HEREDITARY HAEMATOLOGICAL DISORDERS IN THE GREEK POPULATION OF CAPE TOWN.

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TABLE MOUNTAIN CAPE TOWN

ABSTRACT

It is a recognized fact that most well defined population groups have their own typical pattern of inherited disorders. The genetic conditions most commonly found in Greek persons are the thalassaemias, glucose-6-dehydrogenase (G-6-PD) deficiency and, to a lesser extent, certain of the haemoglobinopathies. The clinical and socio-economic consequences of these disorders are significant. In the homozygous state, alpha-thalassaemia is incompatible with life, while thalassaemia major (homozygous beta-thalassaemia) results in a severe anaemia with death usually occurring in the second or third decades. Treatment to prolong the life of these patients is very costly. Alpha- and beta-thalassaemia, when heterozygous, may result in a mild anaemia or be asymptomatic. G-6-PD deficiency results in attacks of haemolysis on ingestion of certain medical preparations and is of far less importance than the thalassaemias. Haemoglobin S is the commonest haemoglobinopathy occurring in Greeks and results in a severe clinical condition when homozygous, as with the thalassaemias. However, the gene frequency is far less than that of the thalassaemias.

The high prevalence of G-6-PD deficiency and haemoglobin S have been demonstrated to be due to the selective advantage they confer against malaria. This same mechanism probably applies to the thalassaemias but has not been proven. Thus, these disorders have been demonstrated to occur more frequently in low-lying areas and places where malaria was endemic in the past. Population movement has made this situation less clearcut in recent times.

Cape Town has a Greek population numbering approximately 5000 persons. As the thalassaemias, particularly, cause a notable public health

problem in Greece, it could be expected that the position would be similar, but on a smaller scale in Cape Town. For these reasons it was decided to undertake a study in Cape Town to ascertain exactly what the position was and what recommendations to make concerning management and prevention.

The study involved the random blood sampling of 5% (250 individuals) of the Greek population. These blood samples then underwent full screening to diagnose any inherited anaemia, including ones not expected to be found in high prevalence. Where warranted, further examinations were performed on the blood to enable a diagnosis to be made. In addition, a brief medical history was obtained from all the respondents and a more detailed history and full physical examination with special emphasis on the haemopoietic system was undertaken in persons found to have any disturbance. Furthermore, family studies were done where possible.

The second part of this thesis concerns the presentation of detailed case reports of known cases of thalassaemia major, individuals with G-6-PD deficiency and subjects found to have hereditary spherocytosis. Included in this section are pedigree studies of families in which these various conditions occur.

Results given include firstly, epidemiological data and thereafter details of the prevalence of the various genetic conditions. It was found that the prevalence of alpha-thalassaemia carriers was 1,2%

beta-thalassaemia heterozygotes	9,2%
G-6-PD deficiency	6,7%
hereditary spherocytosis	1,2%
	(this finding was unexpected)
sickle cell trait	0,4%

Findings which are presented, analysed and discussed include personal, clinical and haematological data.

As a result of this study important theoretical genetic data have been collected which will facilitate the necessary steps to be taken for education of the population at risk, genetic control, clinical treatment and prevention of the serious consequences of these various inherited disorders.

For my parents who made my medical studies possible.

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"The modern haematologist, instead of describing in English what he can see, prefers to describe in Greek what he can't."

Richard Asher, Lancet, 2: 359, 1959.

INTRODUCTION

South Africa, a relatively large country, has a number of ethnically distinct population groups residing within its borders and provides great potential for epidemiological and genetic investigations. Groups as diverse as the tribal African and the European descendants of early immigrants contain smaller sub-groups each with their own particular pattern of inherited disorders and therefore furnish a rich field for study.

The investigation which forms the subject of this thesis is concerned with inherited haematological disorders in the Greek population of Cape Town. It is well established that the genetic disorders which occur most frequently in Mediterranean peoples, including Greeks, are haematological. These are thalassaemia and glucose-6-phosphate dehydrogenase (G-6-PD) deficiency with a high incidence, and the haemoglobinopathies with a lower incidence. It is now almost universally accepted that the high prevalence of these disorders is due to the selective advantage possessed by people with minor forms of these conditions. These individuals were protected against malaria, which in the pre-quinine era was a potentially lethal disease.

Haemoglobin is the oxygen-carrying component of the red blood corpuscle. It is made up of haem, the iron-containing part, and globin, the protein moiety. Globin in turn consists of four chains, 2 alpha-chains and 2 non-alpha chains. The 2 non alpha-chains are designated either beta, gamma or delta.

Normal adults have 97% haemoglobin A (HbA) which consists of 2 alpha and 2 beta-chains, 1,5 to 3,0% haemoglobin A_2 (HbA_2) consisting of 2

alpha and 2 delta-chains, and less than 1,7% haemoglobin F consisting of 2 alpha and 2 gamma-chains.

In fetuses and neonates HbF is the predominant haemoglobin. Shortly after birth, an ill-understood mechanism switches off the gene directing the manufacture of gamma-chains and turns on that for producing beta-chains, with resultant increased production of HbA. The proportion of HbF thence decreases until it reaches adult quantities at about the age of 6 months, when HbA is the predominant haemoglobin. The relative amounts of the various haemoglobins are important as they are disordered in the thalassaemias and provide the most important diagnostic tool.

THE THALASSAEMIAS

The thalassaemias are a heterogenous group of disorders occurring as a result of a quantitative decrease in globin chain production. In beta-thalassaemia there is a decrease in beta-chain synthesis, while in alpha-thalassaemia there is a decrease in alpha-chain production. There can also be disturbed delta-chain production which will be discussed more fully later in the text.

Alpha-thalassaemias

Homozygous alpha-thalassaemia, where there is no alpha-chain production, is incompatible with life and results in a hydrops fetalis.

Heterozygous alpha-thalassaemia is often asymptomatic though it may give rise to a moderate to mild anaemia and is in fact often a diagnostic problem.

Beta-thalassaemias

In homozygous beta-thalassaemia (thalassaemia major), the proportion of HbF and HbA_2 are far greater than normal. The gross chain imbalance causes a haemolytic anaemia and significant ineffective erythropoiesis which results in a severe clinical and haematological disorder. Clinically, the untreated patient is extremely anaemic, growth retarded, may have massive hepatosplenomegaly and classically has a "mongoloid" facies. The prognosis is poor in untreated cases who usually die in childhood of infections or cardiac failure. Treatment is essentially blood transfusion together with an iron chelating agent, as well as appropriate supportive measures. The treatment, however, is not without complications as it adds to the problem of iron overload and resultant haemachromatosis which is, in addition, one of the natural sequelae of the disorder. With this therapy prognosis is improved, but life expectancy is nevertheless limited to the second or third decade. The cause of death is usually cardiac failure occurring as a result of haemachromatosis affecting the heart and exacerbated by anaemia.

Heterozygous beta-thalassaemia (thalassaemia minor, beta-thalassaemia trait) is a far less severe condition. It is often totally asymptomatic though it may also result in an anaemia requiring active management. At times of stress, such as during a concurrent illness, pregnancy or puberty in females, an anaemia may become apparent which on subsequent investigation proves to be thalassaemia minor. Asymptomatic individuals may be diagnosed as having thalassaemia minor during haematological population surveys or during family studies.

Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency

G-6-PD is an enzyme occurring in the red blood cell and is important in maintaining red cell membrane integrity. Red blood cells deficient in G-6-PD haemolyse under certain conditions which include the ingestion of fava beans and certain commonly used drugs such as acetyl salicylic acid preparations, certain anti-malarial medicines and some of the sulphonamide group of drugs. Haemolysis may also occur following certain infections, e.g. infectious hepatitis. G-6-PD deficient infants may have a severe neonatal bilirubinaemia.

Cape Town has a small but significant Greek population of about 5000. Considering that the prevalence of thalassaemia heterozygotes in Greece varies from 6-18% and that of G-6-PD deficiency from 1-41%, depending on the area surveyed, it could be expected that the Greek population of Cape Town would have incidences of these disorders occurring within the above ranges, depending on their origin in Greece.

From the information given above it can be seen that thalassaemia, and to a lesser extent G-6-PD deficiency, carry a notable mortality and morbidity. Furthermore, extrapolating from the prevalence in Greece, it could be expected that these disorders would occur in significant proportions in Cape Town.

The knowledge to be gained from the haematological and genetic investigation which was carried out would have important implications for both the medical authorities and the Greek community of Cape Town.

In summary, the practical value of such an investigation includes:

1. Collection of factual data concerning the incidence of the

disorders; hence, the magnitude of the problem can be assessed.

2. Any change in incidence of the disorders could be noted and discussed.

3. Information as to the incidence and significance of other blood disorders could possibly be gained.

4. Affected individuals could be counselled in terms of prophylaxis, treatment and prognosis.

5. Couples at risk could be counselled concerning the risks involved in procreation, while those not at risk could be reassured.

SECTION I

A REVIEW OF GREECE, ITS PEOPLE AND THEIR EMIGRATION TO SOUTH AFRICA

CHAPTER 1

GREECE

Greece is situated in south-eastern Europe and occupies the southernmost extremity of the Balkan peninsula and the islands of the Aegean sea, numbering about 400 as far south as Crete. It is 131 944 sq. kilometres in area and has a population of approximately 9 million people.

A. PHYSICAL GEOGRAPHY

The physical geography, with special reference to the climate and vegetation, is of significance as it has influenced the nature and distribution of genetic disorders found in persons of Greek origin. Malaria was endemic in southern Europe where the physical geography allowed the vector to thrive. It has been postulated (as will be discussed further on in the text) that the inherited haematological disorders found in Greeks have a marked prevalence due to the protection these disorders provided against malaria.

The sea dominates Greece. The many arms and inlets result in numerous peninsulas and no region in Greece is situated far from the sea. The next most significant feature is the mountains, which cover three-quarters of the surface of Greece. These form a network running from north-west to south-east. Finally, there are lowlands, consisting of a thin, broken coastal strip, narrow valleys, small plains and basins.

There are striking regional variations in climate due to the physical diversity of the land. Basically, the climate is of the Mediterranean

type with the rains occuring in winter. Due to the situation of the mountain ranges, the Ionian coast has a heavier rainfall than the eastern sector. Summers are hot and dry almost everywhere with the average July temperature at sea level being about 27°C. However, the mountains of the interior have some summer rains and the coast is subject to sea breezes.

Vegetation is diverse due to the topographical variations of the land. The mountainous north has deciduous trees, whereas southern Greece has largely scrub. The vegetation of the lowlands consists of trees and shrubs.

