatriya 2, Sindhi Lohana 1, Cutchhi Lohana 1, Parsi 1 and Marathi-speaking Muslim 1. The second group consisted of 36 subjects with a history of haemolytic anaemia; in two subjects with haemoglobinuria, the enzyme was deficient; both of these belonged to the Parsi community.

Mital (personal communication, 1965) from Agra, Uttar Pradesh, examined 162 healthy individuals for glutathione stability using the method of Beutler (1957). The series consisted of doctors, students, technical staff of the Medical College, Agra, and blood donors. All 103 males and 59 females came from Uttar Pradesh. The erythrocytic GSH was unstable in 12 persons (7.4%).

Siniscalco and co-workers (personal communication, 1965) reported an appreciable frequency of G-6-PD deficiency (the exact figure is not yet available) among the tribal communities scattered along and around the Godavari river in the Taluk of Polavaram in Andhra Pradesh. Preliminary results

### Table 5

<table>
<thead>
<tr>
<th>Origin</th>
<th>Number examined</th>
<th>Number showing deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bengali</td>
<td>103</td>
<td>4</td>
</tr>
<tr>
<td>Nepali</td>
<td>25</td>
<td>2</td>
</tr>
<tr>
<td>Uttar Pradeshi and Bihari</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>Muslim</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>Christian</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Punjabi</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Marathi</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>South Indian</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>173</td>
<td>8</td>
</tr>
</tbody>
</table>

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### Table 6

<table>
<thead>
<tr>
<th>Community</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Deficient</td>
</tr>
<tr>
<td>Maharasthra: Hindu</td>
<td>35</td>
<td>0</td>
</tr>
<tr>
<td>Konkan: Hindu—Saraswat</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Parsi</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Christian</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Others</td>
<td>21</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>81</td>
<td>6</td>
</tr>
</tbody>
</table>

<sup>a Reproduced, by permission, from Baxi et al. (1961).</sup>
for part of the above population have already been published (Meera Khan, 1964) and are summarized in Table 7. The results indicate the presence of G-6-PD deficiency in the Godavari valley in the Eastern Ghats of India.

**TABLE 7**

RESULTS OF DYE-DECOLORATION TEST IN TRIBAL AND NON-TRIBAL POPULATIONS IN POLAVARAM

<table>
<thead>
<tr>
<th>Population</th>
<th>Normal</th>
<th>Partially deficient</th>
<th>Partially deficient or deficient</th>
<th>Deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-tribal</td>
<td>238</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Tribal of Thal-lavaram area</td>
<td>115</td>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tribal living in other areas</td>
<td>90</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>


Charlton & Bothwell (1961) examined the blood of 100 Indians resident in Natal, Africa, employing the brilliant cresyl blue decoloration test. These Indians were mostly descendants of immigrants from Madras. None showed any G-6-PD deficiency.

No definitive information is yet available about the type of G-6-PD deficiency. Preliminary observations by Swarup, Ghosh & Chatterjea (unpublished data) indicate that both caucasic and negroid types are found in Bengalis. The preliminary results published by Meera Khan (1964) also indicate the presence of both types of deficiency.

**Comment**

Studies so far carried out indicate that erythrocytic G-6-PD deficiency is present in Indians. Its prevalence in different races and communities varies widely. The highest frequency, about 15%, has been recorded in Paris. The frequency in Bengalis seems to be about 4%. In certain tribal populations of Polavaram in Andhra Pradesh, the frequency appears to be high.

**SOME NOTES ON THE INTER-RELATIONSHIP OF HAEMOGLOBINOPATHY, G-6-PD DEFICIENCY AND CERTAIN ENVIRONMENTAL FACTORS IN INDIA**

**Haemoglobinopathy and malaria**

Interest in the study of a possible relationship between malaria and haemoglobinopathy was aroused by the pioneer observations of Allison (1954), who demonstrated that the majority (13) of 15 subjects with sickle-cell trait studied by him were resistant to induced *Plasmodium falciparum* infection, while in the control group 14 out of 15 showed no resistance. Subsequent studies confirmed that persons with sickle-cell trait are in general resistant to *P. falciparum* infection (Raper, 1955; Edington & Lehmann, 1956); they did not, however, show similar resistance to *P. malariae* (Allison, 1954; Colbourne & Edington, 1956).

