

This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization.

WORLD HEALTH ORGANIZATION
TECHNICAL REPORT SERIES

No. 338

HAEMOGLOBINOPATHIES AND ALLIED DISORDERS

Report of a WHO Scientific Group

WORLD HEALTH ORGANIZATION

GENEVA

1966

WHO SCIENTIFIC GROUP ON
HAEMOGLOBINOPATHIES AND ALLIED DISORDERS

Geneva, 14-20 December 1965

Members :

Dr T. Arends, Department of Experimental Haematology, Venezuelan Institute for Scientific Investigations, Caracas, Venezuela

Dr E. Beutler, Chairman, Division of Medicine, City of Hope Medical Centre, Duarte, Calif., USA (*Rapporteur*)

Dr R. Cabannes, Blood Transfusion and Haematology Centre, Purpan Hospital, Toulouse, France

Professor K. Choremis, Director, St Sophie's Children's Hospital, Athens, Greece (*Vice-Chairman*)

Dr H. Lehmann, Abnormal Haemoglobin Research Unit, Medical Research Council, Department of Biochemistry, Cambridge, England (*Chairman*)

Professor N. Taleb, French Faculty of Medicine and Pharmacy, St Joseph's University, Beirut, Lebanon

Dr S. Tuchinda, Paediatrics Department, Siriraj Hospital, Bangkok, Thailand

Dr J. M. Vandepitte, Department of Bacteriology, St Raphaël Clinic, Louvain, Belgium (*Rapporteur*)

Secretariat :

Dr R. L. Kirk, Chief, Human Genetics, WHO (*Secretary*)

Dr A. B. Raper, Department of Haematology, Bristol Royal Infirmary, Bristol, England (*Temporary Adviser*)

© World Health Organization 1966

Publications of the World Health Organization enjoy copyright protection in accordance with the provisions of Protocol 2 of the Universal Copyright Convention. Nevertheless governmental agencies or learned and professional societies may reproduce data or excerpts or illustrations from them without requesting an authorization from the World Health Organization.

For rights of reproduction or translation of WHO publications *in toto*, application should be made to the Division of Editorial and Reference Services, World Health Organization, Geneva, Switzerland. The World Health Organization welcomes such applications.

PRINTED IN SWITZERLAND

CONTENTS

	Page
1. Introduction	5
2. The abnormal haemoglobins	7
2.1 Biochemical and genetic basis	7
2.2 Classification	7
2.3 Distribution	7
3. The thalassaemias	8
3.1 Biochemical and genetic basis	8
3.2 Classification	8
3.3 Distribution	9
4. Glucose-6-phosphate dehydrogenase (G6PD) deficiencies	10
4.1 Biochemical and genetic basis	10
4.2 Classification	10
4.3 Distribution	11
5. Clinical manifestations of haemoglobinopathies, thalassaemias and G6PD deficiencies	11
5.1 Haemoglobinopathies	12
5.2 Thalassaemias	14
5.3 G6PD deficiencies	16
6. Existing methods of treatment	18
6.1 Sickle-cell anaemia and other forms of sickle-cell disease	18
6.2 Sickle-cell trait	19
6.3 Other haemoglobinopathies	19
6.4 Thalassaemias	19
6.5 G6PD deficiency	20
6.6 Use of G6PD-deficient blood for transfusions	21
7. Need for further urgent research on distribution, clinical manifestations and treatment	22
7.1 Geographical distribution	22
7.2 Clinical effects	24
7.3 Treatment	24
8. The role of malaria in haemoglobinopathies, thalassaemias and G6PD deficiencies	26
8.1 The present status of the malaria hypothesis	26
8.2 Further studies of the malaria hypothesis	28
9. Diagnostic facilities	30
9.1 Techniques and levels of laboratory organization	30
9.2 Requirements for staff and training of personnel	32
Annex 1. World distribution of haemoglobins S, C and E, β -thalassaemia and G6PD deficiency	34
Annex 2. Compounds known to have induced haemolysis of G6PD-deficient red cells	39
Annex 3. Methods for the detection of G6PD variants	40

HAEMOGLOBINOPATHIES AND ALLIED DISORDERS

Report of a WHO Scientific Group

The WHO Scientific Group on Haemoglobinopathies and Allied Disorders met in Geneva from 14 to 20 December 1965. The meeting was opened by Dr L. Kaprio, Director, Division of Public Health Services, who welcomed the participants on behalf of the Director-General. Dr H. Lehmann was elected Chairman of the Group and Professor K. Choremis Vice-Chairman. Dr E. Beutler and Dr J. M. Vandepitte served as Rapporteurs.

1. INTRODUCTION

The three groups of diseases with which this report is concerned all result from inherited abnormalities that affect the function of human red blood cells.

In the *haemoglobinopathies* the actual chemical structure of haemoglobin is abnormal, and the alteration in structure may affect the rate at which haemoglobin is synthesized in the body, or the fate of the red cells that contain abnormal pigment.

The *thalassaemias* represent a group of diseases in which the abnormality lies wholly in the rate at which haemoglobin is synthesized.

The *glucose-6-phosphate dehydrogenase (G6PD) deficiencies* are inherited defects affecting an enzyme whose function is necessary to maintain the full viability of the red cells. Persons with a deficiency of the enzyme are particularly sensitive to the haemolytic effects of certain drugs, especially certain antimalarials, and they are also sensitive to some foodstuffs.

These three types of abnormality are known to occur with appreciable frequency in large areas of the world. Some of the abnormalities are associated with severe clinical disorders. Thus, the haemoglobinopathy known as sickle-cell anaemia affects about 1% of all children born in tropical Africa and causes their early death; it is responsible for a total of approximately 80 000 infant deaths per year. In addition, there are probably as many as 100 000 persons with thalassaemia major in the world, all of whom will die early in life despite repeated blood transfusions, which may

be available to them in countries with advanced methods of treatment. Each year, hundreds of children in countries bordering the Mediterranean suffer acute attacks of favism, severe enough in many cases to cause death if untreated. In various parts of the world, there are millions of persons with G6PD deficiency, for whom the haemolytic effects of certain drugs or environmental chemicals present a potential hazard which must be guarded against constantly—a hazard that may be serious where antimalarial drugs are in routine use.

The diseases dealt with here present, therefore, a series of problems of considerable public health importance. The significance of these problems is often not fully realized, even in countries where they assume their biggest proportions. As other diseases are brought under control, however, the clinical significance of the haemoglobinopathies and the allied disorders will become increasingly apparent.

In the present report, a brief review is given of what is known of the world distribution of these defects. Most attention will be given to those disorders with severe clinical manifestations, such as sickle-cell anaemia and the thalassaemias.

Another interesting fact is that the distribution of abnormal haemoglobins and of G6PD deficiency closely parallels the world distribution of falciparum malaria. For the past ten years, evidence has been accumulating that this relationship is not fortuitous, at least for haemoglobin S (Hb S), but is connected with the relative protection that it affords against falciparum malaria in young children heterozygous for Hb S. Convincing evidence for such a protective effect against malaria in the case of other haemoglobinopathies and G6PD deficiency is still lacking. This is clearly a problem of great importance, and the report sets out the need for further studies in this field and the difficulties that may be met in carrying them out.

Many parts of the world have still not been adequately surveyed for the occurrence of haemoglobin disorders. Further, even in countries where special surveys have been carried out, laboratory facilities for the examination of patients as they present themselves for treatment are often inadequate for correct diagnosis. For this reason, knowledge of the distribution and true incidence of either α - or β -thalassaemia and of G6PD deficiency is still very inadequate, and proposals are made for its improvement.

Attention is drawn also to the gaps in the understanding of the clinical symptoms associated with some of the haemoglobin disorders, and in particular to the urgent need for greatly increased study of the methods of treatment for persons with the more severe forms.

2. THE ABNORMAL HAEMOGLOBINS

2.1 Biochemical and genetic basis

Haemoglobin, the respiratory pigment of erythrocytes, is composed of haem and globin. Globin consists of two pairs of polypeptide chains. In normal adult haemoglobin two of these are designated α -chains and two are designated β -chains. Adult haemoglobin may, therefore, be referred to as $\alpha_2\beta_2$. Foetal haemoglobin contains γ -chains instead of β -chains, so that foetal haemoglobin, Hb F, may be designated $\alpha_2\gamma_2$. Similarly, δ -chains take the place of β -chains in the minor normal component, Hb A₂, ($\alpha_2\delta_2$). The abnormal haemoglobins result from substitution of one amino acid for another in the normal peptide sequence of one of the sub-units of the globin portion of haemoglobin. These abnormalities in amino acid sequence arise through mutations of the genes that determine the structure of the polypeptide chain concerned. Since all the structural genes for the haemoglobin peptide chains are autosomal, they occur in pairs. If one of a gene pair is represented by the mutant chain, the affected individual is heterozygous and his cells will, in general, contain both the structurally normal and the abnormal haemoglobin. If, on the other hand, both genes of a pair have the same abnormality, then the individual is designated as homozygous, and the bulk of the haemoglobin in each cell will be of the abnormal variety. Although many abnormal haemoglobins have been described, this report will deal only with those that are clinically significant and present in large numbers of persons. These are haemoglobins S, C and E.