B. ORIGIN OF THE GREEK PEOPLE

The progenitors of the Greek people are the Minoans and Mycenaens, who came from Asia Minor or the Middle East to Greece between 3000 and 5000 B.C. and developed the first civilizations of ancient Greece on Crete. In 1100 B.C. the Dorians, a bloodthirsty, violent people from the Danube valley, invaded the Peloponnese, the Greek mainland and Crete. This resulted in the indigenous Argives moving east and settling along the Ionian coast of Asia Minor where large cities and centres of artistic and scientific thought were founded.

In the 6th century B.C. Dorian arms, Aegean galleys and Ionian business acumen combined to make Greece pre-eminent from Spain to the Black Sea. In succeeding centuries the Macedonians, Persians, Romans, Byzantines all conquered Greece. The warlike Macedonians came from the north under Philip and subsequently his son, Alexander the Great, founded an empire extending from India to North Africa. In the years after the birth of Christ, Greece was conquered by a variety of nations

Figure 1 - 1 Greek National Dress

including the Vandals, Huns, Slavs, Bulgarians, Franks, Catalans, Venetians, Genoans and Turks. In the Greek War of Independence which lasted from 1821 to 1829 Greece finally freed herself from Turkish domination and became an independent monarchy. It is probable that the various conquerors of ancient and modern Greece contributed to the gene pool of the present day Greek people.

C. SOCIO-ECONOMIC BACKGROUND

Fifty-five percent of Greece's exports are agricultural in nature, although foodstuffs still have to be imported. Greece is not important industrially or in international trade and individuals have a low per capita income. As a result of these poor economic conditions there is a constant emigration from Greece. During the nineteen sixties alone, it has been estimated that approximately one million Greeks emigrated, especially to America, Canada and Australia and to a lesser extent, to South Africa.

The Eastern Orthodox Church is a strong unifying force amongst the Greek community but not to the same extent as the family unit. Close bonds within the family extend into their business and economic interests, thereby further strengthening the family ties.

CHAPTER 2

LESBOS

The majority of the Greek population of Cape Town have their antecedents on the island of Lesbos. Lesbos is one of the five Greek islands situated in the northern Aegean Sea. The others are Lemnos, Chios, Samos and Icaria.

A. PHYSICAL GEOGRAPHY

Lesbos, also known as Mytelene, has an area of 1630 sq. kilometres, a population of 117 400 and is the largest north-east Aegean island. The vegetation is diverse, varying from the luxuriant and wooded region in the south-east around Agiassos, to the barren wilderness region in the south-west. The pine-covered coastal mountains are broken up by valleys containing lush olive groves. The climate is Mediterranean in type.

B. HISTORY AND ORIGINS OF THE POPULATION

The north-east Aegean islands have played an important part in and seen much of the early growth of western civilization. The islanders were originally descended from the Pelasgians and Ionians who fled the Greek mainland following the Dorian invasion of the Peloponnese. From the 8th to the 16th century B.C. Lesbos, together with the other north-east Aegean islands, were in the vanguard of Greek civilization and made important contributions to both science and the arts. However, the great cities which grew up in these regions were too individualistic and fiercely independent to unite even against a common foreign foe and as a result they were doomed

Fig. 2 - 1 Typical town on the island of Lesbos

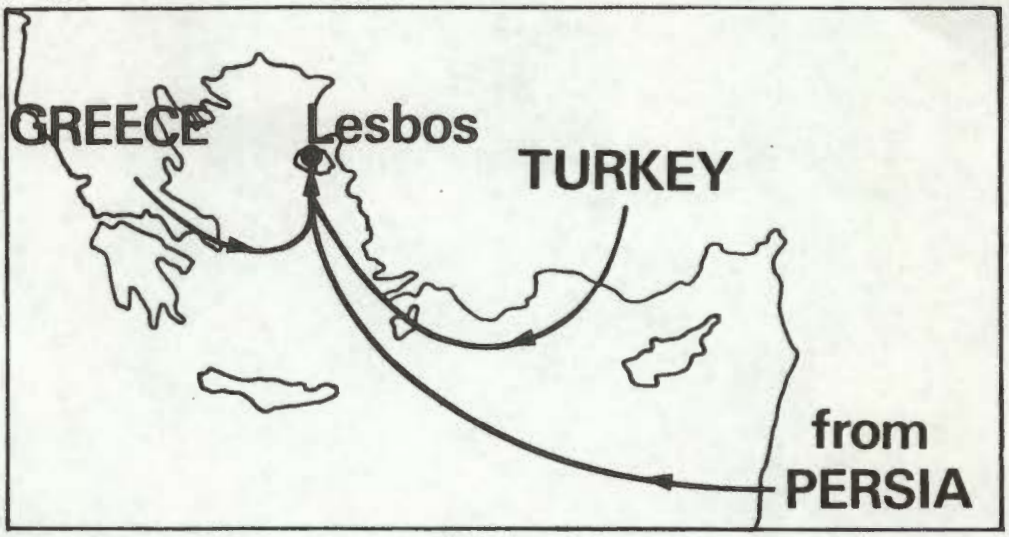


Fig. 2 - 2 Origins of migration to Lesbos

to live under a succession of conquerors. They first fell under Lydian and Persian rule before falling to successive invading armies, including the Byzantines, Crusaders and Genoese. Finally, the Turks held Lesbos from the 15th century. These invaders all contributed to the culture of the islands and to the gene pool of the population. Although the islanders played a courageous and important role in the Greek War of Independence of 1821, it was not until 1927 that Lesbos became a part of Greece.

CHAPTER 3

POPULATION GROUPS OF SOUTH AFRICA

A. SOUTH AFRICA

South Africa has a population of approximately 25,5 million people. This is made up of 18 million African Negroes, 3 million Cape Coloureds and Asiatics and 4,2 million Whites. Of this latter group approximately 60 000 are of Greek origin.

The White section comprise peoples mainly of Dutch and British descent and to a lesser extent French, German, Portuguese, Greek and Italian. The first Whites from Holland arrived in South Africa in 1652 to establish a hospital and refreshment station for ships of the Dutch East India Company. In 1688 the French Huguenots fleeing religious persecution arrived at the Cape and in 1820, after the Napoleonic Wars, 5000 Britons settled in the Eastern Cape. Italian, Greek and Portuguese people came in small numbers on their own initiative in the late nineteenth and early twentieth centuries.

In the early nineteen sixties, when South Africa actively recruited immigrants, larger numbers of these Mediterranean peoples immigrated to South Africa.

B. CAPE PENINSULA

When the first White settlers arrived in the Cape in 1652 they met the Bushmen who were roving hunters and the Hottentots who were herdsmen. Blood of these peoples is still to be found in the Cape Coloured population, a group of mixed ethnic background.

The first Whites to settle in Cape Town were the Dutch, followed by the French Huguenots who were fleeing religious persecution in Europe. After British occupation of the Cape in 1806, many British subjects also settled. By virtue of the city being a major sea port, persons of many nationalities have passed through Cape Town, while others have left some of their genes behind. During South Africa's drive for immigrants in the 1960s Cape Town received peoples of different origins. The overall result is that the present day population is fairly cosmopolitan.

Cape Town at present has a population of about 1,1 million. These are made up of 47% Cape Coloureds, 34% Whites, 10% African Negroes and 8% Asiatics. Included in the White population group are the Greeks, who number about 5000 and represent 1,3% of this group.

CHAPTER 4

THE GREEKS IN SOUTH AFRICA

A. SIZE OF POPULATION, DISTRIBUTION AND ORIGIN

At present the Greek population in South Africa numbers between 55 000 and 65 000, the greatest proportion of whom reside in the large centres. Johannesburg and the Witwatersrand have between 45 000 and 50 000 Greek people. Approximately 20% of these individuals originate from Cyprus, while the rest come from the Peloponnese, the islands of Ithaca, Lesbos and Lemnos and to a lesser extent, the other islands.

Durban has a Greek community of about 2000 individuals, as does Port Elizabeth and small numbers of Greek people are scattered in most towns in South Africa.

As previously mentioned Cape Town, with a population of just over one million people, has a Greek community of approximately 5000. Between 70% and 80% of Cape Town's Greek population originate from Lesbos, while the remainder have their antecedants in Cyprus, Lemnos, Cephalonia, Athens, the Peloponnese and the other islands. The reason for the high proportion of people being from Lesbos is that one of the first Greek people to arrive in Cape Town was from this island and he, finding local circumstances to his liking, brought out his brothers. They in turn brought their cousins to the Cape. This practice snowballed, with individuals encouraging their family and friends to emigrate to Cape Town and resulted in the present pattern of Cape Town's Greek community.