The information available on the relationship between sickle-cell trait and *P. vivax* infection is scanty and inconclusive. Boyd & Stratman-Thomas (1933) observed that Negroes in the United States of America were somewhat more resistant to induced *P. vivax* infection than white people. The concept of haemoglobinopathy was unknown at that time, and no attempt was made to link up this resistance with Hb S trait.

In a study on the relationship between malaria and haemoglobinopathy, it was observed that an Hb E thalassaemia subject was resistant to induced *P. vivax* infection (Chatterjea, Saha, Ray & Chaudhuri, 1956). No such resistance was, however, demonstrable with induced *P. falciparum* infection (Chatterjea, 1959). Further observations on resistance to *P. vivax* infection in a larger series of Hb E thalassaemia cases along with a few other haemoglobinopathic disorders were made by Ray, Chatterjea & Chaudhuri (1964). Results of the study are shown in Table 8. In a series of 11 patients with Hb E thalassaemia, only 2 could be infected, although most of these subjects were very anaemic and in poor general condition; while in the control

**TABLE 8**

RESULTS OF INOCULATION WITH MALARIAL BLOOD (*P. VIVAX*)

<table>
<thead>
<tr>
<th>Haemoglobinopathy</th>
<th>Total No.</th>
<th>Malaria infection induced</th>
<th>No malaria infection induced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb E thalassaemia</td>
<td>11</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Homozygous thalassaemia</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Sickle-cell anaemia</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Hb S disease</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Thalassaemia trait</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Homozygous E disease</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Hb E trait</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Controls</td>
<td>14</td>
<td>13</td>
<td>1</td>
</tr>
</tbody>
</table>
series 13 out of 14 were readily infected. Such infection could not be induced even in a splenectomized child with Hb E thalassaemia. Similar resistance was seen in homozygous thalassaemia and possibly also in thalassaemia trait, but not in subjects with haemoglobin E trait or homozygous Hb E disease. It is further interesting to note that the Hb E thalassaemia subjects were resistant to *P. vivax* but not to *P. falciparum*. The observed resistance to *P. vivax* could not be correlated with any of the following parameters: Hb F content, reticulocyte count, G-6-PD activity or stability of reduced glutathione. It should, however, be mentioned that in a similar study from Thailand, both Hb-E-trait adults and Hb-E-thalassaemia children developed infection with *P. vivax* (Kruatrachue et al., 1961). In a survey of malarial infection in relation to the distribution of Hb E in various age-groups in a malarious area in Thailand, subjects with Hb A and Hb E were found to be equally susceptible to malaria (Kruatrachue et al., 1962).

The resistance of Indian subjects to *P. vivax* and African subjects to *P. falciparum* may be ascribed to the fact that malarial parasites in general do not thrive well in haemoglobinopathic red cells. Well-documented data on the resistance of Africans to *P. vivax* are not available. The relatively scanty data on *P. falciparum* infection in Indian subjects do not completely exclude the possibility of some resistance to this species in addition to the striking resistance to *P. vivax*.

The mechanism of the resistance shown by Hb-E thalassaemia subjects from India is, however, not clear. There is no direct evidence to show that the haemoglobin E gene *per se* has any protective role. It is possible that the thalassaemia gene contributes to the observed resistance. It may be mentioned in this connexion that in the recent study from Thailand (Kruatrachue et al., 1962), G-6-PD deficiency could not always be correlated with resistance to malarial infection.

It would appear on the whole that resistance to malarial infection must involve other factors besides abnormal haemoglobin and G-6-PD deficiency. Only a minority of American Negroes have these traits, but it is generally agreed that many more of them show after inoculation a considerable resistance to several species of malarial parasites, including *P. vivax* and *P. knowlesi*. The differential susceptibility of American Negroes and American whites who had no previous exposure to the disease suggests that other factors, probably under complex or polygenic control, are involved (Livingstone, 1961). In this context, the differential susceptibility of Indian and Thai subjects may be due to other associated factors not directly related to haemoglobin variants.