2.2 Classification

1. *Sickle-cell diseases* will be considered to consist of all disease states where the Hb S gene is present. Clinically, they give rise to haemolytic anaemias of varying severity.

2. *Sickle-cell anaemia* is sickle-cell disease in which the patient is homozygous for the Hb S gene. This state gives rise to a severe haemolytic anaemia.

3. The term "trait" will be used to designate all heterozygotes for an abnormal haemoglobin with Hb A. The AS condition will be referred to as a "trait" even though it is recognized that some pathological changes may be associated with it.

2.3 Distribution

The geographical distributions of Hbs S, C and E are summarized in Annex 1. The data tabulated give only an approximate idea of present knowledge of this distribution, since it has not been possible to cover exhaus-

tively the very large literature on this subject. In addition, some of the figures are not at all representative of the country as a whole, while others refer to population groups drawn from several countries or to minorities or immigrants.

In Africa, the gene for Hb S is widely distributed in a broad equatorial belt extending from ocean to ocean. Its distribution is limited to the north by the desert and the Ethiopian highlands. Southwards it extends approximately to the river Kunene in the west and to the river Zambesi in the east. Madagascar, however, is also included. Other less important foci of this haemoglobin are found in the Mediterranean area, including Arabia, and in the Indian subcontinent.

Hb C has a more restricted distribution. High frequencies are only found in the Volta highlands (northern part of Ghana and Upper Volta). To the east, its distribution does not extend beyond an imaginary line going from the Gulf of Gabès to the Niger delta. There is a gradual decrease in its incidence to the north and to the west, and there tends to be mutual exclusion of haemoglobins S and C because of the unfavourable effect of the interaction of these genes. Hb E, on the other hand, is found primarily in the tropical regions of South-East Asia. In addition to these primary areas of distribution, the abnormal haemoglobins have been detected sporadically in many regions of the world, where their presence may be confined to particular ethnic groups, primarily immigrant populations, as among Negroes in Northern and Central America.

3. THE THALASSAEMIAS

3.1 Biochemical and genetic basis

Like the abnormal haemoglobins, the thalassaemias are hereditary disorders of haemoglobin formation. Indeed, the thalassaemias are often classified as haemoglobinopathies. They differ, however, from the other disorders of haemoglobin formation in that no abnormal haemoglobin chains are formed. Rather, the rate of adult haemoglobin ($\alpha_2\beta_2$) formation is diminished and, as a consequence, various combinations of normal polypeptide chains may exist in abnormal quantity. The thalassaemias are characterized by hypochromic microcytic red cells with increased resistance to lysis by hypotonic solutions. They are now known to comprise a genetically heterogeneous group of disorders of considerable complexity.

3.2 Classification

The clinically most important forms of thalassaemia can be divided into that group in which there is decreased synthesis of the α -chain, known as

α -thalassaemias, and that group in which there is decreased synthesis of the β -chain, known as β -thalassaemias. Like the haemoglobinopathies, the α - and β -thalassaemias may exist either in the heterozygous or in the homozygous state. The genes for thalassaemia may also interact with those of other haemoglobinopathies.

3.2.1 α -Thalassaemia

(a) The heterozygous state is barely diagnosable except through family studies. Hb A₂ may be normal or decreased. Small quantities of Hb Bart's (γ_4) are regularly found in cord blood, and small quantities of Hb H (β_4) are occasionally found in adult heterozygotes.

(b) Homozygous α -thalassaemia is virtually always lethal before birth, resulting in hydrops foetalis, at least in East and South-East Asia.

(c) Hb H disease represents the result of interaction between heterozygous thalassaemia and another gene which, in itself, produces no detectable effects. Hb A₂ is decreased.

3.2.2 β -Thalassaemia

(a) The heterozygous state is generally known as thalassaemia minor and is characterized by an increase of Hb A₂ and less frequently by an increase of Hb F. The two characteristics do not always occur together. The clinical manifestations of thalassaemia minor are very variable, ranging from an asymptomatic state without shortening of red cell survival or splenomegaly to a moderately severe haemolytic state with splenomegaly.

(b) Homozygous β -thalassaemia presents clinically as thalassaemia major (Cooley's anaemia), a disease requiring frequent transfusions and often terminating fatally in early childhood.

(c) The combination of β -thalassaemia with genes for haemoglobins abnormal in the β -chain, such as Hb S, E or C, results in the production of large amounts of the abnormal haemoglobin because of suppression of the normal allele. For example, sickle-cell thalassaemia (microdrepanocytic disease) gives rise to a severe clinical disorder in which more than 70% of the haemoglobin may be of type S.

3.3 Distribution

Because of the difficulties inherent in the detection of α -thalassaemia(s), little information is available regarding the distribution of the mutant gene(s). In South-East Asia the blood of a high proportion of stillborn babies with hydrops foetalis contains predominantly Hb Bart's. Approximately 5% of babies in Thailand have been found to have increased Hb Bart's and are therefore presumably heterozygous for α -thalassaemia.

Among black Africans, 9 to 18% of newborns have increased amounts of Hb Bart's, and among American Negroes, the proportion is 2 to 7%. However, a relationship to hydrops foetalis has not been established, and the fate of homozygotes is not known. The distribution of β -thalassaemia is summarized in Annex 1. These disorders have the greatest known prevalence in the Mediterranean regions and in South-East Asia, but surveys have disclosed the presence of heterozygotes in many areas all over the world.

4. GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCIES

4.1 Biochemical and genetic basis

Glucose-6-phosphate dehydrogenase is an important enzyme in the metabolism of glucose by the red blood cell and other tissues. It represents the first step in the oxidative metabolism of glucose through the hexose monophosphate pathway. Approximately 10 years ago it was recognized that deficiency of this enzyme was responsible for sensitivity of certain American Negro subjects to the haemolytic effect of primaquine. This enzyme deficiency, unlike the haemoglobinopathies, is inherited as a sex-linked gene. It is expressed fully in the male, but expression in female heterozygotes varies greatly. Some heterozygous females are entirely normal, some have intermediate enzyme activity, and some are grossly enzyme deficient. Estimates of the gene frequency must therefore be based on surveys carried out on males.

4.2 Classification

More recently it has become apparent that a very extensive genetic polymorphism exists for this enzyme. A particular variant may be described in terms of (1) quantitation of its enzymatic activity; (2) its electrophoretic mobility, and (3) its biochemical characteristics (specifically, its Michaelis constant (K_m) for triphosphopyridine nucleotide (TPN) and G6P and its relative affinity for 2-deoxyglucose-6-phosphate).

The most prevalent type of enzyme in both Caucasian and black African populations is electrophoretic type B. In Caucasian populations the enzyme that is formed in G6PD deficiency has the same mobility as the normal B type and has been called B(-). Enzyme-deficient black Africans differ from enzyme-deficient Caucasians in that the degree of enzyme deficiency is not as severe. This is true, not only in the erythrocytes, but also in other tissues. Tissue levels in those Caucasian populations that have been investigated are moderately decreased; in American Negro populations they are

normal, or nearly so. Further, the enzyme present in enzyme-deficient Africans shows an increased electrophoretic mobility in a variety of alkaline buffers and has been designated A(—). In addition, there is an appreciable group of Negro subjects—approximately 10% of American Negroes—who also possess an enzyme of high electrophoretic mobility but who are not enzyme deficient. These are designated A(+), or simply A.

Aside from these common variants, about which there appears to be general agreement, many other variants have been described. Because of the lack of standardization of methods used in characterizing enzyme variants, it is not at all certain that all the “new” variants described are, in fact, additional variants: some may be merely previously described variants studied under different experimental conditions, for example, at a different pH or in a different buffer system.

4.3 Distribution

To date, the vast majority of surveys have been concerned only with the level of enzyme activity, and not with the electrophoretic types. The available data on G6PD deficiency are summarized in Annex 1. It is apparent that G6PD deficiency is prevalent in an area very similar to, but wider than, the zones in which the abnormal haemoglobins and thalassaemias are found. It should be emphasized, however, although it is not indicated in the tabular data, that different genetic variants are prevalent in different geographical areas. Thus, the enzyme deficiency found in Africa is biochemically, and therefore undoubtedly genetically, distinct from the type found in areas such as Sardinia, Greece and among the Sephardic Jews. Very little information is available regarding the type of enzyme deficiency prevalent in East and South-East Asia.

5. CLINICAL MANIFESTATIONS OF HAEMOGLOBINOPATHIES, THALASSAEMIAS AND G6PD DEFICIENCIES

The abnormal haemoglobins, thalassaemia states, and G6PD deficiency are responsible for clinical disorders in a very large segment of the world's population. The severity of these disorders varies, and while some cause prenatal death—as appears to be the case with homozygous *a*-thalassaemia—others cause only the mildest symptoms or none at all. Precise diagnosis of each abnormal state may depend upon sophisticated biochemical or genetic investigations. Such investigations have made it clear that a fairly characteristic clinical syndrome may be expected for many of these disorders. Although the classification presented here is basically a

biochemical-genetic one, it is recognized that, especially in the thalassaemias, a clinical classification is important and may be very adequate in the management of the individual case. Only those disorders that are most important clinically and most prevalent are considered here.