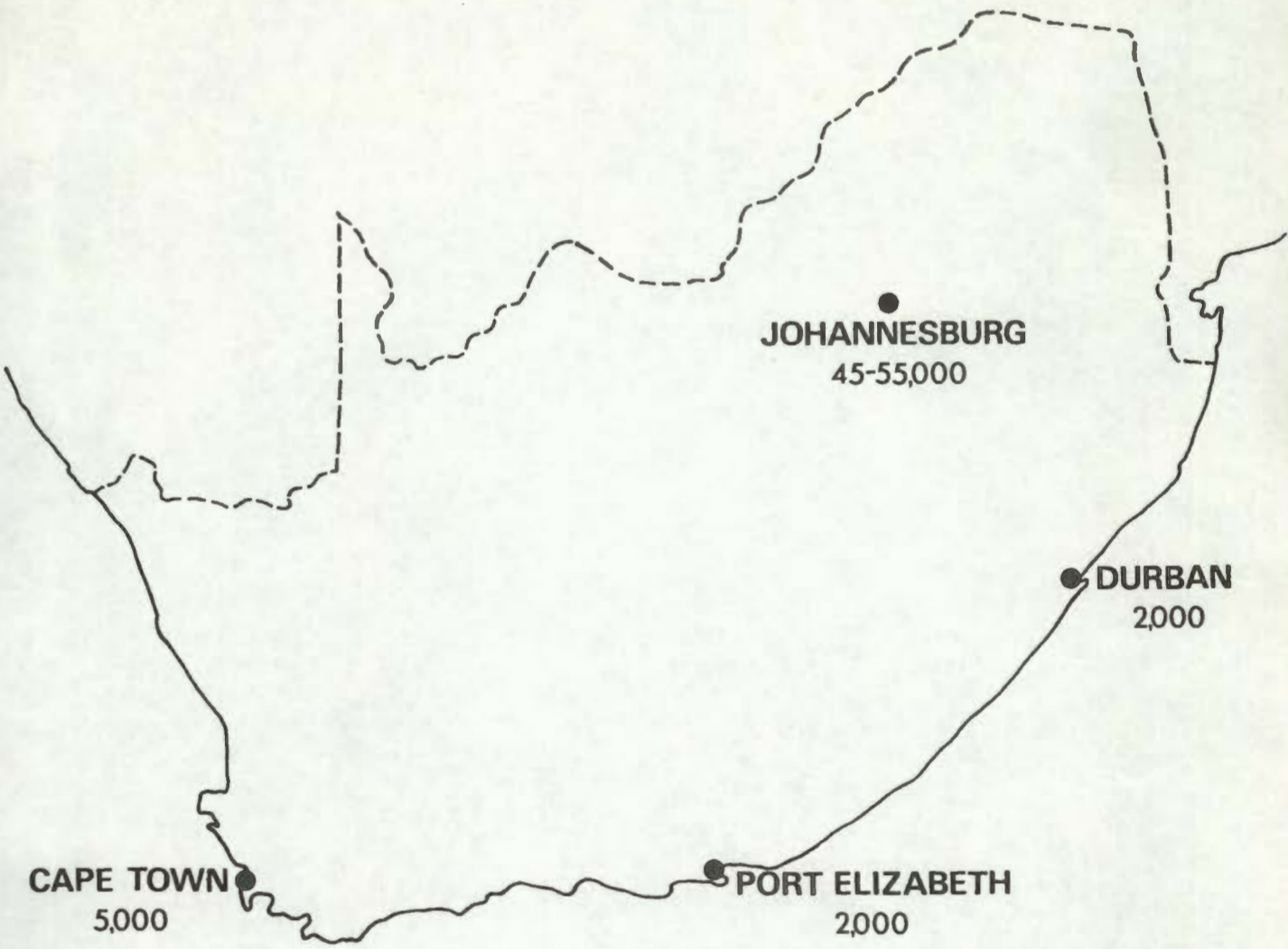


Fig. 4 - 1 Distribution of the Greek population in the major centres of South Africa

B. HISTORY

The Greeks in South Africa can be traced back to about 1850. The first visitors were seamen, most of whom did not stay long in Cape Town before returning to Greece. With the discovery of diamonds and gold, more Greek people (as with other population groups) were attracted to South Africa and by 1902 Cape Town's Greek community numbered about 1000. This population fluctuated as many went north to the goldfields of the Witwatersrand.

In 1900 a Greek Orthodox priest from Crete was sent to Cape Town to establish a Greek community and thereafter the church played an important and leading role. In 1903 the constitution of the community was formulated and the foundation stone for the first Greek Orthodox church in South Africa was laid. The church was completed in 1904 and is still in use today. In the following decade additional communities were established in Johannesburg and Pretoria.

By 1915 there were about 1500 Greeks in Cape Town but with the coming of the First World War in 1914, many of Cape Town's Greeks returned to help defend Greece. The result was that by 1925 there were only approximately 250 Greeks in Cape Town. At this time the number of foreigners allowed into South Africa was restricted, which further depleted Cape Town's Greek population.

The population subsequently numbered about 1000 by 1960. In 1961 immigration offices were opened in several European countries, including Greece, to find and recruit suitable immigrants for South Africa. The result was that Cape Town's Greek population grew rapidly from 1000 in 1960 to about 5000 in 1975.

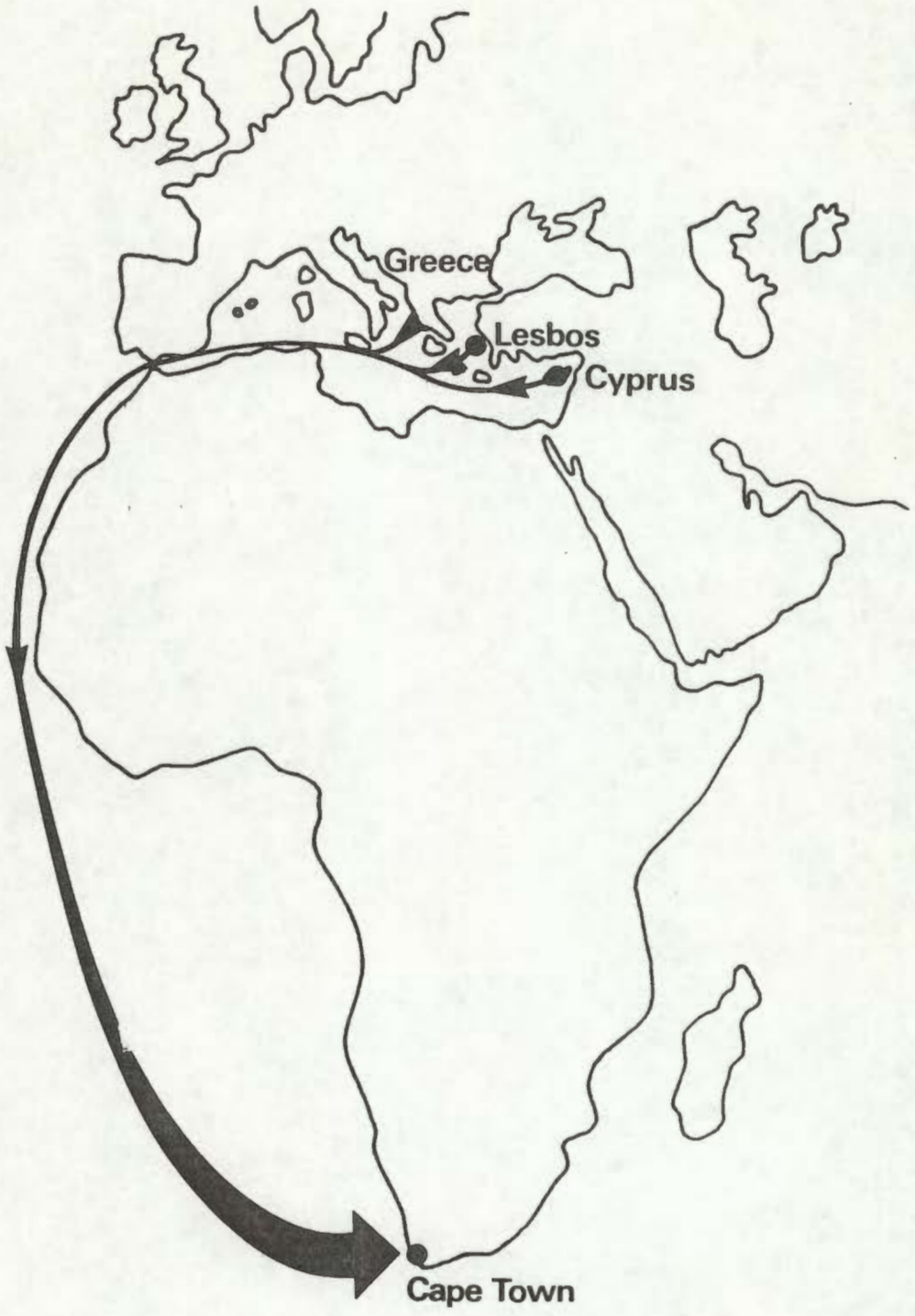


Fig. 4 - 2 Migration from Greece to South Africa

Fig. 4 - 3 Greek Orthodox Church, Cape Town

At present the Greek population is static, with few new immigrants coming to Cape Town. The reason for this situation is that Anglo-Saxon immigrants are presently favoured, while South Africa's current political situation is not encouraging to potential immigrants. However, Cape Town has recently acquired some Greeks who have emigrated from Rhodesia as a result of that country's internal and external problems.

C. LIFE STYLE

Of the 40 Greek families in Cape Town in 1925, almost all owned tearoom businesses. The situation at present is far more diverse, with members of the Cape Town's Greek community being in the professions, in commerce and employed as artisans. However, a large proportion still own cafes and work long, inconvenient hours.

The main congregating places for members of the Greek community are the church on Sundays and at the Greek cinema. Greek films are shown weekly at a local cinema on Sunday evenings, which is very popular and a favourite meeting venue.

Individuals of the Greek community tend to marry within the community and often marry spouses from the same island and town. There are exceptions to this rule, especially amongst the South African born generations.

SECTION II

A HISTORICAL REVIEW OF THE INHERITED HAEMATOLOGICAL DISORDERS KNOWN TO BE FOUND IN GREEKS

CHAPTER 5

THE THALASSAEMIAS

Although thalassaemia has only been defined relatively recently, it has been present as a clinical entity for a long time. Zaino (1964), after studying the skulls of ancient man and their radiographs and recognizing the characteristic changes of thalassaemia, has come to the conclusion that the condition originated over 50 000 years ago in the now inundated Mediterranean valley south of Italy and Greece and thence spread to the rest of the world.

Cooley and Lee (1925) reported 5 children with anaemia, hepatosplenomegaly, skin pigmentation, thickening of the long bones and skull, decreased red cell osmotic fragility and leucocytosis. While the heterogenous group of childhood anaemias had previously been grouped together under the general term "Van Jaksch's anaemia", Cooley, in writing a critical review of these in 1927, clearly defined those cases he had investigated as a specific separate condition.

Thalassa (θαλασσα) means "sea" in Greek and on this basis Whipple and Bradford (1930) coined the term "thalassaemia". They noted the Mediterranean distribution of the disorder and suggested that a metabolic defect might be responsible.

At about the same time cases of haemolytic jaundice with decreased osmotic fragility were reported in the Italian literature. They became known as "La malattia di Rietti-Greppi-Micheli" with the haematological aspects being emphasized to distinguish it from familial acholuric jaundice (Rietti, 1925; Greppi, 1928; Micheli, Rietti and Momigliano,

1935).

The first claim that thalassaemia was inherited, followed a report of a child with thalassaemia major whose parents had mild red cell abnormalities (Caminopetros, 1938). Hereafter many cases of both syndromes were reported in the American and European literature. Wintrobe, et al (1940) described a haemolytic anaemia which they considered to be a mild form of Cooley's anaemia in 14 members of 3 Italian families.