Caminoportes (1938) observed that in a Mediterranean anaemia subject who had a coincidental malarial infection there was improvement of anaemia. Impressed with this observation, he inoculated seven such patients with malaria and reported that it had a beneficial effect on the anaemia. In the study reported above no such beneficial effect was observed. On the contrary, malarial infection led to clinical and haematological deterioration.

**Thalassaemia, Hb E and iron deficiency**

To illustrate the theory of balanced polymorphism as a genetic equilibrator in a population, Haldane (1949) suggested that the thalassaemia trait may offer resistance to the development of iron deficiency. This theory received support from Sijpstei*1955*, who wrote: "It is surmised that mild chronic anaemia that results from thalassaemia minor may give rise to an increased absorption of iron. It is pointed out that this might be of great importance to the women in a community where a high infant mortality rate has to be compensated for by a large number of births." To test the validity of this hypothesis, 120 subjects suffering from varying degrees of iron-deficiency anaemia were examined in order to determine the incidence of the haemoglobinopathic trait in them as compared with the incidence in the general population (Chatterjea, 1964). The relevant data are shown in Table 9.

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Thalassaemia trait (%)</th>
<th>Hb E trait (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron-deficiency anaemia</td>
<td>120</td>
<td>4.4</td>
<td>5.3</td>
</tr>
<tr>
<td>Normal anaemia</td>
<td>700</td>
<td>3.7</td>
<td>3.9</td>
</tr>
</tbody>
</table>

In the general Bengali population, 7.6% had a haemoglobinopathic trait—3.7% thalassaemia trait and 3.9% Hb E trait (Chatterjea, Swarup et al., 1957). In the iron-deficiency group, 9.7% showed a haemoglobinopathic trait—4.4% thalassaemia trait and 5.3% Hb E trait. The degree of anaemia did not appear to bear any relationship to the haemoglobinopathic...
pathic traits. It should also be pointed out that Sijpestein's hypothesis, based on the assumption that iron absorption is increased in subjects with thalassaemia trait, is not borne out by recent studies on iron absorption (Bannerman, 1961; Erlandson et al., 1962).

To find out whether iron deficiency per se produces any significant change in the proportion of Hb A₂ and Hb F, 91 cases of iron-deficiency anaemia were investigated. The results are shown in Table 10; there was no significant alteration in either of these components (Chatterjea, 1964).

### TABLE 10

<table>
<thead>
<tr>
<th>Hb A₂ AND Hb F IN IRON-DEFICIENCY ANAEMIA</th>
<th>Hb A₂ (%)</th>
<th>Hb F (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>Normal (20)</td>
<td>3.1</td>
<td>1.5-5.2</td>
</tr>
<tr>
<td>Thalassaemia trait (63)</td>
<td>13.5</td>
<td>3.5-24.2</td>
</tr>
<tr>
<td>Iron deficiency (91)</td>
<td>3.8</td>
<td>0.5-6.0</td>
</tr>
</tbody>
</table>

**Effect of iron deficiency on the production of haemoglobin E**

In subjects with Hb E trait associated with iron deficiency, the proportion of Hb E was found to be considerably lower than that ordinarily found in a normal case of Hb E trait (Swarup et al., 1964a). With an Hb level of about 4.0 g per 100 ml of blood, the proportion of Hb E was only about 15%. Following iron therapy, the proportion of Hb E increased as the Hb level improved. When the Hb level rose to 13.0 g per 100 ml of blood, the Hb E content was between 35% and 45%. It appeared that iron deficiency had more effect in decreasing the production of Hb E than that of Hb A.

**G-6-PD deficiency and iron deficiency**

In a group of 20 Bengalis with iron deficiency, low G-6-PD was found in only one instance. Although this was only a small series, the results suggest that the incidence of G-6-PD deficiency in iron-deficient subjects is not different from that in the normal Bengali population (Chatterjea, 1964).

**Erythrocytic G-6-PD deficiency and stability of reduced glutathione (GHS) in haemoglobin E and thalassaemia**

Simultaneous estimation of GSH stability and G-6-PD activity in thalassaemia trait, Hb E trait and Hb E thalassaemia provided interesting data which may be briefly summarized.