5.1 Haemoglobinopathies

5.1.1 *Homozygous disorders*

(a) *Sickle-cell anaemia (S-S)*

Sickle-cell anaemia presents as a severe haemolytic disorder punctuated by two types of crises—painful (thrombotic or vascular occlusive) and aplastic. Vascular occlusive crises are the most common. Aplastic crises appear to occur more frequently in some areas than in others; they are, for example, more frequent in Greece and in Africa than in the USA. This may be because of the higher incidence of infection, which complicates the course of the disease in Africa. In West Africa, crises appear frequently to precede the onset of the rainy season, in the early autumn, with a second peak before the onset of the heavier rains in February and April. Hyposthenuria is commonly present in sickle-cell anaemia. There is considerable variability in the life-span of patients with this disorder in various parts of the world. Early mortality appears to be the rule in East and Central Africa, but longer survivals are observed in West Africa and the West Indies. In the USA, survival into adult life is common.

The reason for the variation in severity of sickle-cell anaemia is not at all clear. Undoubtedly, the general state of hygiene and the availability of medical care—in particular the availability of transfusions—play an important role. It is possible that the amelioration in the course of the disease that usually occurs is the result of splenic fibrosis (“autosplenectomy”); or it may be an expression of the subjects’ fitness in other respects. There seems to exist a rough inverse correlation between the severity of the disease and the levels of foetal haemoglobin in the peripheral blood. It is not known whether or not genetic factors regulate the relative amounts of S and F haemoglobin found in subjects with sickle-cell disease. It has been suggested that G6PD deficiency may confer protection against the clinical effects of the homozygous sickling state. However, the isolated report of this effect has not been confirmed and is not considered to be convincing.

(b) *Hb C disease*

This disorder gives rise to a mild haemolytic anaemia. A high proportion of target cells are found, and moderate splenomegaly is common. Many patients appear to live out a full life-span. Neurological disturbances with EEG abnormalities have been found in North Africa.

(c) *Hb E disease*

Homozygous Hb E disease resembles Hb C disease. It varies in severity from an asymptomatic state to a mild haemolytic anaemia. Splenomegaly is not common. Target cells are present in large numbers. Under conditions of stress, more severe anaemia may occur.

5.1.2 *Traits*

(a) *Sickle-cell trait*

In the vast majority of carriers, the sickle-cell trait appears to be innocuous. However, it has become apparent that, very infrequently, certain clinical disorders are associated with this heterozygous state. Splenic infarction during high-altitude flight in non-pressurized aircraft has occasionally been reported. Haematuria without any other cause occurs occasionally in individuals with the sickle-cell trait and appears to be a more frequent finding in such individuals than in normal subjects. Such haematuria may be due to a set of local circumstances within the kidney which predispose to *in vivo* sickling. These include: (1) marked arterial oxygen desaturation, and (2) the hyperosmolarity which is encountered in certain portions of the renal circulation. In addition to haematuria, hyposthenuria occurs in subjects with the sickle-cell trait. It has also been suggested that there may be an increased incidence of urinary infection in pregnancy in subjects with the sickle-cell trait. It is emphasized that complications of the sickle-cell trait are exceedingly rare, and that mortality and morbidity in affected subjects are not perceptibly different from those in subjects without this trait.

(b) *C and E traits*

These have no known clinical effects.

5.1.3 *Mixed haemoglobinopathies*

(a) *S-C disease*

In general, Hb S-C disease has a similar, but somewhat milder, clinical course than sickle-cell anaemia. In S-C disease, thromboses have been reported to be somewhat more common, giving rise especially to haematuria and aseptic necrosis of the femoral head. Vascular abnormalities of the retina are common. It has been reported that maternal mortality, particularly shortly following delivery, is greater in S-C disease than in S-S disease. Other investigators have not confirmed this, and further investigations of the relative effects of S-S disease and S-C disease on the pregnant woman would be worth while.

(b) *S-D disease*

S-D disease is a rare and milder form of sickle-cell anaemia.

(c) *S-E disease*

S-E disease is also rare and similar to, but milder than, sickle-cell anaemia.

5.2 Thalassaemias

5.2.1 α -Thalassaemias

(a) *Homozygous α -thalassaemia*

As seen in South-East Asia, homozygous α -thalassaemia is believed to be lethal, resulting in hydrops foetalis. On the other hand, although evidence is accumulating that, among black Africans and American Negroes, there is a high incidence of the heterozygous state for α -thalassaemia, no corresponding incidence of hydrops foetalis with large amounts of Hb Bart's has been reported. The fate of homozygotes for this presumed α -thalassaemia in this population group is unknown. The possibility that it results in earlier foetal wastage must be given consideration.

(b) *Heterozygous α -thalassaemia*

It has been reported that newborns with increased Hb Bart's in the cord blood develop hypochromia and microcytosis. Presumably this represents an expression of the heterozygous state. There is unfortunately little further information regarding the clinical consequences of the heterozygous state for this haemoglobinopathy. There are also few data regarding the clinical consequences of the doubly heterozygous state for α - and β -thalassaemia, although the few patients who have been studied appeared to suffer from mild thalassaemia major.

(c) *Hb H disease*

Hb H disease is a mild to moderate haemolytic disease. Intra-erythrocytic inclusion bodies are regularly present. Patients with this disorder suffer from fatigue, hepatosplenomegaly, frequent infection, and fever. Aplastic crises commonly follow infection.

5.2.2 β -Thalassaemia

(a) *Homozygous β -thalassaemia*

Homozygous β -thalassaemia presents clinically as thalassaemia major. There is some variability in the degree of severity of this disorder, but all children affected require frequent blood transfusions, have marked splenomegaly and occasional fever, and have bony abnormalities related to encroachment on the marrow space by proliferating bone marrow. It is very unusual for children with this disease to reach adult life.

(b) *Heterozygous β -thalassaemia*

The severity of β -thalassaemia minor is quite variable, but ordinarily it presents clinically as a mildly symptomatic state. It is characterized by hypochromia and microcytosis of red cells. The haemoglobin concentration of the peripheral blood is usually decreased. Red cell survival varies from normal to slightly shortened. Hyperbilirubinaemia and splenomegaly may occur in the more severe forms of the disorder but are unusual. There is no obvious change in the life-span of individuals with β -thalassaemia. However, no careful quantitative studies have been carried out.

The differentiation of thalassaemia minor from iron-deficiency anaemia poses a problem of great practical importance. The concentration of Hb A₂ is increased in the great majority of β -thalassaemia heterozygotes, and is therefore perhaps the best single differential diagnostic criterion. However, in many areas, facilities for the determination of Hb A₂ concentration are not available. If it is not possible to perform Hb A₂ determinations, estimation of the plasma iron and iron-binding capacity and appraisal of the bone-marrow iron may be very helpful. In thalassaemia minor, the plasma iron and iron-binding capacity are usually, but not always, normal, and bone-marrow iron is usually present. In contrast, in iron-deficiency anaemia, the plasma iron concentration is decreased, the iron-binding capacity is increased and bone-marrow iron is absent.

Elevated red cell counts occur frequently in thalassaemia minor but are not usually found in iron deficiency, at least in adults. In blood smears prepared from subjects with thalassaemia minor, cells containing foetal haemoglobin may regularly be demonstrated with the acid-elution technique. This is true even in the large number of cases in which no elevation of the foetal haemoglobin of the blood, as measured by alkali denaturation, is present. The use of the acid-elution technique on blood smears is useful, even in children as young as one year of age, since in iron deficiency anaemia, foetal red cells are absent after this time.

Osmotic fragility tests are not considered to be of value in the differential diagnosis of thalassaemia minor and iron-deficiency anaemia. Careful study of osmotic fragility curves, however, may show differences between subjects with these disorders, since multimodal populations of cells have been found in subjects with thalassaemia. A short course of oral iron therapy may be useful for differential diagnosis.

(c) *Heterozygotes for β -thalassaemia and abnormal haemoglobin*

(i) *S-thalassaemia*

The combination of the gene for Hb S and that for β -thalassaemia gives rise to a serious haemolytic disease. Most frequently, this disorder closely resembles sickle-cell anaemia, but occasionally, features of the clinical picture of thalassaemia have been observed. In such instances,

great enlargement of the spleen may be present and splenectomy may be required.

(ii) *C-thalassaemia and E-thalassaemia*

C- and E-thalassaemia present as thalassaemia major syndromes. Patients with thalassaemia Hb C and E diseases are chronically ill, have hepatosplenomegaly, repeated respiratory infection, frequent bouts of fever, and intermittent jaundice. The severity of the anaemia is very variable. Infections and minor illnesses may induce crises that may cause death.

5.3 G6PD deficiencies

Different variants of G6PD deficiency appear to produce different clinical effects. Some of these effects are discussed in detail below.

5.3.1 *A(-) subjects*

(a) *Favism*

Favism is described below in section 5.3.2. It has not been reported in the African type of deficiency. It is not certain whether or not this is due to lack of exposure to fava beans, lack of additional required factors, or because of the milder nature of the enzymatic defect.

(b) *Neonatal hyperbilirubinaemia*

Neonatal hyperbilirubinaemia has been reported in the African type of G6PD deficiency.

(c) *Drug-induced haemolytic anaemia*

Extensive experimental surveys of drugs to which G6PD-deficient subjects may be sensitive have been made. Compounds that have been shown by experiment or by clinical observation to produce haemolysis are listed in Annex 2. The most commonly used haemolytic drugs are nitrofurantoin and the 8-aminoquinoline antimalarials.