Rietti (1946) in a review of the literature equated the Rietti-Greppi-Micheli syndrome with the cases reported by Wintrobe. Subsequently, after formal genetic investigations, it was established that Cooley's anaemia represented the homozygous state of a partially dominant autosomal gene (i.e. heterozygotes may manifest some of the symptoms and signs of the disorder) and that the cases reported by Wintrobe, et al (1940) and the Rietti-Greppi-Micheli syndrome represented the heterozygous state. Family studies now revealed that the parents of patients with Cooley's anaemia had grades of haematological abnormalities which varied from a moderately severe haemolytic anaemia to minor changes such as a slight increase in osmotic fragility (Silvestroni and Bianco, 1949). Other names for the syndrome at this time included target cell anaemia (Damashek, 1940), familial microcytic anaemia (Strauss, Daland and Fox, 1941), Mediterranean haemopathic syndromes (Chini and Valeri, 1949) and hereditary eliptocytosis (Committee for the clarification of the nomenclature, 1950).

The discovery by Pauling, et al (1949) that sickle cell disease is caused by an inherited defect in the globin portion of the haemoglobin led to intensified study of the molecular basis of the haemoglobin

molecule in health and disease. The study of abnormal haemoglobin variants associated with thalassaemia and of the various haemoglobin fractions led to the conclusion that thalassaemia is, in fact, a heterogenous group of genetic disorders of haemoglobin synthesis (Itano, 1957; Ingram and Stretton, 1959).

As a result of the improved techniques in molecular biology, isolation of messenger ribonucleic acid (mRNA) has now become feasible and Benz and Forget (1971) and Nienhuis and Anderson (1971) have demonstrated that a defect of mRNA is responsible for decreased synthesis of beta-chains in beta-thalassaemia. Similarly, Ottolengi, et al (1974) proved that a gene delection is usually the fundamental abnormality in alpha-thalassaemia.

The most recent progress in the study of the thalassaemias has been in the application of the present status of knowledge. A major step has been made with the discovery that beta-chain synthesis is activated at a low level as early as 8 weeks gestation (Wood and Weatherall, 1973) and that the beta-thalassaemia defect is expressed during early life (Kan, et al, 1975). A second important development has been the improvement of techniques enabling fetal blood to be sampled (Hobbins and Mahoney, 1974; Kan, et al, 1974). This progress has now led to certain specialized centres being able to diagnose thalassaemia major antenatally early enough to terminate an affected pregnancy (Fairweather, et al, 1978).

The present state of knowledge concerning the molecular basis of these disorders is presented and discussed in further detail in Section III.

CHAPTER 6

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

The precipitation of haemolytic crises after exposure to certain environmental agents has long been recognized and it is likely that G-6-PD deficiency was known in ancient times. In the 6th century B.C. Pythagoras forbade his disciples to eat fava beans as he asserted that his grandfather had been reincarnated as a bean (Tingey, 1961). However, it is possible that he empirically recognized the problem of favism! In the 5th century B.C. favism was mentioned by Herodotus (Jacobs, 1950). At the present time older Greek physicians remember their teachers recommending that army recruits should not be issued with blankets until they were aired and free of the fumes of mothballs as they contain naphthalene which induces haemolysis in G-6-PD deficient individuals (Kirkman, 1971).

As early as 1926 Cordes reported the occurrence of acute haemolytic anaemia amongst workers treated with plasmochin for malaria. In World War 2 when troops of different ethnic groups were given primaquine it was noticeable that the red blood cells of Negroes underwent haemolysis far more commonly than in other persons. This was further substantiated by Earle, et al (1948) who reported that 5 to 10% of American Negroes suffered from pamaquin-induced haemolysis, although this complication occurred rarely in Caucasians. Hockwald, et al (1952) made similar observations during an evaluation of the less toxic drug, primaquine. They also noted that the severity of the haemolysis was related to the amount of the drug administered.

Using prison volunteers in a malaria research unit at Joliet, U.S.A.

Dern, et al (1954) showed that primaquine sensitivity is due to an intrinsic defect of the red blood cells. They removed blood samples from sensitive individuals, labelled their red cells with ^{51}Cr, a radioactive isotope of chromium, and then transfused them into a non-sensitive person. Primaquine was then administered and hemolysis of the ^{51}Cr labelled (sensitive) red cells was shown to occur. A reciprocal experiment in which ^{51}Cr labelled non-sensitive red cells was transferred into a sensitive individual who was then given primaquine, was performed. These cells did not undergo rapid hemolysis. It was thus proved that the defect in a sensitive person lay in the red cell itself.

Dern, Beutler and Alving (1954) demonstrated that haemolysis is self-limiting in that clinical recovery occurs even if the daily dose of the "trigger" drug is continued.

Beutler and co-workers (1955) discovered the first biochemical abnormality in the drug-sensitive cells. This was a low content of reduced glutathione (GSH). Flanagan, et al (1958) found that the amount of GSH in the red cells of sensitive persons fell just before these red cells underwent destruction after the ingestion of primaquine. This observation, besides being an important clue to the biochemical basis of the haemolytic process, also led to an in vitro test for primaquine sensitivity (Beutler, 1957).

Glutathione reductase and reduced nicotinamide adenine dinucleotide phosphate (NADPH) are necessary to maintain glutathione in the reduced state in the red cells of man. NADPH is generated in the erythrocytes by G-6-PD. Carson, et al (1956) demonstrated that the activity of G-6-PD is markedly reduced in the red cells of primaquine sensitive

individuals. This represents a double handicap in ability to generate NADPH, for the product of G-6-PD becomes the substrate for 6-phosphogluconate dehydrogenase (6-PGD), the other principal NADPH generating enzyme of human erythrocytes.

Sansoni and Segni (1956) and Szeinberg, et al (1957) noted that drug-induced haemolytic anaemia and favism tend to occur in the same persons and that their red cells had a low GSH content. Panizon (1958) demonstrated that sulfanilamide had a similar haemolytic effect. Thereafter, other drugs including nitrofurantoin, azulfidine and acetanilid were found to cause haemolysis in sensitive individuals (Kirkman, 1971).

Kimbro, Sacks and Torbert (1957) and Zinkham and Childs (1957) recorded that drugs causing haemolysis in sensitive individuals are either oxidation-reduction compounds or converted to oxidation-reduction compounds during metabolism.

The early investigation subjects were predominantly men (soldiers, prisoners and labourers) and it was only in 1958 that Childs, et al, demonstrated that the abnormality was transmitted in an X-linked pattern with intermediate dominance, which implies that some of the genetic heterozygotes might suffer to a variable extent the effects of the disorder. Males fell into two separate categories - those with high or low levels of G-6-PD; whereas females from affected families had levels which varied from the highest to the lowest male values. Some had values independent of their fathers' values, while daughters of affected males often had intermediate or low values and fully affected females had affected sons. Similar conclusions concerning the mode of transmission were drawn from work on Caucasians of Mediterranean origin by Zinkham, Lenhard and Childs (1958).

In 1960, Motulsky and Allison separately noted that the global geographic distribution of G-6-PD deficiency and falciparum malaria coincided and proposed that G-6-PD deficiency conferred some selective advantage against this type of malaria. This correlation has since been demonstrated in local areas in Greece (Stomatoyannopoulos and Fessas, 1964) and in Cyprus (Plato, Rucknagel and Gershowitz, 1964). The most comprehensive work illustrating this relationship was presented by Siniscalco (1966) on Sardinia, where malaria was endemic in coastal areas until the 1940s. The Sardinian population is relatively stable and these investigators found that individuals in the coastal areas have a far higher gene frequency of G-6-PD deficiency than people in the malaria-free higher altitude regions. The best evidence of the resistance of G-6-PD deficient cells to the malarial parasite was presented by Luzzatto, Usanga and Reddy (1969). Using the phenomenon of X-chromosome inactivation in females, they demonstrated that G-6-PD deficient red cells contain fewer malarial parasites than do normal red cells. Women who were heterozygous for G-6-PD deficiency (and thus had 2 cell lines) and were infected with malaria, were found to have fewer malarial parasites in their G-6-PD deficient red cells than their normal red cells.

At present more than 100 different types of G-6-PD have been recognized and in the majority defective activity can occur. Each year more of these are diagnosed but, apart from this progress, no particularly significant recent discoveries regarding this condition have been made.

SECTION III

A DETAILED REVIEW OF THE INHERITED HAEMATOLOGICAL DISORDERS FOUND IN THE GREEK POPULATION OF CAPE TOWN

THE THALASSAEMIAS - A CLASSIFICATION

CLASSIFICATION

1. Alpha-Thalassaemias

   A. Alpha-Thalassaemia 1

   B. Alpha-Thalassaemia 2

   C. Chain termination mutants
      e.g. Haemoglobin Constant Spring

2. Beta-Thalassaemias and delta-beta-Thalassaemias

   A. $Beta^{+}$ Thalassaemia

   B. $Beta^{0}$ Thalassaemia

   C. Delta-beta Thalassaemia

   D. Non alpha-chain fusion syndromes
      e.g. Haemoglobin Lepore

3. Hereditary persistence of fetal haemoglobin

   A. With homogenous distribution of fetal haemoglobin

   B. With heterogenous distribution of fetal haemoglobin among red cells.

CHAPTER 7

THE ALPHA-THALASSAEMIAS

1. HISTORICAL INTRODUCTION AND MOLECULAR BASIS

The alpha-thalassaemias are a distinct group of hereditary haematological disorders caused by a defect in alpha-chain production. All the haemoglobin is equally affected and there is no alteration in the relative amounts of haemoglobins A, A_2 and F produced. This latter fact results in problems in the diagnosis of the carrier state of alpha-thalassaemia and difficulty in defining the genetic transmission of these disorders.

The initial reports on this disorder emanated almost simultaneously from Greece and the United States (Gouttas, et al, 1955; Rigas, Koler and Osgood, 1955). Haemoglobin H was shortly thereafter demonstrated to be a tetramer of beta chains (Jones, et al, 1959). At about this time, a new haemoglobin variant, designated haemoglobin Bart's, was discovered in a baby at St. Bartholomew's Hospital, London (Ager and Lehman, 1958). This haemoglobin was characterized as being a tetramer of gamma-chains with a molecular formula (Hunt and Lehman, 1959). It was now suggested that γ_4 and β_4 were formed as a result of decreased alpha-chain production, thus resulting in haemoglobin Bart's and haemoglobin H (Ingram and Stratton, 1959). The discovery that adults with haemoglobin H disease often have traces of haemoglobin Bart's in their red cells lent further weight to this hypothesis (Fessas, 1960). Finally, a decreased rate of production of alpha-chains was demonstrated by studies of in vitro alpha- and beta-chain production by Weatherall, Clegg and Naughton (1965).