Deficiency of the erythrocytic enzyme G-6-PD leads to an instability of erythrocytic GSH. In the series investigated at the School of Tropical Medicine, Calcutta, the frequency of G-6-PD deficiency was as follows: normal 4.0%, Hb E trait 20%, thalassaemia trait 20%. All these patients had instability of erythrocytic GSH. An interesting feature was that in all these groups, a proportion had unstable GSH with normal activities of G-6-PD and glutathione reductase. In Hb E thalassaemia, 29.6% had unstable GSH; in 7.8% the instability could be explained on the basis of G-6-PD deficiency; in the remaining 21.8% the instability remained unexplained; deficiency of glutathione reductase was involved in an occasional case only (Swarup et al., 1963; Swarup et al., 1962; Chatterjea et al., 1963). In Hb E thalassaemia, methaemoglobin levels were elevated in 14 out of 15 subjects, elevation being marked in 8 cases. In 7 of these 8 cases there was also an instability of erythrocytic GSH, indicating either a defect in the NADP-linked methaemoglobin reductase system or unavailability of reduced NADP. In view of normal G-6-PD and glutathione reductase activities, it is possible that a graded deficiency of NADP is the basic cause of GSH instability. Thus, a mild deficiency of NADP would result only in slight elevation of methaemoglobin, GSH remaining stable. In severe deficiency of NADP, elevation of methaemoglobin is pronounced and GSH also becomes unstable (Swarup et al., 1964b).

Regarding the significance of these findings, it should be mentioned that the pattern of GSH instability with normal amounts of G-6-PD and glutathione reductase is by no means only found in Hb E thalassaemia. A similar pattern has since been noted in hereditary spherocytosis, porphyria erythropoietica, nutritional macrocytic anaemia and autoimmune haemolytic anaemia. It is possible that GSH instability due to NADP deficiency is but a non-specific reflection of unstable red cells whose life span has been shortened by a variety of unfavourable genetic or environmental influences. In Hb E thalassaemia, this instability may thus be secondary to the known anomaly in globin synthesis. In fact, GSH is stable in 70.4% of the subjects and the observed instability has no significant correlation with the severity of the anaemia. Therefore, at the present state of our knowledge we should keep an open mind and wait for more detailed information on the relevant intra-erythrocytic metabolic cycles. A coherent
theory of the evolution and biogenesis of the polypeptide chains and relevant enzyme systems must emerge before we can hope to explain these findings.

Defective generation of thromboplasts in Hb E thalassaemia and Hb E disease

In a large proportion of subjects with Hb E thalassaemia, thromboplastin generation as assessed by the method of Biggs and Douglas was grossly defective. The poor generation of thromboplastin was associated with a defect of serum in all cases; an associated plasma defect was also present in a proportion of the cases. A similar abnormality was also found in Hb E disease (Basu et al., 1965, 1966).

The observed abnormality could be detected in one of the parents. The abnormality in thromboplastin generation could not, however, be correlated with any other clinical, haematological or biological parameter. The absence of any significant bleeding manifestation in the presence of such a gross abnormality in the generation of thromboplastin presents an intriguing situation. It was further interesting to note that prothrombin consumption was normal in all such cases. These findings suggest that the cause of the abnormality is to be found in the in vitro generation of thromboplastin as estimated under the test conditions. The adequacy of the prothrombin consumption may indicate that thromboplastin generation, through poor during the first six minutes of examination, is ultimately adequate.

II. NEPAL ¹

THALASSAEMIA

The thalassaemia syndrome is occasionally encountered by clinicians in Nepalese Gurkha subjects, but there are only a few reports of such cases in the literature. Brain & Vella (1958) reported an instance of Hb H-α thalassaemia in a Nepalese woman aged 24. A second case of a similar nature was described, in a pregnant Gurkha woman in Malaya (Weatherall & Vella, 1960).

An apparent case of thalassaemia in homozygous form was recorded in a female Gurkha child by Weatherall & Vella (1960). Family studies revealed increased amounts of Hb A₂ in the parents and siblings. Red cell morphology in the parents was compatible with thalassaemia trait. This family provided evidence for the presence of β thalassaemia.