(d) *Haemolysis induced by infections*

Infections appear clinically to precipitate haemolysis in subjects with G6PD deficiency. However, this has not been investigated experimentally.

5.3.2 *B(-) subjects*

(a) *Favism*

Favism is a severe acute haemolytic anaemia which results from ingestion or other contact with the fava bean and perhaps with other closely

related legumes. It is most common in younger boys, and occurs primarily in the spring. It is the most serious consequence of G6PD deficiency, and may result in death.

All patients with favism have G6PD deficiency; however, all G6PD-deficient patients are not sensitive to the haemolytic effect of the fava bean. It would appear that another factor in addition to G6PD deficiency is required for favism to occur. The nature of this factor is not known, but it may be immunological, hereditary, or both.

The attacks of favism, even in a sensitive subject, appear to be sporadic. Sensitive individuals have often eaten the beans frequently prior to their first attack and, at least in some instances, persons who have suffered from favism are reported to have eaten fava beans with impunity subsequently. Green beans are more likely to cause the disorder than dried or cooked beans. Further, there may be heterogeneity among the beans themselves. It is possible, for example, that the haemolytic factor is unstable. Further studies of the factors required for sensitivity to fava beans are required. Such investigations could be carried out in areas in which favism is prevalent, such as in Italy, Greece, or Lebanon.

(b) Neonatal hyperbilirubinaemia

A high proportion of full-term infants developing non-physiological hyperbilirubinaemia several days after birth, with or without kernicterus, in Italy, Greece and in East and South-East Asia, are G6PD-deficient. G6PD deficiency does not appear to be associated with hyperbilirubinaemia in Israel. The reason for the discrepancy is unknown and requires further investigation. Additional genetic factors have been suspected. A high percentage of newborns with pyknoctosis have been found to be G6PD-deficient.

(c) Drug-induced haemolytic anaemia

G6PD-deficient subjects are especially sensitive to the haemolytic effects of a large variety of drugs. While the spectrum of drugs causing such anaemia has been studied in detail in subjects with the A(—) type of deficiency, very few experimental studies have been carried out in subjects with the B(—) type of deficiency. Many of the available data are based upon clinical impressions in individuals who receive drugs for the treatment of some other disease which may, in itself, have been responsible for the haemolytic reactions. The available information regarding sensitivity due to drugs in B(—) subjects is summarized in Annex 2. The most commonly used offending drugs are nitrofurantoin and the 8-aminoquinoline antimalarials. Occasionally, especially in the case of 8-aminoquinoline antimalarials, administration of a haemolytic drug to a patient who is known to be G6PD-deficient may be imperative. In contrast to the A(—) type of deficiency, it is not at all certain that the B(—) type of deficiency

gives rise to a self-limited haemolytic disorder. The drugs listed in Annex 2 must therefore be administered only if absolutely necessary, and considerable caution must be exercised.

(d) Haemolysis induced by infections

Infections, particularly with viral agents, including those causing hepatitis and influenza-like illnesses, appear to be associated with haemolytic episodes in G6PD-deficient subjects.

5.3.3 Other variants

Rare genetic variants of G6PD, such as Chicago I and Oklahoma I, result in non-spherocytic congenital haemolytic anaemia, even in the absence of drug ingestion. Such cases are rare and do not represent an important public health problem.

6. EXISTING METHODS OF TREATMENT

6.1 Sickle-cell anaemia and other forms of sickle-cell disease

6.1.1 *General and long-term management*

In the care of patients with sickle-cell disease, attention must be paid to general health factors such as sanitation, protection from exposure to cold, and treatment of infections as they arise. Immunization against the commonly occurring diseases is recommended. Good nutrition should be maintained in these patients, since they are particularly likely to develop megaloblastosis. The anaemia responds to folic acid treatment when megaloblastosis is present. Otherwise the peripheral blood haemoglobin level can be corrected only by blood transfusions. Their volume and frequency are dictated chiefly by the clinical situation, and it is regarded as unnecessary to maintain the haemoglobin level above fixed arbitrary values. Narcotics and sedatives must be used judiciously in patients with sickle-cell disease, since the chronic nature of their complaints makes them particularly liable to addiction or habituation. Dactylitis, when it occurs, has sometimes been treated with corticosteroids. Other measures that have been found to be useful by some investigators include long-term alkalization with oral bicarbonate, the prophylactic use of antibiotics against infection, suppressive treatment with chloroquine in malarious areas, and repeated small blood transfusions. The latter treatment has been undertaken in an attempt to introduce sufficient numbers of non-sickling erythrocytes into the circulation in order to lessen the tenacity of any sickle-thrombi which may be formed.

6.1.2 *Treatment of crises*

(a) *Aplastic crises*

Aplastic crises are treated by blood transfusion. If examination of the bone marrow reveals megaloblastic transformation, the administration of folic acid is recommended.

(b) *Vascular occlusive (painful) crises*

Many therapies have been recommended for the management of vascular occlusive crises, the most common type of crisis in sickle-cell disease. The very multiplicity of treatments attests to the fact that none is completely satisfactory. Agents that have been recommended to prevent coagulation of blood when aggregates of sickle cells form in small blood vessels include magnesium and anticoagulants such as heparin and dicoumarol. In order to mitigate the effects of the sickling process and thus prevent or treat crises, the administration of alkali, promazine or other phenothiazines, vasodilators (such as sodium nitrite or tolazoline), lumbosacral plexus block (for priapism), plasma expanders, *p*-aminopropiophenone, potassium salts or diaminodiphenyl sulfone have all been suggested. In addition, the use of hydration and the administration of oxygen and transfusion have been advocated.

6.2 **Sickle-cell trait**

The sickle-cell trait is generally asymptomatic and does not usually require treatment. Intractable haematuria may require blood transfusion. It is important to exercise caution while patients with the sickle-cell trait are receiving anaesthesia and to make certain that they remain as well oxygenated as possible. Tourniquets should be used with caution.

6.3 **Other haemoglobinopathies**

The homozygous state for haemoglobin C and E does not generally require treatment. In a few cases where anaemia has been more marked, splenectomy has been thought to be beneficial.

6.4 **Thalassaemias**

6.4.1 *Thalassaemia major (homozygous β -thalassaemia), E- β -thalassaemia, C- β -thalassaemia, and Hb H disease*

As in sickle-cell disease, patients with thalassaemia major must be given good general supportive care. The role of adjuvant hormones, such as thyroid and anabolic steroids, is uncertain. In addition, it is almost inva-

riably necessary to give these patients repeated blood transfusions. Relatively fresh blood is preferable for this purpose. Bone changes and malnutrition may be avoided by giving sufficient blood to raise the haemoglobin level to 9 or 10 g/100 ml. Because the transfusion of such large amounts leads to an increased iron overload, and because of the difficulty in obtaining blood, some clinicians are content to achieve lower levels. There are some patients, including most of those with Hb H disease, who seem to benefit from splenectomy. Indications are an increased destruction of red cells and an increased requirement for blood transfusions or need for relief from the pressure caused by the spleen. Splenectomy is in no sense a routine treatment, and, for immunological reasons, even if indicated, it is best deferred until the patient is over four years of age. The problem of iron overload has assumed increasing importance since better medical care of children with thalassaemia major has permitted them to live for longer periods of time. It is particularly important for patients with thalassaemia major not to be given medicinal iron, either by the oral or parenteral route. Excess iron may be removed by the administration of desferrioxamine or diethylenetriaminepentaacetate (DPTA).

6.4.2 *Thalassaemia minor*

Thalassaemia minor does not require therapy in the vast majority of instances. It is important to recognize that iron is useless in the therapy of this condition. The anaemia may become clinically significant under conditions of stress, such as pregnancy, infection, malnutrition, or under therapy with agents such as pyrimethamine.

6.5 G6PD deficiency

6.5.1 *G6PD deficiency of the A(-) type*

Haemolytic diseases in individuals with the A(-) type of G6PD deficiency rarely represent a serious therapeutic problem. Blood transfusion is not ordinarily indicated, since the anaemia is relatively mild and tends to be self-limited. Occasionally, when the patient has been exposed to drugs with exceptional haemolytic potency, such as naphthalene or pamaquine, blood transfusions may be required to correct the rapidly developing anaemia and hypovolaemia. When transfusions are given, the blood administered should first be tested to make certain that it is not G6PD-deficient. Subjects known to be G6PD-deficient should not ordinarily be given drugs known to cause haemolysis (see Annex 2). Occasionally, especially in the case of 8-aminoquinoline antimalarials, it may be necessary to administer a haemolytic drug to a patient known to be G6PD-deficient. Because these drugs destroy only the older members of the red cell population, modified dosage schedules, which, in effect, gradually destroy the

most sensitive erythrocytes, may be used when such drugs are given to enzyme-deficient subjects. One such schedule, for example, calls for the administration of primaquine in eight weekly doses of 45 mg instead of the daily administration of 30 mg of the drug.