Haemoglobin Bart's and Hb H disease are the two main clinical disorders resulting from a defect of alpha-chain production. These result from the interaction of the alpha-thalassaemia genes:

(A) alpha-thalassaemia 1 gene, which results in complete abolition of alpha-chain synthesis;

(B) alpha-thalassaemia 2 gene, a milder gene resulting in partial reduction in alpha-chain synthesis;

(C) Hb Constant Spring, an alpha-chain variant synthesized in very low amounts.

(A) Alpha-thalassaemia 1

Infants with Hb Bart's hydrops syndrome produce no alpha-chains (Weatherall, Clegg and Wong, 1970). It has now been demonstrated by Taylor et al (1974) and Ottolenghi et al (1974) that the absence of alpha-chains in this syndrome is due to absence of functional alpha-chain mRNA. Experiments involving hybridization of DNA prepared from the liver of an alpha-thalassaemia 1 homozygote with purified copy DNA probes made from normal globin mRNA, together with viral reverse transcriptase, showed that alpha-chain genes are absent from the chromosomes of the affected babies.

In summary, therefore, alpha-thalassaemia 1 is the result of a gene deletion of the alpha-chain genes.

(B) Alpha-thalassaemia 2

Good evidence suggests that human alpha-chain genes are duplicated (Lehman and Lang, 1974, Rucknagel and Winter, 1974). If one considers that alpha-thalassaemia 1 results from a deletion of both of the genes, it is reasonable to assume that alpha-thalassaemia 2

is a deletion of one of the genes. Thus, an individual suffering from Hb H disease would be heterozygous for both alpha-thalassaemia 1, where no alpha-chains are present, and for alpha-thalassaemia 2, where one functional chain gene (locus) is present.

Experiments involving the measurement of alpha-chain mRNA in the cells of patients with Hb H disease have indicated that the amount of alpha-chain mRNA is decreased in these individuals (Benz, Swerdlow and Forget, 1973; Pritchard, et al, 1974). Kan, et al (1973) demonstrated that the kinetics of hybridization of Hb H disease DNA are compatible with the loss of 3 out of 4 genes. Since alpha-thalassaemia 1 DNA has no alpha-genes, this indicates that the alpha-thalassaemia 2 DNA is lacking one of the pair of alpha-genes on the affected chromosome.

(C) Chain Termination Mutants

Patients with Hb H disease from various race groups have been found to have small quantities of an alpha-chain haemoglobin variant. This variant was first characterized in a family from Constant Spring, Jamaica, hence the name Hb Constant Spring. It has been found that patients with one alpha-thalassaemia 1 gene and the gene for Hb Constant Spring have typical Hb H disease. Thus, this mutation behaves in the same way as the alpha-thalassaemia 2 gene. Hb Constant Spring has an elongated chain with 31 extra residues at the C- terminal end, due to a single base change in the mRNA.

As a result of different base substitutions, further haemoglobin variants with differing substitutions at position 142, but with the same additional amino acid residues in the rest of their chains have been recognised; hence Haemoglobin Icaria (Clegg, et al, 1974) and

Haemoglobin Koya Dora (De Jong, Khan and Bernini, 1975). Still other hypothetical variants remain to be discovered.

As there is no alpha-Constant Spring chain synthesis in reticulocytes, it has been suggested (Clegg and Weatherall, 1976) that alpha-Constant Spring mRNA is relatively unstable. This would explain why chain-termination mutant haemoglobins are synthesized only in small quantities.

2. CLINICAL ASPECTS

(A) Haemoglobin Bart's Hydrops Fetalis Syndrome

This lethal syndrome results from the homozygous alpha-thalassaemia 1 state, in which no alpha-chains are produced. The infants, usually born prematurely, have low birthweights and 50% are stillborn. The remainder die within the first hour of life. The gross findings are those of generalized oedema, pallor, hepatosplenomegaly and ascites.

As no cases of haemoglobin Bart's hydrops fetalis were found in the course of the survey, this subject will not be discussed in detail.

(B) Heterozygous Alpha-Thalassaemia

Individuals with this disorder may have haemoglobin H disease (described below) or be totally asymptomatic and only diagnosed on screening programmes. In this latter group, clinical and haematological findings may be minimal and diagnosis may prove to be extremely difficult.

This disorder, like haemoglobin Bart's hydrops fetalis, has a particularly high incidence in South East Asia (Wasi et al, 1969),

Yeminite and Iraqi Jews (Zaizov and Matoth, 1972) and in Shiite Arabs (Pembrey, et al, 1975). Other high incidence areas include Greece and Cyprus (Fessas, 1969).

Haemoglobin H disease is discussed below but it should be borne in mind that findings enumerated may occur in nil or varying degrees in those persons with heterozygous alpha-thalassaemia (i.e. carrying 1 or 2 of the implicated genes).

(i) Clinical Manifestations

The clinical expression of the disorder may be extremely variable, from a virtually asymptomatic state to the picture of homozygous thalassaemia. The majority of patients, however, have the appearance of thalassaemia intermedia. Features include pallor, hepatosplenomegaly and, in a significant proportion, mongoloid facies and bone changes similar to that of homozygous beta-thalassaemia.

(ii) Haematological Manifestations

Haemoglobin varies from 3-13g% but usually is in the 7-10g% range. There is usually a moderate reticulocytosis, varying from 4-5%. Peripheral blood smear reveals microcytosis, hypochromia, anisocytosis and poikilocytosis.

Incubation of red cells with brilliant cresyl blue for 30 minutes at room temperature reveals inclusion bodies in some of the cells. This is precipitated haemoglobin H as a result of the redox action of the dye. In addition, numerous Heinz bodies are found after splenectomy (Weatherall and Clegg, 1972).

(iii) Haemoglobin Pattern

On starch gel electrophoresis at alkaline pH, haemoglobin H moves at a speed similar to haemoglobin I and more slowly than haemoglobin A. On electrophoresis at pH 6,5 haemoglobin H can clearly be distinguished from other haemoglobin variants by its anodal migration (Weatherall and Clegg, 1972). Most patients with haemoglobin H disease have an additional haemoglobin component. This component moves more slowly than haemoglobin H at pH 8,6 and nearer to the cathode than haemoglobin H at pH 6,5. This fraction is haemoglobin Bart's (Ramot, et al, 1959).

The total amount of haemoglobin H in the red cells of patients with haemoglobin H disease varies from 4-30%. Wasi, et al (1969) in their series found an average of 8%. In addition, they found an average of 4,8% haemoglobin Bart's in their subjects.

A third component, consisting of delta-chains, has also been isolated from patients with haemoglobin H disease. This component has not been fully characterized (Dance, Heuhns and Beavers, 1963).

The levels of haemoglobin H, Bart's and A_2 vary greatly in haemoglobin H disease. Weatherall and Clegg (1972) have suggested that this may depend on the activity of the gamma locus. Alpha-chains have a greater affinity for beta- than gamma chains and thus the more active the gamma locus is, the more haemoglobin Bart's as compared to haemoglobin H will be present.

(C) Chain Termination Mutants

Haemoglobin Constant Spring, the most fully characterized chain termination mutant, is most frequently diagnosed in association with

alpha-thalassaemia 1 to produce typical haemoglobin H disease clinically (Clegg and Weatherall, 1976).

In Thailand, 40% of haemoglobin H disease results from the interaction of an alpha-thalassaemia 1 gene and a haemoglobin Constant Spring gene (Weatherall and Clegg, 1975). This occurs to a lesser extent in other populations.

CHAPTER 8

THE BETA-THALASSAEMIAS

In broad terms the beta-thalassaemias can be defined as a heterogenous group of inherited haematological disorders occurring as a result of decreased synthesis of the beta-globin chain.

HISTORICAL INTRODUCTION AND MOLECULAR BASIS

In 1954 Pauling first suggested a relationship between the clinical disorder, thalassaemia, and globin synthesis. Ingram and Stretton (1959) noting that some of the thalassaemias interact with some of the haemoglobin variants, such as haemoglobin S, and have raised levels of haemoglobin F and A_2, suggested that the defect may be in beta-chain production. They thus named this group of disorders the beta-thalassaemias.

From about this time, the great heterogeneity of the thalassaemia disorders was noted. It was observed that there may be a total (beta^0) or partial (beta$^+$) deficiency of beta-chains. In addition, the synthesis of delta-chains may be involved, resulting in the delta-beta-thalassaemias in which the HbA_2 level is not raised. Finally, there may also be fusion of delta- and beta-chains, resulting in the haemoglobin Lepore syndromes. To make matters more complex, it has been discovered that the above broad groups can be further subdivided at a molecular level. For example, in beta^0-thalassaemia (Ferrara) beta-chain mRNA is present, though in small quantities, whereas in classical beta^0-thalassaemia there is no beta-chain mRNA.

Nevertheless, it is still further complicated by the claim that beta-

chain synthesis can be induced in the Ferrara type by the ribosome-free supernatant fraction from normal reticulocytes or by blood transfusion (Conconi, et al, 1975). It has also been suggested that although there is a total absence of mRNA in beta^{o}-thalassaemia (Pritchard, et al, 1974), at least in some cases a highly unstable mRNA is synthesized (Clegg and Weatherall, 1974; Weatherall and Clegg, 1975). Studies have revealed that both the beta^{o} and beta^{+} genes are present in Greeks. However, it has not been ascertained whether the beta^{o} is of the classical or Ferrara type (Kattamis, et al, 1978).

In summary, although important advances have been made in the understanding of the molecular basis of the beta-thalassaemias, the situation has not been fully clarified and much remains to be elucidated.

I. THALASSAEMIA MAJOR

This disorder was first known as Cooley's anaemia, and may also be termed homozygous beta-thalassaemia. When the term thalassaemia major is used in the text, the author will be referring to homozygous beta-thalassaemia or homozygous delta-beta-thalassaemia.

At birth, infants with this disorder are completely normal. Their problems begin when haemoglobin A takes over from haemoglobin F as the major component of haemoglobin at about 6 months of age.

A. CLINICAL FINDINGS

Initial presenting symptoms are failure to thrive, recurrent infections, diarrhoea, pallor and feeding problems. Later, anaemia and hepatosplenomegaly, per se, may be discovered at a physical examination for other reasons and suggest the diagnosis. If the physician has a high index of suspicion the diagnosis is often made in the first year of life.