In 134 Nepalese examined in Calcutta, 13.4% appeared, from composite haematological data and/or Hb A₂ level, to be thalassaemia-variant carriers (Swarup, Ghosh & Chatterjea, unpublished observations). Some were professional blood donors; the possibility of iron deficiency partly contributing to this apparently high frequency cannot therefore be excluded.

As part of an investigation into the health of the Sherpas undertaken during the course of the British Shola Khuma Expedition in the spring of 1959, venous blood samples were obtained from 129 inhabitants of Khumbu villages out of an estimated population of 2500. All samples were examined for haemoglobin variants by standard techniques (Jackson, Lehmann & Sharir, 1960). One case of β-thalassaemia trait was found. It should be noted that the Sherpas are racially quite distinct from the Gurkhas. The Sherpas live on the southern watershed of the main Himalayan range in north-eastern Nepal in villages lying mostly at altitudes between 10 000 ft and 12 000 ft (about 3000 and 3600 m). They are of pure Tibetan ancestry.

ABNORMAL HAEMOGLOBINS

Haemoglobin E

The search for this abnormal haemoglobin in 199 Nepalese soldiers and later in many others gave no positive result (Aksoy et al., 1955; Lehmann, 1959). Vella (1962), however, reported 3 instances of Hb E in a series of 557 Gurkhas examined in Singapore. In a series of 109 Nepalese examined in Calcutta there was one instance of Hb E trait (Chatterjea, 1959).

Haemoglobin H

Two instances of Hb H in heterozygous form, along with thalassaemia trait, were reported in Gurkha women examined in Singapore by Brain & Vella (1958).

G-6-PD DEFICIENCY

A series of 30 Nepalese males selected at random from the professional donors to the Central Blood Bank, Calcutta, were examined for erythrocytic GSH

¹ Most studies have been carried out on Nepalese temporarily resident outside Nepal.
stability according to the method of Beutler (1957) and for G-6-PD activity according to the assay procedure of Marks (1958). In 3 subjects, G-6-PD was deficient and GSH unstable (Swarup, Ghosh & Chatterjea, unpublished observations). Vella (1961) in his examination of 14 Nepalese blood samples in Singapore did not, however, find any instance of such deficiency.

III. BHUTAN

In the large series of haemoglobinopathic disorders examined in the School of Tropical Medicine, Calcutta, there was one instance of homozygous thalassaemia in a Bhutani child. The available literature contains no other reference regarding the presence of abnormal haemoglobin or G-6-PD deficiency in Bhutan.

IV. PAKISTAN

THALASSAEMIA AND HAEMOGLOBINOPATHY

The available literature contains few references to Pakistan in this connexion. Khaleque (1961), from Dacca, described a Muslim family of East Pakistan in which 2 children out of 10 siblings were suffering from typical Hb E thalassaemia with Hb E and Hb F. The father showed Hb E trait and the mother had thalassaemia trait with an increased level of Hb A2.

It should be noted that the Muslims in Bengal and in East Pakistan belong to a common racial stock. As already mentioned in the review of haemoglobinopathic patterns in Bengal, the series investigated at the School of Tropical Medicine, Calcutta, included a large number of Muslims. A total of 424 unrelated Muslims were investigated. They included patients referred to the haematology clinic for refractory anaemia and splenomegaly as well as some apparently normal subjects. Some of these patients were residents and citizens of East Pakistan who came to Calcutta for treatment and in some cases the parents were still residents and/or citizens of Pakistan. In this series there were 50 typical instances of Hb E thalassaemia and 25 instances of Hb E trait (Swarup, Ghosh & Chatterjea, 1958, unpublished observations). Hb E was also reported by Vella (1963) in East Pakistanis in Malaya.

Thus the Muslims in East Pakistan certainly have both thalassaemia and Hb E.

Regarding Hb S, there are a few reports relating to its presence in the Muslim population of India. These have already been referred to earlier in the account of Hb S in India, and will be briefly summarized here. Khandelwal & Paithankar (1961) found one case of sickle-cell anaemia in a study of 20 Muslims. Lele et al. (1962) recorded one instance of sickle-cell anaemia in a 9-year-old Muslim boy of Aurangabad. Sharma et al. (1963) reported one case of Hb S thalassaemia in a Muslim from Saurashtra. Shukla & Solanki (1958) in their survey on 68 Muslims and Sukumaran and co-workers (1956) in their miscellaneous group which included some Muslims did not, however, find any evidence of sickling.