6.5.2 *G6PD deficiency of the B(-) type*

Favism is the most serious haemolytic state encountered in this type of G6PD deficiency, and mortality rates of the order of 10% have been reported. With the judicious use of blood transfusions, the mortality in patients who reach hospital has been reduced virtually to zero. Not all patients need to be given transfusions, but the physician must be aware of the potentially explosive nature of the haemolytic reaction occurring in these patients. The haemoglobin concentration of the blood should be measured every few hours during an attack, and transfusion given promptly if needed. The blood used should not be G6PD-deficient. Some authorities suggest that fresh blood may be preferable. If blood is not available, infusions of saline or plasma expanders may be given. The patient's electrolyte balance should be maintained, and hyperkalaemia should be treated. Haemodialysis may be required if anuria occurs. Haemolytic disease of the newborn due to G6PD deficiency should be managed in the same manner as haemolytic disease of the newborn due to Rh or ABO blood group incompatibility.

Administration of vitamin K derivatives, especially in large doses, should be avoided, particularly in areas in which G6PD deficiency is known to be prevalent. The administration of chloramphenicol and sulfonamides, which are not only potentially haemolytic but also interfere with bilirubin conjugation by the liver, should be avoided. Patients should not ordinarily be given the drugs listed in Annex 2.

6.5.3 *Other variants of G6PD deficiency*

Patients with non-spherocytic congenital haemolytic anaemia due to G6PD deficiency should be counselled to avoid the drugs listed in Annex 2. Splenectomy is of little or no benefit in this condition.

6.6 Use of G6PD-deficient blood for transfusions

It is noted above that G6PD-deficient blood should not be used for transfusions given to G6PD-deficient patients undergoing haemolytic reactions due to eating fava beans or to drug ingestion. For general purposes, however, G6PD-deficient blood may be considered adequate for transfusion. While it is recognized that ingestion of haemolytic drugs will result in the destruction of transfused G6PD-deficient cells, it is unlikely that any patient will receive more than 500 ml of such blood, representing

200 ml of red cells. Since, on the average, only about half the transfused cells would be susceptible to destruction upon drug ingestion, it is unlikely that more than 100 ml of red cells would be destroyed, and this destruction would generally be spread over a period of several days. When this relatively minor risk is balanced against the restriction in blood supplies and increased technical problems that would occur were G6PD-deficient blood excluded, the use of G6PD-deficient blood for general transfusion purposes seems warranted.

7. NEED FOR FURTHER URGENT RESEARCH ON DISTRIBUTION, CLINICAL MANIFESTATIONS AND TREATMENT

7.1 Geographical distribution

As is clear from Annex 1 and sections 2.3, 3.3, and 4.3, a great deal of information has accumulated during the last twenty years on the world distribution of the abnormal haemoglobins, the thalassaemias and G6PD deficiencies. Despite this, knowledge of the distribution of these disorders in many areas of the world is still inadequate. This is true for the abnormal haemoglobins, but the gaps in knowledge are even more apparent for the thalassaemias and G6PD deficiencies.

In the case of G6PD deficiencies, attempts should be made in future surveys to deal not only with the presence or absence of enzyme deficiency in a region but also to give descriptions of the type of enzyme deficiency encountered on the basis of the criteria enumerated in section 4.2. Such information would be not only of considerable anthropological but also of clinical interest, since it is known that some of the different biochemical variants of G6PD deficiency differ in their clinical manifestations.

The Group believes that attention should be directed primarily to those areas in which it may be anticipated that previously unrecognized public health problems exist. The following areas are recommended for more thorough surveys :

AFRICA

West Africa between Tibesti and South Senegal
Volta highlands (North Ghana and Upper Volta)

The interaction between haemoglobins C and S can be studied in several populations in this area.

Ethiopia
Central African Republic
Malawi

Angola	}	Surveys in these four countries may give further information on the southern limit of distribution of the gene for Hb S.
Basutoland		
Mozambique		
Swaziland		

SOUTH AMERICA AND THE CARIBBEAN

Nothing is known about the presence of haemoglobinopathies in many parts of this great region. Urgent research is required in some population groups that are disappearing.

ASIA

Detailed studies have been made in parts of Asia, but large areas remain almost completely unexplored. Further studies on the thalassaemias and G6PD deficiency are urgently needed.

Indian subcontinent

Abnormal haemoglobins, particularly among tribal populations: thalassaemias in the north; G6PD deficiency in all areas. All of these require investigation.

South-East Asia

More studies are required in most areas except Thailand.

China

As a result of studies on emigrant populations, thalassaemia is known to be present, especially in the south-east. Thorough studies in all areas are required.

Middle East

High frequencies of thalassaemias and G6PD deficiency in some populations and some cases of haemoglobinopathies have been reported. More detailed studies are required.

EUROPE

Bulgaria	}	Detailed studies have already been made in these countries, but more thorough investigations are still required.
Greece		
Italy		
Roumania		
Spain		
Yugoslavia		
USSR—the area around the Caspian Sea.		

OCEANIA

Except for New Guinea, large parts of Oceania remain relatively unexplored.

7.2 Clinical effects

The clinical consequences of the abnormal haemoglobins, thalassaemias and G6PD deficiencies vary considerably from patient to patient. To what extent this is due to various environmental factors, such as diet, or previous and current infections, or to other genetic factors, is still not entirely clear. Attention has been drawn already to the almost complete ignorance of the causative agent in cases of favism and to the role that different mutations might play in affecting the severity of thalassaemia. With respect to the haemolytic effects of various drugs on G6PD-deficient individuals, most systematic work has been carried out on persons of the A(—) type. Further study of persons with the B(—) type is needed, and such work will have to be extended to the various mutants of G6PD where these are found to occur in any large section of the human population.

7.3 Treatment

Since the treatment of the haemoglobinopathies and thalassaemia major leaves a great deal to be desired, attention has been given both to the possibility of preventing the occurrence of these disorders and to ways of treating them more effectively when they arise. Further research in these areas is required very urgently; it may be classified under the following headings.

7.3.1 *Chemical alteration of haemoglobin*

Chemical alteration of haemoglobin—by oxidizing haemoglobin to methaemoglobin or by converting it to carboxyhaemoglobin by treatment with carbon monoxide—has so far not been very useful in ameliorating sickle-cell anaemia. It is possible, however, that the production of other minor chemical alterations of the haemoglobin molecule may affect tactoid formation sufficiently to produce clinical benefit, and further studies may be worth while.

7.3.2 *Drugs influencing the sickle-cell crisis*

Both the occurrence and the course of the sickle-cell crises are notoriously variable, and claims have been made for the value of a number of drugs in reducing their frequency and intensity. The Group stresses that the value of any given agent can be established only by the most meticulous clinical studies, and that more trials are urgently needed. One experimental design adequate for such studies is to divide a group of patients at random into a treated and an untreated group and to administer the treatment for a period of six months or one year to the first group, after which the two groups are “crossed over”, i.e., the treatment is then administered to the

former control group for six months or one year, while the group already treated becomes the control group.

7.3.3 *Drugs influencing thalassaemia patients receiving transfusion therapy*

One of the problems considered to be important in maintaining thalassaemia patients on an extended blood transfusion regimen is the heavy iron load imposed on the patient. Extensive studies with desferrioxamine have already been carried out and have shown that appreciable amounts of iron may be removed from iron-loaded subjects by this means. Treatment with DTPA has been the subject of less thorough investigation but seems quite promising in the therapy of thalassaemia major. In particular, the drug may be added directly to blood prior to infusion, thus combining the necessary transfusional and iron-removing therapies. Furthermore, it is probable that the cost of DTPA will be substantially lower than the cost of desferrioxamine. For these reasons more extensive investigation of the utility of DTPA in the therapy of thalassaemia major appears indicated. Adequate clinical trials are needed here also.

7.3.4 *Genetic counselling*

The possibility of preventing the occurrence of these hereditary diseases in their homozygous form by appropriate genetic counselling has been considered. In many countries such counselling would depend upon an adequate programme of social education. Further studies should be made of the feasibility of such counselling, and information should be sought from countries such as Italy, where programmes of this kind are under way.

7.3.5 *Influencing the change from γ - to β -chain synthesis*

If the organism of a person with sickle-cell disease could be induced to synthesize γ -chains instead of β -chains, then a harmless disorder would result. Much needs to be learned about repression and de-repression of genes. Basic work on topics such as the role of histones and RNA in the repression and activation of the genetic material needs to be done and might lead to practical means for repression of the β -S gene and de-repression of the γ -gene. At the same time, it is possible that clinical studies of the effects of various factors, such as antimetabolites, on the relative rates of γ - and β -chain synthesis might prove of value in the management of patients with sickle-cell disease.

7.3.6 *Marrow transplantation*

Except for rare successes, extensive attempts to transplant bone marrow from one individual into another have been failures, even when the recipient was treated aggressively with immuno-suppressive agents. Studies on the

typing of somatic antigens and/or new immuno-suppressive techniques could be of great value in devising new treatments for the haemoglobinopathies and thalassaemia as well as many other hereditary disorders. If the diagnosis of homozygous haemoglobinopathies or thalassaemias could be made in the prenatal period, the possibility that immunological tolerance might be induced by injection of donor tissue *in utero* could be considered.

7.3.7 Genetic surgery

The possibility of changing man's genetic material by replacing deleterious genes by their normal counterparts seems remote at the moment. Nonetheless, basic research in this area in animal systems is to be encouraged.