With increasing age, there will be stunting of growth which may, however, be avoided by early vigorous therapy. Pallor and jaundice are usually present and increased pigmentation may be a feature. Typical "mongoloid" facies develop as a result of bone marrow hyperplasia which Weatherall and Clegg (1972) have suggested might more appropriately be termed "thalassaemic" facies (Fig. 20 - 1), so characteristic are the changes. There is bossing of the skull and prominence of the malar eminences with depression of the nasal bridge and hypertrophy of the maxillae, resulting in protrusion of the upper teeth (Logothetis, et al, 1971). Recently, digital clubbing has been

reported in a significant proportion of thalassaemia major patients. This appears to be related to the severity of iron overload and hepatic dysfunction (Sinniah, et al, 1978).

B. RADIOLOGICAL FINDINGS

The skeletal changes in thalassaemia major are due to overgrowth of the bone marrow. The erythroid hyperplasia is a response to increased destruction of defective red cells. Marrow hypertrophy widens the medullary cavities and increased intramedullary pressure results in atrophy of the spongiosa and corticalis. The shafts of the long and short tubular bones are osteoporotic. The cortex is thinned and normal bone modelling is lost. The changes are usually most marked in the short tubular bones of the hands and feet and in the femora (Fig. 20 - 6).

In the skull, marrow hyperplasia results in widening of the diploic space and thinning of the outer table. The diploic trabeculae may assume a position perpendicular to the inner table, presenting a radial pattern and thus resulting in the characteristic "hair-on-end" appearance (Fig. 20 - 11) (Mosely, 1962).

Overgrowth of marrow in the maxillary, sphenoidal and temporal bones, besides leading to the mongoloid or rodent facies and maloclusion of the jaws, may result in significant underpneumatization of the paranasal and mastoid sinuses (Caffey, 1957). Due to expansion of the maxillae the orbits may be displaced laterally, producing hypertelorism.

Changes in the vertebral column are usually limited to osteoporosis. Pathological fractures of affected bones may occur and may in fact be

the presenting symptom (Modell, 1976).

Abdominal lymphangiography may reveal nodal filling defects in para-aortic and iliac lymph glands. When correlated with histologic findings on nodes removed at splenectomy, these filling defects are demonstrated to be haemosiderin deposits and foci of extra-medullary erythropoiesis (Parsons, 1977).

C. HAEMATOLOGICAL FINDINGS

Untreated individuals are always markedly anaemic with a haemoglobin of approximately 4 g/100 ml. The anaemia is a hypochromic, microcytic type with a low mean cell haemoglobin (MCH) and a low mean cell volume (MCV). Peripheral blood smear reveals hypochromia, anisopoikilocytosis, many mishapen microcytes, reticulocytes, basophilic stippling and characteristically many target cells.

After splenectomy the red cell characteristically undergoes more severe morphological changes and takes on bizarre forms (Nathan and Gunn, 1966). The anisopoikilocytosis is more marked, there are teardrop cells and more nucleated cells are present. After staining with methyl violet inclusion bodies are found in both reticulocytes and nucleated red cells (Fessas, 1963). Fessas has found that these inclusion bodies are probably denatured, aggregated alpha-chains (1966). Study of these inclusions has revealed that the amount of precipitation correlates well with the clinical severity of the disorder.

There is usually a moderate leucocytosis and normal platelet count (Weatherall and Clegg, 1972). However, the spleen may sequester platelets and hypersplenism may result in thrombocytopenia, causing a

bleeding tendency.

D. COMPLICATIONS

The initial disturbance of growth, manifest in early childhood, occurs in the presence of grossly active bone marrow. The striking clinical feature is lack of muscle tissue, which gives the limbs a stick-like appearance. There is also decreased exercise tolerance and weakness at this stage (Modell, 1976). Retarded linear growth is significant at the age of approximately 9 years. The degree of anaemia shows only slight correlation with the severity of retarded height. Systemic abnormalities, however, appear to reflect the growth retardation far more closely (Logothetis, et al, 1972). Secondary sexual characteristics have been found to be delayed or absent in patients with thalassaemia major. Some reported patients have had gonadal dysfunction with normal pituitary function, whereas in others the gonadal dysfunction has been secondary to pituitary dysfunction (Lassman, et al, 1974).

The abovementioned endocrine abnormalities are due to iron overload and result in haemachromatosis and iron deposition in endocrine organs. Further endocrine abnormalities occurring via this mechanism include diabetes mellitus from iron deposition in the pancreas, hypoparathyroidism (Oberklaid, 1975), hypothyroidism and adrenal insufficiency (Lassman, et al, 1974).

The cephalofacial deformities leading to the mongoloid facies usually become manifest after the first year of life. The maxillary hypertrophy leads to protrusion of the incisors and may cause significant dental malocclusion. Susceptibility to infections is commonly a

problem and rare organisms have been reported in an increased incidence (Blum, Viant and Duchy, 1969).

Splenic enlargement occurring as a result of increased workload because of the splenic breakdown of defective red blood corpuscles and iron deposition may cause hypersplenism, followed by neutropenia and thrombocytopenia. Platelet sequestration by an enlarged spleen will worsen the thrombocytopenia. Trauma to an hypertrophied spleen, with the risk of rupture, may result in a life-threatening situation.

Now that patients with thalassaemia major survive into the second and third decade, the problems of haemosiderosis are being met more frequently. The precise relationship between hypertransfusion regimes and development of haemosiderosis has as yet not been fully clarified. However, some investigators believe that hypertransfusion results in the earlier development of siderosis. The most serious complication of iron overload is iron deposition in the myocardium. This leads to gradual cardiac enlargement and eventual cardiac failure. Anaemia, too, is a contributory factor. Iron deposition in the liver may result in a diffuse nodular cirrhosis. Attacks of pericarditis, similar in type to acute benign non-specific pericarditis frequently occur (Weatherall and Clegg, 1972) especially after splenectomy.

The most recent complications reported in this condition have been neurological. Eight cases of spinal compression found to be caused by regions of extramedullary erythropoiesis have been reported in the literature (Cross, et al, 1977). This has also occurred in the past in 1 non-Greek patient in Cape Town (Goldberg, 1976). Transient motor seizures, including some associated with temporary hemiparesis, which have resolved rapidly and been thought to be due to cortical vein throm-

bosis, have been reported (Sinniah, et al, 1977). These patients have usually been dehydrated as a result of vomiting or diarrhoea from an intercurrent illness. A neurological syndrome which is probably a side effect of blood transfusion has recently been described in the literature. This consists of hypertension, convulsion and cerebral haemorrhage (Wasi, et al, 1978). It has therefore been recommended that care be taken when giving rapid blood transfusion to patients adapted to longstanding anaemia.

Psychological tensions often develop within the family of a patient with thalassaemia major. The patient may express resentment while the parents may experience guilt feelings. This puts considerable stress on the family unit.

E. PROGNOSIS

Before the clinical entity of thalassaemia was recognized and understood, patients with thalassaemia major died in childhood. Death was usually the result of anaemia or infection. The use of antibiotics and blood transfusion has considerably increased the lifespan of patients with this condition. Presently the most commonly used regimes of hypertransfusion in association with chelating agents has added years to the life expectancy of these patients. However, they still eventually succumb in the second or third decades, usually as a cumulative result of iron overload. The most frequent cause of death, as mentioned previously, is cardiac failure.

As the thalassaemia syndromes are so heterogenous it is to be expected that there are individuals with a relatively mild form of thalassaemia major. There are reports in the literature of long survival in this disorder (Apostolidis and Avgoustakes, 1973; Eichner, 1976).

F. BIOCHEMICAL FINDINGS

There is almost invariably a raised bilirubin level in patients with thalassaemia major due to increased rate of haemolysis. It is usually in the 1-3mg/100 ml range. Serum glutamic oxaloacetic transaminase (SGOT) and lactic dehydrogenase (LDH) are also raised, probably reflecting hepatic damage. The serum iron is raised with a saturated iron binding capacity. Serum calcium as well as magnesium and zinc may be low (Erlandson, et al, 1964).

G. HAEMOGLOBIN PATTERN

The haemoglobin pattern in patients with thalassaemia major is characterized by decreased haemoglobin A and raised haemoglobins F and A_2. The levels of haemoglobin F and A_2 may show considerable variation. Patients possessing the beta^0 thalassaemia gene appear to produce no haemoglobin A. Their haemoglobin is constituted solely of haemoglobin A_2 and F (Fessas and Karaklis, 1962).

Haemoglobin F, which is alkali resistant (Vecchio, 1946), occurs usually in the 10 to 90% range. Using the modified acid elution technique of Kleihauer, Braun and Betke (1957), it has been found that the fetal haemoglobin is heterogeneously distributed in the cells (Frazer and Raper, 1961). No relationship has been found between the level of fetal haemoglobin and the severity of thalassaemia major. The turnover of haemoglobin F has been demonstrated to be slower than that of haemoglobin A and A_2. This is probably due to the longer survival of cells with a high level of haemoglobin F (Nathan and Gunn, 1966).

Haemoglobin A_2 levels may vary considerably in thalassaemia major. The range is usually from 1,4-13 per cent. The level of haemoglobin A_2 does

not parallel the severity of the disorder (Fessas, 1959). Low levels of haemoglobin A_2 in patients with thalassaemia major, where the parents have raised haemoglobin A_2 implies occurrence of the doubly heterozygous state for beta- and delta-beta-thalassaemia genes. In cases where there is high haemoglobin F, the haemoglobin A_2 may appear within normal limits, but there is nevertheless a decreased haemoglobin A/haemoglobin A_2 ratio (Kunkel, et al, 1957).

Fresh haemolysates prepared from patients with thalassaemia major contain trace amounts of a haemoglobin, which moves more slowly than haemoglobin A_2 and disappears on the addition of haemoglobin H (Fessas and Loukopoulos, 1964). This fraction probably consists of free alpha-chains.

In vitro experiments of haemoglobin production have demonstrated that there is decreased production of beta-chains in beta-thalassaemia. There is thus a relative increase of alpha chains which are unstable and precipitate rapidly to become associated with the red cell membrane (Weatherall, et al, 1969; Modell, et al, 1969). This latter fact is of great importance in considering the pathophysiology of the disorder.

H. PATHOPHYSIOLOGY

The anaemia of thalassaemia is a result of ineffective erythroporesis and shortened red cell survival. There is extensive intramedullary destruction of red cell precursors (Finch, et al, 1970), with a rapid turnover of haemoglobin A. The turnover of haemoglobin F is far lower (Gabuzda, Nathan and Gardner, 1963). Red cell survival studies using ^{51}Cr labelling techniques indicate a reduced survival time ranging from 7 to 22 days (Sturgeon and Firth, 1957). The in-

effective erythroporesis leads to gross erythroid hyperplasia, which in turn gives rise to many of the clinical features of the disorder.