Vella (1959) in his survey of 2500 Indians in Malaya found instances of Hb E trait in a Tamil-Muslim father originating from Nagapatnam and in his son. A survey was recently conducted in North-Western Pakistan among Pathans. The study was organized by Dr M. A. Stern of Oxford University and the samples were examined by Lehmann and Kynoch in Cambridge. A total of 130 samples were examined. Seven showed an elevated level of Hb A2 (β-thalassaemia) and Hb D Punjab was found in one (Lehmann, personal communication, 1965).

Lehmann and co-workers (1961) reported sickle-cell trait in a Pathan. Later, Bolton and co-workers (1964) examined the blood of 4 Brahis, 6 Sindis, 9 Baluchis, 18 Pathans and 18 men of miscellaneous origins attending the out-patient department of the Church Missionary Society Hospital, Quetta. Paper electrophoresis at pH 8.6 and pH 8.9 gave evidence of the following abnormal haemoglobins: Hb A and Hb J in one Sindi and two Pathans; Hb A and Hb D in one Brahui and two Baluchis; and possibly Hb J and Hb D in one Pathan. Sickling tests were negative and Hb A2 was within the normal range.

G-6-PD DEFICIENCY

In 17 Muslims examined in the School of Tropical Medicine, Calcutta, there was one instance of G-6-PD
deficiency (see Table 5). In a parallel series of 15 cases of haemoglobinuria examined at the above centre, there were 2 Muslims who showed significant enzyme deficiency (Swarup, Ghosh & Chatterjea, 1962, unpublished observations). Among the above-mentioned 130 Pathans in North-Western Pakistan there were 9 instances of G-6-PD deficiency.

In an examination of 196 apparently healthy persons (112 male and 84 female) in Karachi, partial deficiency was found in 6 cases and gross deficiency in 4 (Salim, 1966).

V. CEYLON

THALASSAEMIA

De Silva & Weeratunge (1951) were apparently the first to report thalassaemia in Sinhalese children. Thalassaemia, either in homozygous form or in combination with Hb E, appears to be the commonest type of haemoglobinopathy in Ceylon. In a series of 36 cases of such disorder investigated with appropriate techniques by De Silva and co-workers (1959), there were 9 instances of thalassaemia major and 11 instances of Hb E thalassaemia. All the cases of homozygous thalassaemia were in Sinhalese, while two of the families with Hb E thalassaemia were Muslims of mixed Moorish descent. The thalassaemia appears in general to be of the β type (De Silva, personal communication, 1965), though one family with α-thalassaemia has been found (Nagaratnam, to be published; cited by De Silva). No other data relating to the prevalence or distribution of thalassaemia in different parts of the country or in different communities are available.

ABNORMAL HAEMOGLOBINS

Haemoglobin E

Graff et al. (1955) were the first to report Hb E from Ceylon. They examined blood samples from 9 pure or almost pure Veddas, 2 of whom showed Hb E in heterozygous form. Their studies on the basis of blood grouping and typing studies further indicate that these Veddas are related to the Todas and Kotas of South India rather than to the Veddoids of India. Later studies in Veddas suggest a variable frequency of Hb E, from 4% to 10% (Lehmann, 1956; De Silva et al., 1959). Sporadic instances have been reported in Sinhalese subjects (Nagaratnam et al., 1958). Vella (1963) reported instances of Hb E in Sinhalese in Malaya.

De Silva and co-workers (1959) reported 11 cases with Hb E thalassaemia. They came from 4 Sinhalese and 2 Muslim families and comprised 8 females and 3 males. It is possible that the Sinhalese originally migrated from Bengal (India) 2500 years ago and that the Indian Muslims also came from Bengal. No Hb E has so far been found in the Tamils in Ceylon (De Silva, personal communication, 1965).