8. THE ROLE OF MALARIA IN HAEMOGLOBINOPATHIES, THALASSAEMIAS AND G6PD DEFICIENCIES

It is reasonable to suppose that, when a gene that is severely deleterious in the homozygous form reaches a high frequency in a population, it must be selected by conferring some sort of advantage to individuals who carry the gene in the heterozygous combination. Such an advantage might reveal itself either in increased survival of the heterozygotes up to the age of reproduction or through increased fertility of marriages involving at least one heterozygote.

The first clear demonstration that such a balancing mechanism was operating in a human population was made with respect to the gene for sickle-cell anaemia. Here it was suggested that persons heterozygous for the Hb S gene were less likely to die from falciparum malaria than persons carrying genes for only Hb A. Investigations have been extended to study the possible selective advantage of other traits referred to in this report, principally Hb C and Hb E heterozygotes, the carriers of G6PD deficiency and of β -thalassaemia. These studies have been concerned mainly with the role of falciparum malaria as the selective agent, sometimes with other species of *Plasmodium*, and more rarely with other disease agents or environmental conditions.

8.1 The present status of the malaria hypothesis

Evidence for the selective action of malarial infection has been sought by several methods. The geographical distribution of *P. falciparum* has been correlated with that of the frequency of the gene under consideration. Parasite densities, parasite rates, and in some instances proportional

SUMMARY OF AVAILABLE DATA ON THE MALARIA HYPOTHESIS

Genetic character	Evidence of protective effect in malaria infection (<i>P. falciparum</i>)					
	Geographical correlation	Age stratification	Fertility of women	Parasite rate ; parasite density	Lower mortality rate	Induced <i>in vivo</i> infections
Hb S	High correlation with <i>P. falciparum</i> distribution	Suggestive evidence	Suggestive evidence in some surveys	Very suggestive evidence in infants ; not conclusive in older children or adults	Highly suggestive evidence in infants, suggestive in children ; highly significant reduction in cerebral malaria	Inconclusive studies
Hb C	Restricted geographically, but in <i>falciparum</i> area	Limited data available	No data available	Evidence not conclusive	Insufficient data	No data available
Hb E	Reasonable correlation with <i>P. falciparum</i> distribution	Limited data available	No data available	Evidence not in favour of any relationship	Probable in infants	No data available
Thalassaemia α and β	Suggestive evidence, but far from conclusive	Limited data available	Limited evidence (β) more work needed	No data available	No data available	No data available
G6PD deficiency	As for Hb S, but further accurate studies needed, differentiating type	Not in favour	Limited evidence (B-), more work needed	Evidence not conclusive ; generally negative	Data not conclusive	Studies negative

mortality, have been compared in trait carriers and in normal subjects. Induced malarial infections have been studied in volunteers carrying either the sickle-cell trait or G6PD deficiency and in normal controls. In each case, the aim has been to establish (or refute) a protective effect of the gene in question against malarial infection, a positive result being taken to indicate that malaria is at least one of the factors maintaining the gene at a high frequency. Data have also been collected to determine whether trait carriers have a higher fertility than normal, and whether age stratification of the trait suggests early death of subjects who do not carry the trait. The general results of these studies (with Hb S, C and E, β -thalassaemia, and G6PD deficiency) are summarized in the table on this page.

Taken together, the evidence supporting the selective effect of falciparum malaria upon the Hb S gene now seems to be fairly convincing. The failure so far to obtain similarly convincing evidence in the case of the Hb C, Hb E, β -thalassaemia and G6PD-deficiency genes cannot yet be considered as excluding malaria as a selective factor in their favour. In any case, with the exception of the gene for β -thalassaemia, the deleterious effect of these genes in the homozygous state is considerably less than that of the Hb S gene; hence the selective advantage required to maintain them at an elevated level may be very small, and positive data may be correspondingly more difficult to obtain. For β -thalassaemia, however, which is at least as lethal as the Hb S gene in the homozygous state, selection in favour of heterozygotes must be intense, yet nothing but some suggestive geographical evidence has been adduced to implicate falciparum malaria as the selective agent.

8.2 Further studies of the malaria hypothesis

An understanding of the mechanism by which genes are maintained in human populations under different environmental conditions, including disease, is of great importance, particularly when such genes are present in high frequency in large segments of the population. Further studies on the relationship between various agents, in particular malaria parasites (including *P. vivax*, *P. malariae*, and *P. ovale*, as well as *P. falciparum*), and the frequency of the abnormal haemoglobins and G6PD genes are highly desirable. Such studies will need to be planned carefully to avoid the pitfalls that have made difficult the interpretation of many of the previous investigations.

8.2.1 Studies on geographical distribution

In section 7.1, attention was drawn to the need for further studies on the distribution of abnormal haemoglobins, thalassaemias and G6PD deficiencies, and a list of areas where such studies are needed was given. It seems unlikely, however, that this approach will greatly extend knowledge of the selective forces operating on the frequency of these genes. Further investigations of G6PD deficiency may be worth while, since most of the available data do not provide information regarding the genetic type of deficiency found, nor have previous studies concerned themselves extensively with distribution of electrophoretic variants. Geographic studies should pay attention to details of the topographic distribution in small areas, as for example in the careful studies in Sardinia, and particularly to factors that may have disturbed the frequency of the gene in certain populations, such as immigration, war or other catastrophic events.

8.2.2 *Course of malaria in subjects with and without the mutant gene*

(a) *Volunteer subjects deliberately infected*

Results of such studies in the past have been equivocal, largely for two reasons: with falciparum infections it is not possible to use children in the age-group where the effect is likely to be greatest, and in adults the parasitaemia has to be controlled at levels lower than those where the effect may become significant. It is possible that, if species of plasmodia other than falciparum are involved in maintaining one or other of the genes in a selective balance, infection of human volunteers may yield some clues.

(b) *Studies on children living in regions where malaria is endemic*

Analysis of parasite rates and counts in such children has contributed much useful information in relation to the sickle-cell trait. There is still a need for further investigations in respect of Hb C, Hb E, β -thalassaemia and G6PD deficiency. Such investigations would be best confined to children between four months and four years of age and to places where insecticides or drugs are unlikely to interfere with the incidence or course of infections. These studies depend on the supposition that parasite rates and counts bear some relation to the mortality from malaria; there is limited evidence to support this hypothesis, and it will need validation in further studies.

(c) *Relation of the frequency of mutant genes in the heterozygous state to incidence of, and mortality from, cerebral malaria*

Studies of this kind are considered to be valuable; they have been made in respect of Hb S, but could well be extended to populations where the genes for Hb C, Hb E, β -thalassaemia, and G6PD deficiency are prevalent. In such studies it is important not only to ascertain carefully the genetic constitution of the subjects, but also to confirm the cause of death at post-mortem. Screening methods for G6PD are still in the process of development, and certain difficulties are not generally appreciated; a more detailed discussion of the techniques is given in Annex 3. Further, because the anaemia resulting from the malarial infection may give rise to false positive results for G6PD deficiency, assessment of the G6PD genotype is possibly best deferred until after treatment of the infection. Finally, the effect of the different variants of G6PD deficiency has to be taken into account. It is possible that the effect of the A(—) enzyme on malaria may not be the same, for example, as that of the B(—) enzyme.

8.2.3 *In vitro culture of malarial parasites*

Recent progress in the *in vitro* culture of malaria parasites will make possible the exploration of the metabolic relationship between the para-

sites and human red cells with various haemoglobin or enzyme constitutions. This approach is one that could possibly yield valuable results.

8.2.4 *Studies of malaria in animal species*

The existence of haemoglobin variants in some species of animals that may act as hosts to malaria parasites suggests that it may be possible to establish laboratory populations in which critical tests of the malaria hypothesis could be made. It should be remembered, however, that the results of studies in animals can, at best, only be suggestive of the situation in human populations.

8.2.5 *Relaxation of selection*

It may be expected that genes whose frequency has been maintained in a population through the operation of positive selection due to a disease factor will become less frequent in succeeding generations if the selection is relaxed. The study of such effects in human populations is extremely difficult, and so far it has not been possible to provide convincing evidence that such a change is taking place. However, in populations where the frequency of the gene being selected is high, and where the homozygous state is very deleterious, it may be possible to demonstrate such an effect over a relatively short period of time after the effective reduction in incidence of malaria. Certain areas in Africa and also in Greece may be worth investigation from this point of view.

9. DIAGNOSTIC FACILITIES

9.1 **Techniques and levels of laboratory organization**

9.1.1 *Local and mobile laboratories*

The laboratory methods involved in the diagnosis of the clinically important haemoglobinopathies are relatively simple, and only inexpensive equipment is needed. This should be made available in every hospital in those countries where these diseases are prevalent. Most abnormal haemoglobins can easily be detected and recognized by a combination of elementary haematological techniques and paper electrophoresis of haemoglobin. This basic type of laboratory may be attached to a rural hospital, but in areas where large distances create particular problems an adequately equipped mobile laboratory would better fit the local needs. Such laboratories could be used exclusively for diagnosis and surveys on haemoglobinopathies and allied disorders, although in most situations they would be combined with other kinds of epidemiological and therapeutic work.

In remote rural hospitals where no power supply is available and in mobile laboratories, high-voltage dry batteries can provide electric current.