The problems at a cellular level are caused by globin chain imbalance. Decreased beta-chain synthesis leads to relative excess alpha-chains. These alpha-chains are unstable and precipitate to form inclusions which damage the red cell membrane. Cells with large inclusions have little haemoglobin and are broken down in the bone marrow. Red cell precursors, having relatively large amounts of gamma-chains, are largely protected from chain imbalance, as the gamma-chains combine with the alpha-chains to produce fetal haemoglobin. Under these conditions the inclusion bodies will be small with little damage to the cell membrane. Inclusion containing red cells reaching the peripheral blood are sequestered in the spleen. In the spleen the inclusions are removed by splenic pitting and then returned to the circulation. Hence, in patients who have had a splenectomy, large numbers of red cells with inclusions may be seen in the peripheral blood.

The problem of whether iron is absorbed from the gut in increased amounts or not has not yet been clarified. Nevertheless, iron appears to accumulate in the thalassaemic normoblast in larger than normal amounts. It takes the form of ferritin molecules or siderosomes (Fessas and Loukopoulos, 1974). Accumulation of iron in the mitochondria might interfere with their function in maintaining the energy metabolism of the normoblast and in participating in haem synthesis.

An increase in potassium flux has been detected in inclusion bearing erythrocytes in thalassaemia major. This has also been found with haemoglobin H disease (Nathan, et al, 1969). This phenomenon has been

attributed to a selective permeability of the membrane to potassium following depletion of energy sources (Gunn, Silvers and Rosse, 1972). The altered potassium permeability appears to be less important than the noxious effect of haemoglobin precipitation to the red cells (Knox-Macaulay, et al, 1972).

I. TREATMENT

1. BLOOD TRANSFUSION

Before the early 1960's most patients with thalassaemia major were given blood transfusions only when their haemoglobin had reached the low level of approximately 5 g/100 ml, for fear of causing early iron overload and death. However, with this regime patients suffer all the problems of the disease as well as of the treatment (Modell, 1976). Wolman (1964) suggested that if a patient's haemoglobin was kept in the normal range he could live a normal life without necessarily shortening his survival. This resulted in a general change of policy and the hypertransfusion regime is now practiced almost universally (Necheles, 1974). Thus patients who were debilitated and symptomatic are now normal until they are about 12 years of age, when they tend to show the signs and symptoms of iron overload. Patients on these regimes who were previously troubled by recurrent and serious chest infections no longer suffer these complications. Initial growth is normal (Hyman, et al, 1976), bone changes regress, heart size decreases and gastro-intestinal iron absorption is suppressed (Modell, 1976). The patient's well-being plays a significant part in relieving the family of much stress. In addition, this regime suppresses bone marrow hypertrophy, thus promoting a more normal life without the facial stigmata of the condition (Weiner, et al, 1978).

The long term prognosis ultimately depends on the rate of progression of haemosiderosis. Iron overload as a result of high level transfusion undoubtedly occurs. However, there is no evidence that toxicity appears earlier. As a possible reason it has been suggested (Necheles, Beard and Allen, 1969) that hypoxia potentiates the toxic effects of iron overload. It has also been suggested that the reduction in gastro-intestinal iron absorption offsets the increase due to high level transfusion. Recent reports indicate that iron overload can be significantly decreased by transfusing only the youngest 50 per cent of donor red cells and this will therefore further improve the outlook in this situation (Corash, et al, 1976).

2. CHELATING AGENTS

Desferrioxamine, produced by streptomyces piloxes, is the most widely used chelating agent in chronic iron overload. Desferrioxamine is only of use when a patient is already iron overloaded. This usually occurs at the age of about 8 years, when approximately 100 units of blood have been received. Modell (1976) recommends Desferrioxamine in doses of 0,5 g to 1 g intra-muscularly daily. However, Desferrioxamine can also be used intravenously (Retsky, 1976). About two-thirds of the iron bound by Desferrioxamine is excreted in the urine and one-third in the stool (Modell and Matthews, 1976).

The most recent advances in the treatment of thalassaemia major have demonstrated that constant infusion of desferrioxamine either by the intravenous or subcutaneous route, is more efficient than when given in an intramuscular dose. When given intravenously this treatment is 79 per cent as effective as subcutaenously and the latter route is

now considered the treatment of choice (Graziáno, et al, 1978).

Desferrioxamine is given over an 8 hour period by subcutaneous infusion with a pump and results in little inconvenience to the patient. This mode of treatment has been found to be 2,4 times as efficient as intramuscular treatment (Cohen and Schwartz, 1978). It has been found that this regime decreases inorganic iron absorbtion from the gut and that iron excretion is greater and occurs from an earlier age (Pippard, et al, 1977). This is compared to intramuscular treatment which is only effective from about 5 years of age when the patient is already severely iron overloaded (Necheles, et al, 1974; Modell, 1976). As a result of this progress it is now possible to approach iron balance in these individuals. However, it is not known yet whether cardiac siderosis is reversed (Weiner, et al, 1978). These recent advances will clearly improve the prognosis and increase the life expectancy of thalassaemic major patients.

Vitamin C is usually given in conjunction with desferrioxamine, as it has been found that it enhances the excretion of iron in response to desferrioxamine (Wapnick, et al, 1969). In iron overload, increased catabolism of ascorbic acid has been reported. In addition to ascorbic acid deficiency it appears that there is some disorder of iron release (Charlton and Bothwell, 1976). Hence, increased efficiency of iron excretion is obtained by using desferrioxamine together with vitamin C.

D.T.P.A. (diethylenetriaminepentaacetic acid), a chelating agent, has also been used in the treatment of iron overload. However, disadvantages such as hyperpyrexial reactions (Letsky, 1976) and non-specificity for iron have been noted. As a result of this latter quality, other

possibilities of antenatal diagnosis. Fetal blood may be obtained from blind needling of the placenta or by aspirating blood from surface placental vessels (Hobbins and Mahoney, 1974). Fetal blood cells are then analysed for the presence of beta-chain synthesis. This technique has been successfully applied by Kan, et al (1975).

The most recent significant advances in the study of the thalassaemias have had important benefits in making antenatal diagnosis of the condition possible. The thalassaemia model has been of use in DNA and RNA engineering and hence progress in this field has resulted in progress in the understanding of the molecular defect in the thalassaemias with practical applications in antenatal diagnosis.

As mentioned above, homozygous beta-thalassaemia has now been successfully diagnosed antenatally (Fairweather, et al, 1978), but with a high risk of miscarriage or damage of about 10 per cent to the fetus. This risk is nevertheless acceptable compared to the tragedy of a child with the disorder. Alpha-thalassaemia has been shown to be due to a gene deletion. Cultured amniotic cells display this abnormality and, in fact, open up the possibilities of antenatal diagnosis by amniocentesis instead of fetal blood sampling, with the high associated risk. This has been successfully undertaken recently (Wong, et al, 1978). Antenatal diagnosis of sickle cell anaemia has recently been achieved by amniocentesis (Kan and Dozy, 1978) and it is hoped that in the future all the thalassaemias will be diagnosed in this very specialized way.

It must be emphasized that although there is such spectacular progress in this field it is only large specialized centres that are able to undertake this work. This service, therefore, cannot at present be

cations such as magnesium and calcium are depleted.

2,3-dihydroxybenzoic acid (2,3-DHB), an oral chelating agent, is at present under investigation. Initial reports suggest that it will be of use when administered in combination with desferrioxamine (Graziano, Grady and Cerani, 1974; Grady, 1976).

3. SPLENECTOMY

Judicious selection of patients for splenectomy is of great importance due to the associated risks of the surgical procedure.

The most important indication for splenectomy is increase in blood requirements. Causative mechanisms are sequestration of red cells in a non-circulating splenic pool, dilutional anaemia as a result of increased plasma volume and hypersplenism. The discomfort caused by a large spleen and risk of rupture following trauma are contributory factors.

Risks associated with splenectomy include severe infection occurring post-operatively in the very young, increased iron load on the liver and increased incidence of recurrent pericarditis (Fessas and Loukopoulos, 1974).

4. PREVENTION

In most centres, until recently, the only method available for the prevention of new cases of thalassaemia major was to recommend that two thalassaemia heterozygotes should not procreate. However, the discovery that abnormalities of the beta-chain can be detected by the study of haemoglobin synthesis (Kan, et al, 1972) opened up the

offered to all who need it, though it is envisaged that this will be possible in the not too distant future.

5. FUTURE POSSIBILITIES

i) Bone Marrow Transplantation

This method of management is at present used in cases of resistant aplastic anaemias. However, the procedure still carries a significant morbidity and mortality, but if perfected it may well be an important tool in the treatment of thalassaemia major.

ii) Gene Manipulation

It has been demonstrated that sickle-cell anaemia in Saudi Arabs is clinically, as well as haematologically, a very mild disorder (Perrine, et al, 1978). It is thought this may be due to the protective effect conferred by the high concentrations of fetal haemoglobin (Hbf) found in the disorder amongst this population group (Perrine, et al, 1972).

Relatively large amounts of Hbf have also been shown to be beneficial in beta-thalassaemia. Persons who have a gene for the hereditary persistence of fetal haemoglobin as well as beta-thalassaemia have a milder condition than those who only inherit thalassaemia (Wood, Weatherall and Clegg, 1976). Furthermore, those forms of beta-thalassaemia in which the defect results in increased levels of Hbf are generally milder than those in patients with limited Hbf production (Wood, Clegg and Weatherall, 1977).

The mechanisms regulating HbF production in these disorders are at present not clear but are of a genetic nature. It is not possible to switch on or off the genes for globin chain synthesis, but when this is achieved benefit may be derived from increasing the levels of HbF in individuals suffering from thalassaemia major.

II. HETEROZYGOUS BETA-THALASSAEMIA

Heterozygous beta-thalassaemia demonstrates the heterogeneity of the thalassaemia syndromes in that the clinical state, haematological findings and haemoglobin patterns may vary considerably. At one end of the spectrum patients have symptomatic anaemia and at the other end individuals are totally asymptomatic. The more severe forms are more likely to occur in Mediterranean as opposed to Negro populations.