Haemoglobin S

Wickremasinghe and co-workers (cited by De Silva et al., 1962) reported Hb S in three unrelated families in a village in the Eastern Province. In another village approximately 30 miles (50 km) away, De Silva and co-workers (1962) found a Sinhalese family with Hb S. The propositus, a 7-year-old girl with a moderately severe degree of anaemia and 44.4% of Hb S, was possibly an instance of Hb S thalassaemia, Hb S being inherited from the father and thalassaemia from the mother. In three generations of this family of which 17 members were investigated, there were 8 instances of sickle-cell trait, 5 instances of thalassaemia trait and 1 instance of Hb E trait. It is of interest to note that the two villages in question are situated near an old military fort which had accommodated Negro soldiers since the sixteenth century. It should also be mentioned that since 1955, De Silva and his colleagues had been investigating all suspected cases of haemoglobinopathy with standard techniques of electrophoresis, column chromatography, sickling test and alkali denaturation test. So far this was the only instance where Hb S was found.

Wickeremasinghe & Ponnuswamy (cited by De Silva et al., 1962) investigated over 2000 specimens of blood from Sinhalese without finding any Hb S. Sickling tests carried out on over 150 specimens of blood obtained from a random selection of cases of all types of anaemia attending the Paediatric Department of the University of Ceylon gave no positive findings either (De Silva et al., 1962).

G-6-PD DEFICIENCY

No published account is available for reference. De Silva (personal communication, 1965) screened 56 samples of blood from cases of neonatal jaundice with negative results.
VI. CONCLUSIONS

The Indian subcontinent is a rich reservoir of thalassaemia and various abnormal haemoglobins. Several abnormal haemoglobins and some types of genetic interactions were first reported in Indians. The many genetic variations and different endogamous populations which still exist here provide wide scope for intensive exploration and close study from different aspects.

Present data on the distribution of abnormal haemoglobins and G-6-PD deficiency are derived from work carried out in a few centres. Many parts of the country and many communities remain unstudied. Available data on possible correlations between these genetic disorders and environmental factors are too inadequate to permit any definite conclusion. Results of the studies in Calcutta (Ray, Chatterjea & Chaudhuri, 1964) indicate that in Hb E thalassaemia, there is significant resistance to induced infection with Plasmodium vivax.

It is therefore suggested that:

1. Future studies should aim at gathering definite information on the incidence of thalassaemia, abnormal haemoglobins and G-6-PD deficiency in different parts of the country and in various castes and communities. Particular emphasis should be laid on carrying out investigations in areas and communities not yet covered by previous studies. Rural populations, particularly the endogamous groups, need to be investigated.

2. The research design must be based on standard statistical principles. Diagnostic criteria should be firmly laid down and uniformly followed. Care should be taken to differentiate the thalassaemia trait from iron deficiency which is so common in India, particularly in women. Quantitation of Hb A₂ is essential for this purpose, and in some instances it may be necessary to estimate the serum iron level as well. The electrophoretic method should be standardized so that abnormal haemoglobins separate as clearly as possible. In field studies, G-6-PD may be estimated with the dye decoloration technique of Motulsky & Campbell-Kraut (1961) following the standard criteria of interpretation. There should be facilities for checking doubtful results by assay of G-6-PD according to the method of Kornberg & Horekar (1955) as modified by Marks (1958).

3. In addition to investigations in the general population, the following sectional populations may be conveniently studied and analysed according to their ethnic background: (a) schoolchildren; (b) industrial workers, (c) blood donors, (d) hospital in- and out-patients.

4. For rapid collection of essential data, there should be at least four fully equipped regional centres. Each centre should have mobile units for collecting blood samples and for making the necessary field examinations on the spot.

5. For correlative studies and elucidation of relevant problems in population genetics, a thorough examination of the subjects concerned must be made for any associated disorder, overt or occult. In view of the claims already made for the influence of malaria on natural selection, it would be worthwhile to carry out investigations in populations which differ widely in their malaria experience. The possibility of any relationship between these genetic parameters and other common microbial infections like smallpox, cholera, typhoid fever or parasitic infestations like hookworm may be considered.

6. For successful completion of any programme, it will essential to provide additional staff, equipment, chemicals, transport facilities and incidental expenses.