9.1.2 *District or regional laboratories*

Laboratories of district or regional hospitals could extend their diagnostic possibilities by adding more refined techniques to the methods previously mentioned. For instance, in areas where β -thalassaemia is a health hazard, electrophoresis for semi-quantitative estimation of Hb A₂ could be included, while in countries where favism is prevalent, one of the screening techniques for G6PD deficiency should be made available (Annex 3).

9.1.3 *National laboratories*

In addition to these two types of diagnostic laboratory, most countries will need a laboratory especially equipped not only to function as a reference and research centre for the country as a whole, but also to serve as a teaching laboratory where technicians for laboratories at the district or regional level may receive training. As such a laboratory will have to be integrated into an already existing institution (transfusion centre, Pasteur Institute, medical school, public health laboratory, paediatric clinic, etc.) there should be no need for duplication of costly equipment. Facilities should be available for techniques such as quantitative assay of G6PD, quantitative determination of haemoglobin fractions, techniques for study of unusual variants, consultation for genetic and statistical problems, and other, more highly specialized, functions.

Some laboratories of this type could well become more highly specialized to serve the needs of a whole group of countries. Such area laboratories should be developed in collaboration with other area laboratories collaborating with WHO. This would have the advantage of co-ordinating activities in a variety of different fields of research and would help to establish high-level research institutes in areas where these do not already exist or to strengthen those already in existence.

9.1.4 *International centres*

A few institutions that have already been engaged for several years on more basic research work in the field of haemoglobinopathies or G6PD deficiency already exist in different parts of the world. Some of these have grown into reference centres to which laboratories of remote countries have submitted their problems. Such laboratories are equipped for the most advanced type of biochemical and other basic research. They can, for example, carry out the determination of the amino acid sequence of proteins, make molecular weight determinations, and perform the most

complex calculations of gene frequencies, necessitating the use of a computer. There is probably no need for the erection of new costly reference centres of that type. The modest funds available could be more usefully spent in helping the already existing centres to expand their activities and in enabling interested scientists of less advanced countries to visit those centres for specialized technical training. Concerted action, involving co-operation between different institutions, having complementary skills and interests, could give excellent results.

The World Health Organization has recently established an International Reference Centre for Abnormal Haemoglobins at the Department of Biochemistry, University of Cambridge, England. It is hoped that this laboratory will play an important role in handling difficult problems in the identification of new haemoglobin variants as well as in providing advanced training and co-ordination.

9.2 Requirements for staff and training of personnel

Haemoglobinopathies occur in countries where an acute shortage of skilled medical personnel already exists. However, it is urgent that the laboratory diagnosis of these disorders be made more generally available. If this is to be accomplished with the minimal possible addition of staff, adequate training in the necessary techniques must be made available to existing personnel.

9.2.1 Requirements at the local level

Rural hospitals generally have one or more laboratory technicians, and there should be no need to increase their numbers. Only equipment and a short course of training should be provided. In the future, technicians should receive this training before certification. In the meantime, however, supplementary training must be given to the technicians already at work. Short collective courses (maximum 2 weeks) could be organized at the second-level laboratory. Others will find it more convenient to send individual technicians to an already established centre for a short period of informal training.

9.2.2 Requirements at the regional level

No useful work can be done at this level without the existence at each regional hospital laboratory of at least one highly qualified technician fully engaged in the diagnosis of haemoglobinopathies. The training of this technician should be the responsibility of the national centre and he/she could be placed under the supervision of the regional clinical pathologist or paediatrician.

9.2.3 *Requirements at the national level*

The national centre should be run by a clinical pathologist or an equivalent person helped by a staff of two or three qualified technicians according to the local needs. The responsibilities of this centre should include helping the regional hospital laboratories in establishing their own diagnostic services by providing them with qualified technicians trained at the national centre. In some countries, a laboratory course will have to be organized for this special purpose. Qualified technicians from neighbouring countries could be invited to join such a course.

It is recommended that regular contacts between specialists from different parts of the world through the organization of seminars and provision of funds for exchange of research workers should be further encouraged.

Annex 1

**WORLD DISTRIBUTION OF HAEMOGLOBINS S, C AND E,
 β -THALASSAEMIA AND G6PD DEFICIENCY ***

Country	Estimated population (1000s)	Percentage frequency of persons with				Comments
		Hb S	Hb C	β -Thalassaemia	G6PD d (males)	
Africa						
Algeria	11 600	1.5	2	3	< 1	Sahara, 4% Hb S, 4-8% Hb C
Angola	5 084	4-35	0	—	17-27	Hb S not found in Bushmen
Basutoland	729	—	—	—	—	
Bechuanaland	548	—	—	—	3	
Burundi	2 650	1-27	0	—	2-6	
Cameroon	5 103	6-28	< 1	—	20	
Central African Republic	1 320	—	—	—	—	
Chad	2 800	20	< 1	—	—	
Congo (Brazzaville)	826	26	0	Sp.	Sp.	
Congo (Democratic Republic)	15 300	4-36	< 1	0.3	6-23	Data in pygmies: Hb S frequency variable, average 27%; G6PD deficiency: 4%
Dahomey	2 250	17	2-5	—	—	
Equatorial Guinea (Rio Muni)	263	—	—	—	—	
Ethiopia	22 200	< 1	—	—	0	
French Somaliland	80	< 1	—	—	—	
Gabon	454	13-25	< 1	—	—	
Gambia	324	4-23	0.6-3.6	< 1	12-22	
Ghana	7 340	8.3-23	8-19	< 1	24	In north, Hb C > Hb S; in south, Hb S > Hb C
Guinea	3 420	8.5-20	0.7-3.6	—	—	
Ivory Coast	3 750	4-20	4	Sp.	—	
Kenya	9 104	0-25	0	< 1	2-25	
Liberia	1 041	0.7-28	0-3.6	—	—	
Libya	1 559	0	—	—	1	
Madagascar	6 180	3-22	0	—	14-16	
Malawi	3 753	Sp.	—	—	—	
Mali	4 394	10-20	4.8	—	—	
Mauritania	780	5-10	1.5-2	—	—	
Morocco	12 959	0-1.5	0-1.5	Sp.	—	
Mozambique	6 872	1-40	—	—	—	
Niger	3 193	20.6	7.7	3	—	
Nigeria	55 670	18-32	1.6-6.2	< 1	10-27	
Portuguese Guinea	525	0.3-19.5	0-3.6	—	—	
Reunion	382	—	—	—	—	
Rwanda	3 018	1-5	0	—	2-6	
Senegal	3 400	5-12	1-2	Sp.	5-12	
Sierra Leone	2 190	27	3.5	—	—	
Somalia	2 300	—	—	—	—	

* For notes see end of table, page 38.

Country	Estimated population (1000s)	Percentage frequency of persons with				Comments
		Hb S	Hb C	β -Thalassaemia	G6PD d (males)	
Africa (continued)						
South Africa	17 474	< 1	0	—	3-9	Bushmen: Hb S = 0, G6PD d = 3.4%
South-West Africa	554	< 1	—	—	—	
Southern Rhodesia	4 140	10	—	—	—	The figure for β -thalassaemia refers to Arabs only
Sudan	13 180	2-18	0	6	—	
Swaziland	285	0	—	—	4	
Togo	1 603	23	10	—	—	
Tunisia	4 494	2	< 1	3-4	—	
Uganda	7 190	2-40	0	—	15	
United Arab Republic	27 963	< 1	0	Sp.	—	
United Republic of Tanzania	9 990	5-40	0	—	2-28	
Tanganyika	325					
Zanzibar						
Upper Volta	4 716	3.5-9.3	14-25	—	—	
Zambia	3 600	10-20	0	—	—	
Americas						
Argentina	22 045	Sp.	—	Sp.	—	β -thalassaemia in 4-8% of Indians (highlands)
Bolivia	3 653	0	0	—	< 1	
Brazil	78 809	0-13	1	Sp.	0	Figure for G6PD deficiency in Indians only
British Honduras	103	0-20	2.5	—	—	
Canada	19 237	—	—	—	< 1	
Chile	8 492	< 1	0	Sp.	< 1	
Colombia	15 434	0-9	2.8	Sp.	Sp.	
Costa Rica	1 391	—	—	Sp.	—	
Cuba	7 336	5-7	0.5	Sp.	—	
Curaçao	—	5-9	4-8	Sp.	12.2	
Dominican Republic	3 452	10	< 1	—	—	
Ecuador	4 877	—	—	—	—	
El Salvador	2 824	< 1	—	—	—	
French Guiana	—	4-15	0-6	4-11	0	
Guadeloupe	306	0-8	—	—	—	
Guatemala	4 304	—	—	—	—	
Guyana	628	0-15	3?	Sp.	—	
Haiti	4 551	—	—	—	—	
Honduras	2 092	—	—	—	—	
Jamaica	1 728	6-9	3-4	Sp.	—	
Martinique	—	6-9	3-4	—	—	
Mexico	39 643	0-10	Sp.	Sp.	0-4	Figure for G6PD deficiency in Indians only
Nicaragua	1 597	—	—	—	0	
Panama	1 210	8	—	—	—	
Paraguay	1 949	—	—	—	—	