7. In view of the increasing awareness of the problems related to haemoglobinopathic disorders and in view of the limited facilities available for their diagnosis and identification, there is a need for the organization of training programmes.

RÉSUMÉ

Les hémoglobinopathies et les troubles qui leur sont apparentés ont été l'objet de très nombreuses publications au cours des deux dernières décennies. L'auteur se livre, dans cet article, à une revue critique des informations disponibles sur la distribution de ces groupes de maladies dans la péninsule indienne, où les anomalies hémoglobiniques — dont plusieurs types ont été décrits pour la première fois chez des Indiens — et la thalassémie sont particulièrement répandues.

La thalassémie est le trouble le plus fréquemment
Bird, E. H. Beutler, 854

Baxi, A. J., Baxi, P. & Barkhan, J. N. & Batabyal, A.
decrite et du

Bhoutan et a Ceylan. Les hemoglobines anormales le

A. C. signalees:

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D. de

les races et les collectivites, existe sous ses deux types, caucasolde et negroide. Les donnees actuellement dispo-
nibles sur des correlations possibles entre les hemo-
globinopathies et divers facteurs de milieu n'autorisent aucune conclusion valable.

REFERENCES


Beutler, E. (1959) Blood, 14, 103


Bolton, J. P., Harrison, B. D. W., Lehmann, H. & Peal, J. (1964) Man, 64, 113

Brain, M. C. & Vella, F. (1958) Lancet, 1, 192


observé; on l’a décrite en Inde, au Pakistan, au Népal, au Bhoutan et à Ceylan. Les hémoglobines anormales le

plus souvent rencontrées sont Hb S, Hb D et Hb E. C’est
dans les tribus aborigènes du Gujerat et du Pendjab
que l’on a constaté le plus grand nombre de cas d’hémoglo-
binose S, tandis que dans celles du Bengale, de

l’Assam et du Népal les hémoglobines E sont très
répandues. Des hémoglobines rares ont également été
signalées: Hb F, Hb H, Hb J, Hb K, Hb L, Hb M. Les

nombreuses variations génétiques et l’existence au sein
de la population indienne de diverses collectivités endo-
games offrent un vaste champ d’exploration et d’étude
approfondie de ces troubles.

Une enquête a montré dans des cas de thalassémie
assocée à une hémoglobine E chez les Indiens une
résistance notable à l’infection provoquée à Plasmodium
vivax. Dans certaines populations, un pourcentage
important des individus présente une carence des érythro-
cytes en glucose-6-phosphate déshydrégénase. Cette
anomalie génétique, dont la distribution varie suivant
les races et les collectivités, existe sous ses deux types,
caucasolde et négroïde. Les données actuellement dispo-
nibles sur des corrélations possibles entre les hémoglobi-
nopathies et divers facteurs de milieu n’autorisent aucune
conclusion valable.
Chaudhuri, K. C. (1947) Indian J. Pediat., 14, 76
De Silva, C. C. & Weeratunegee, C. E. S. (1951) Arch. Dis. Childh., 26, 224
Ghai, O. P. (1958) Indian J. Child Hlth, 7, 364
Ghose, S. (1965) In: Proceedings of the 155th Clinical Meeting of the School of Tropical Medicine, Calcutta
Haldane, J. B. S. (1949) Ricerca sci., Suppl., p. 75
Lehmann, H. & Sukumaran, P. K. (1956) Man, 56, 95
Marks, P. A. (1958) Science, 127, 1338
Meera Khan, P. (1964) J. Genet, 59, 14
Mukherji, M. (1938) Indian J. Pediat., 5, 1
Patel, D. & Bhende, Y. M. (1939) Indian J. Pediat., 6, 217
Pirzada, M. & Kapur, P. N. (1951) Indian med. Gaz., 86, 150
Sijpestein, J. A. K. (1955) Enige vormen van erelijke nietspheroeytafra anaemia bij kinderen, Groningen, Oppenheimm
Vella, F. (1958) Indian J. med. Sci., 12, 290
Vella, F. (1961) Experientia (Basel), 17, 181
Vella, F. (1962) Oceanica, 32, 219