Country	Estimated population (1000s)	Percentage frequency of persons with				Comments	
		Hb S	Hb C	β -Thalassaemia	G6PD d (males)		
Americas (continued)							
Peru	11 357	Sp.	—	—	0	Figure for G6PD deficiency in Indians only	
Puerto Rico	2 572	5-7	0.4-1	Sp.	0-4		
Surinam	345	0-22	2.6	1-2	7-20		
Trinidad		10	—	—	13		
USA	192 119	0-10	0-3	Sp.	0-11		High figures apply to Negro population
Uruguay	2 682	Sp.	—	—	—		
Venezuela	8 427	1-13	1-3	1	2-12		
Asia							
Aden	225	Sp.	—	—	—	Hb E frequency predominantly in Veddas Data obtained from expatriate Chinese	
Afghanistan	15 227	0	—	—	Sp.		
Bahrain	160	—	—	—	—		
Brunei	93	0	10	Sp.	6.3		
Burma	24 229	0	15-16	Sp.	—		
Cambodia	5 900	0	8-32	Sp.	—		
Ceylon	10 965	< 1	15	Sp.	—		
China	686 400	—	0-2.7	Sp.	2-5		
China (Taiwan)	12 070	0	0	Sp.	2.9-5.4		
Cyprus	587	< 1	0	> 5	0-10.6		
Hong Kong	3 692	0	Sp.	Sp.	3.7-5.5		
India	471 627	< 1	3.9	3.7	4-19	In some tribal populations, frequencies of Hb S and β -thalassaemia of up to 40%. Hb E frequency refers to Bengalis; would appear to be low in other parts of the country	
Indonesia	100 045	< 1	0-20	Sp.	1.1		
West Irian	750	0	0	0-25	8		
Iran	22 860	< 1	—	Sp.	12		
Iraq	7 004	< 1	—	—	9-15		
Israel	2 475	< 1	—	2.7-20	0.4-58	Figure for Hb S refers to Arabs only. High figures for β -thalassaemia only in eastern Jews, absent in occidental Jews	
Japan	96 906	0	0	< 1	< 1		
Jordan	1 827	< 1	0	< 1	—		
Korea							
North Korea	10 700	—	—	—	—		
Republic of Korea	27 633	—	—	—	—		
Kuwait	426	—	—	Sp.	—		
Laos	1 925	—	—	—	—		
Lebanon	2 200	< 1	0	1.7	3		

Country	Estimated population (1000s)	Percentage frequency of persons with				Comments
		Hb S	Hb E	β -Thalassaemia	G6PD d (males)	
Asia (continued)						
Macao		< 1	2.5	1.5	—	
Malaysia						
Malaya and Singapore	7 810	0	0-27	Sp.	2-17	The figure of 17% for G6PD deficiency refers to Malayan aborigines
Sabah	+1 820	0	0-5	Sp.	0-24.2	
Sarawak	820	0	0-5	Sp.	0-11.6	
Mongolia	1 019	—	—	—	—	
Muscat & Oman	565	< 1	—	Sp.	—	
Nepal	9 700	0	< 1	Sp.	—	
Pakistan	100 762	< 1	—	Sp.	Sp.	
Palestine (Gaza)	289	—	—	—	—	
Philippines	31 270	0	< 1	1	5-12.7	
Portuguese Timor	543	0	< 1	—	—	
Ryukyu Islands	915	—	—	—	—	
Saudi Arabia	—	0-11	0	—	0-65	Hb S and G6PD deficiency are absent in Bedouins; high frequencies are in Shiites living in malarious oases
Sikkim	167	—	—	—	—	
Syria	5 399	< 1	—	Sp.	—	
Thailand	29 700	0	10-40	3.3-5	7-20	
Turkey	31 118	0-17	Sp.	Sp.	1-11	High figures for Hb S and for G6PD deficiency are mainly in Eti-Turks
Viet Nam						
North Viet Nam	17 800	—	—	—	—	
Republic of Viet Nam	15 715	0	2	—	—	
Yemen	—	Sp.	—	—	—	
Europe						
Albania	1 762					
Austria	7 172					
Belgium	9 378					
Bulgaria	8 144					
Czechoslovakia	14 058					
Denmark	4 720					
Finland	4 586					
France	48 440	0	0	Sp.	Sp.	A high incidence of β -thalassaemia was found in a Basque village (15 of 52 persons examined)
Germany						
Eastern Germany	16 095					
Federal Republic	55 430			Sp.		
Greece	8 480	0-32	0	4-20	1-32	There is mutual exclusion between Hb S and β -thalassaemia. In some regions a mild non-African type of G6PD deficiency exists. Average frequencies: β -thalassaemia, 7.4%; G6PD deficiency, 5.5%

Country	Estimated population (1000s)	Percentage frequency of persons with				Comments
		Hb S	Hb E	β -Thalassaemia	G6PD d (males)	
Europe (continued)						
Hungary	10 119					
Iceland	185					
Ireland	2 849					
Italy	50 762			0-30	< 1	In Sardinia: β -thalassaemia, 4-28%; G6PD deficiency, 3-35 (mean: 13.9)
Malta	328			Sp.		
Netherlands	12 124			< 1	Sp.	
Norway	3 667				Sp.	Unusual variant of G6PD deficiency
Poland	31 161			< 1	Sp.	
Portugal	9 107	< 1	0	0.47	1	
Romania	18 927					
Spain	31 339			Sp.	Sp.	
Sweden	7 661					
Switzerland	6 000				Sp.	Unusual variant of G6PD deficiency
United Kingdom	54 213	0	0	< 1	Sp.	
Yugoslavia	19 279			Sp.	1	
USSR	224 764	Sp.	—	Sp.	—	Sporadic occurrence of Hb S and β -thalassaemia recorded in southern USSR
Byelorussia	8 434					
Ukraine	44 344					
Oceania (selected countries)						
Australia	11 136	0	0	Sp.	0	Sporadic occurrence of β -thalassaemia reported in Europeans only
Fiji	449	—	—	—	—	
Micronesia					0-9	
New Guinea (Australia)	1 539	0	< 1	0-25	0-30	
New Zealand	2 594	0	0	Sp.	—	Sporadic occurrence of β -thalassaemia reported in Europeans only
Papua	562	—	—	—	—	

NOTES:

When several rates are available, only the extreme figures are given; usually they relate to different population groups.

— = no information available.

Sp. = sporadic cases recorded but no statistics available.

Annex 2**COMPOUNDS KNOWN TO HAVE INDUCED HAEMOLYSIS
OF G6PD-DEFICIENT RED CELLS****Analgesics**

Acetanilide
Acetylsalicylic acid ^a
Phenacetin
Phenazone
Aminophenazone

Sulfonamides and sulfones

Sulfanilamide
Sulfapyridine
Acetylsulfanilamide
Sulfacetamide
Sulfafurazole ^a
Thiazosulfone
Salazosulfapyridine
Aldesulfone sodium
Sulfamethoxypyridazine

Antimalarials

Primaquine
Pamaquine
Pentaquine
Mepacrine ^a
Quinocide

Antibacterial agents other than sulfonamides

Furazolidone
Nitrofurantoin
Chloramphenicol ^b
Para-aminosalicylic acid

Miscellaneous

Naphthalene
Vitamin K (water-soluble analogues)
Probenecid ^c
Trinitrotoluene
Methylene blue
Dimercaprol (BAL)
Phenylhydrazine
Quinine ^b
Quinidine ^b

^a Slightly haemolytic in Africans, but only in very large doses.

^b Haemolytic in Caucasians, but not in Africans.

^c Previously reported as haemolytic when given together with para-aminosalicylic acid to an African. Recently shown to be non-haemolytic in G6PD-deficient Bantus.

Annex 3

METHODS FOR THE DETECTION OF G6PD VARIANTS

Several effective screening methods have been described for the detection of G6PD deficiency in affected males. Techniques that have been used successfully in population surveys include the brilliant cresyl blue decolorization test, the methaemoglobin reduction test, and the MTT-linked spot test.¹ A new screening test which depends upon the fluorescence of reduced nicotinic adenine dinucleotide phosphate (NADPH)² has recently been developed and may also prove to be very useful for screening purposes.

Certain difficulties inherent in G6PD screening must be emphasized:

1. The expression of female heterozygotes is quite variable, because their peripheral blood contains varying proportions of normal and enzyme-deficient erythrocytes. Accordingly, no screening test is considered adequate for the detection of female heterozygotes.

2. The brilliant cresyl blue decolorization test is affected by anaemia. All the screening tests are influenced by states that produce a predominantly young red cell population, at least in the A(-) type of enzyme deficiency. Such states include malaria and drug-induced haemolytic anaemia itself.

3. It should be emphasized that G6PD screening methods depend not only upon activity of G6PD, but also upon other enzymes that may be present in various concentrations in blood samples. For this reason, none of the screening tests should ever be considered equivalent to a quantitative assay of G6PD.

Electrophoretic typing of G6PD variants has been carried out by starch-gel electrophoresis. Unfortunately, there has not been standardization of pH, buffer strength, or buffer composition. Thus, it is difficult to compare the results of different groups of investigators, and standardization of methods is badly needed.

¹ The chemical name of MTT is 3-(4,5-dimethylthiazolyl-1,2)-2,3-diphenyltetrazolium bromide.

² Also known as reduced triphosphopyridine nucleotide (TPNH).