A. CLINICAL MANIFESTATIONS

Patients most severely affected have a chronic anaemia, which at periods of stress or pregnancy may require blood transfusion. They may present with symptoms of anaemia, jaundice or leg ulcers (Weatherall and Clegg, 1972). Upper abdominal pain might be the result of gall stones or perisplenitis (Bannerman and Callender, 1961). This form of the disorder is also known as thalassaemia intermedia. The milder symptomatic form of the disorder was known as "Malatia Rietti-Greppi-Micheli" by the early Italian writers. The greatest proportion of individuals are asymptomatic and may never be diagnosed.

B. RADIOLOGICAL MANIFESTATIONS

Patients with thalassaemia intermedia, the most severe form of the heterozygous state, may have the roentgenographic findings characteristic of thalassaemia major, while those with a mild phenotypic expression of the gene have completely normal radiographs.

C. HAEMATOLOGICAL MANIFESTATIONS

Most thalassaemia heterozygotes are not anaemic. Characteristically one finds a reduced mean cell volume (MCV), a low mean cell haemoglobin (MCH) with a somewhat raised red cell count.

Osmotic fragility tends to be reduced to varying degrees, which is amplified by incubating for 24 hours.

Peripheral blood smear findings vary from normal to changes similar to those seen in thalassaemia major. Red cell changes include hypochromia, microcytosis, poikilocytosis, basophilic stippling and the presence of target cells. Abnormalities tend to be more severe in the Mediterranean, as opposed to the Negro form of the disorder.

Red cell survival time is unimpaired, though ^{51}Cr half-life times have been reported as marginally below normal (Malamos, et al, 1961).

D. COMPLICATIONS

Complications occur essentially in the severely affected thalassaemia heterozygote. These include gall stones and chronic leg ulcers. Patients with thalassaemia minor may be significantly anaemic during pregnancy. Folic acid, as well as iron deficiency, may exacerbate the natural tendency to anaemia.

E. PROGNOSIS

The prognosis for most thalassaemia heterozygotes is complete normality. In patients with thalassaemia intermedia the life span will be variably decreased.

F. HAEMOGLOBIN PATTERN

1. HAEMOGLOBIN A_2

The most specific and characteristic finding in beta-thalassaemia heterozygotes is a raised level of haemoglobin A_2. This was first observed by Kunkel, et al (1957), who found a mean level of 5,11% ($\pm$ 1,35%) in 34 thalassaemia heterozygotes, as compared to 2,54% ($\pm$ 0,35%) in 300 normal individuals. Raised haemoglobin A_2 is found otherwise only in some of the unstable haemoglobinopathies and therefore this finding is an important diagnostic parameter (Weatherall and Clegg, 1972).

Iron deficiency has been found to reduce the haemoglobin A_2 level which then rises on correcting the iron deficiency. This can thus mask a raised haemoglobin A_2 (Wasi, Disthasonghan and Na-Nakorn, 1968).

Following the investigation of chain variants, it has been found that there is increased delta-chain production at both loci in a patient heterozygous for beta-thalassaemia although only one of beta-chain loci is affected (Huisman, Punt and Schaad, 1961).

Significant intrafamilial segregation for levels of haemoglobin A_2 in beta-thalassaemia heterozygotes has been noted (Weatherall, 1964). This suggests a definite genetic factor in the level of haemoglobin A_2.

It has been noted that there is no correlation between the haemoglobin A_2 level and the total haemoglobin level, packed cell volume, red cell abnormalities, haemoglobin F or any other associated haematological finding (Fessas, 1959).

2. HAEMOGLOBIN F

Fifty per cent of thalassaemia heterozygotes have no increase of haemoglobin F while the remainder have levels between 1,5-5%. However, occasional cases have been reported with haemoglobin F in excess of 5% (Weatherall, 1964).

The haemoglobin F is distributed quite heterogenously within the red cells when assessed by the Kleihauer technique (Kleihauer, Braun and Betke, 1957).

As with haemoglobin A_2, there is no correlation between the haemoglobin F level and red cell morphology, severity of anaemia or level of haemoglobin A_2. There is also significant intrafamilial segregation for haemoglobin F values.

DELTA-BETA-THALASSAEMIAS

There is a defect of both beta and delta-chain synthesis in delta-beta-thalassaemia. The diagnosis is thus dependent on haemoglobin F values and haematological parameters. The delta-beta-thalassaemias were formerly known as F-thalassaemia, normal haemoglobin A_2 or beta-thalassaemia type 2.

III. HOMOZYGOUS DELTA-BETA-THALASSAEMIA

A. CLINICAL FINDINGS

This disorder is not as severe as homozygous beta-thalassaemia and survival into adult life can be expected. Clinically, one finds a moderate anaemia, mild jaundice and splenomegaly. These findings are not invariable.

B. HAEMATOLOGICAL FINDINGS

Moderate anaemia with a haemoglobin in the 9-12 g% range is typical. The MCV and MCH are reduced. Peripheral blood smear is similar to that of thalassaemia intermedia.

C. HAEMOGLOBIN PATTERN

There is no beta or delta-chain synthesis. The haemoglobin thus consists entirely of haemoglobin F.

IV. HETEROZYGOUS DELTA-BETA-THALASSAEMIA

Delta-beta-thalassaemia carriers are clinically normal. Haematologically they have the same mild abnormalities as in beta-thalassaemia heterozygotes.

A. HAEMOGLOBIN PATTERN

Haemoglobin A_2 is within normal limits. Haemoglobin F is usually increased in the 5 to 20% range (Zuelzer, Robinson and Booker, 1961). As with the beta-thalassaemia there is intrafamilial segregation of

haemoglobin F levels.

The diagnosis of this disorder, however, may be difficult as occasionally individuals may not have the characteristic increase in HbF and have normal levels for both HbA_2 and HbF (Weatherall and Clegg, 1972; Fessas and Loukopoulos, 1974, and Silvestroni, et al, 1978). These heterozygotes are usually diagnosed ultimately on alpha/beta chain ratio analysis during the investigation of a child with homozygous beta-thalassaemia.

The following thalassaemia syndromes are found in association with abnormal haemoglobins:

Heterozygosity for both delta-beta and beta-thalassaemia (Stomatoyannopoulos, Fessas and Papayannopoulou, 1969)
Haemoglobin S-beta-thalassaemia (Silvestroni and Bianco, 1946)
Haemoglobin S-delta-beta-thalassaemia
Haemoglobin C-beta-thalassaemia (Dunston, et al, 1972)
Haemoglobin E thalassaemia (Wasi, et al, 1969)
Haemoglobin D-beta-thalassaemia (Hynes and Lehmann,1956)
Haemoglobin (Schwartz, et al, 1957)
Haemoglobin J-beta-thalassaemia (Sydenstryker, et al, 1961)

NON ALPHA-CHAIN FUSION SYNDROMES

Haemoglobin Lepore is the classical example of the non alpha-chain fusion syndromes. It was discovered in 1958 (Gerald and Diamond) and defined chemically by Baglioni in 1962. Baglioni suggested that haemoglobin Lepore consisted of normal alpha-chain combined with chains comprising of both delta- and beta-chains. This he postulated resulted from unequal crossing at the closely linked delta and beta loci,

resulting in a delta-beta fusion gene. Haemoglobin Lepore is named after the Italian family in which it was first discovered.

V. HOMOZYGOUS HAEMOGLOBIN LEPORE

Clinical, radiological and haematological findings

The clinical, radiological and haematological findings in patients with the homozygous state for haemoglobin Lepore are similar and indistinguishable from homozygous beta-thalassaemia.

HAEMOGLOBIN ELECTROPHORETIC PATTERN

On electrophoresis haemoglobin Lepore is slow-moving, migrating to a position similar to haemoglobin S. Characteristically, patients have 70-90% haemoglobin F and 10-30% of haemoglobin Lepore. There is a total absence of haemoglobin A and A_2.

VI. HETEROZYGOUS HAEMOGLOBIN LEPORE

Clinical and haematological findings

Patients with heterozygous haemoglobin Lepore are asymptomatic. Haematologic changes are similar to those of mild heterozygous beta-thalassaemia.

HAEMOGLOBIN ELECTROPHORETIC PATTERN

Individuals heterozygous for haemoglobin Lepore have 5-15% haemoglobin Lepore, 1,5-15% haemoglobin F and normal levels of haemoglobin A_2.

HEREDITARY PERSISTENCE OF FETAL HAEMOGLOBIN

Hereditary persistence of fetal haemoglobin (HPFH) was first described by Eddington and Lehmann in 1955. It is a condition characterized by the synthesis of haemoglobin F into adult life without any major haematological abnormalities. It is presently felt that HPFH is an extremely mild form of thalassaemia with good compensation of decreased beta-chain production by gamma chains, resulting in minimal chain imbalance (Weatherall and Clegg, 1972).

VII. HOMOGENOUS DISTRIBUTION OF FETAL HAEMOGLOBIN

Negro type HPFH
Homozygous state
Clinical findings

Patients with this disorder are usually diagnosed when being treated for an intercurrent illness. They tend to be mildly polycythaemic. This is probably due to the increased oxygen affinity of haemoglobin F.

HAEMATOLOGICAL FINDINGS

Peripheral blood smear shows minor abnormalities. These include anisopoikilocytosis, microcytosis and many target cells.

HAEMOGLOBIN PATTERN

Haemoglobin consists solely of haemoglobin F with haemoglobins A and A_2 being totally absent.

Kan, et al (1975), utilizing cDNA x DNA hybridization studies, have shown HPFH results from a deletion of the beta-gene.

VIII. HETEROZYGOUS STATE

There are no clinical or haematological abnormalities associated in patients with this disorder. Individuals have levels of fetal haemoglobin ranging from 15 to 35%. There tends to be intra-familial segregation of fetal haemoglobin levels.

HPFH has been well reported in association with haemoglobin S and C. There have been minimal clinical problems with these individuals.

HPFH, in association with beta-thalassaemia, has resulted in the clinical and haematological picture of beta-thalassaemia minor.

IX. GREEK TYPE

No patients have as of yet been reported in the homozygous state.

HETEROZYGOUS STATE

Individuals with this disorder are clinically and haematologically normal. Haemoglobin F levels range from 10 to 20%. A further difference from the Negro type is a high level of haemoglobin A_2.

HAEMOGLOBIN KENYA

Haemoglobin Kenya has non alpha-chains consisting of the N-terminal residues of the gamma-chain fused to the C- terminal residues of the beta-chain. This has probably resulted from unequal crossing over, with the loss of the whole of the delta-chain locus and those portions

of the gamma- and beta-loci coding for the C- terminal end of the gamma-chain and the N- terminal end of the beta-chain, respectively. Heterozygotes have about 10% haemoglobin F.

X. HETEROGENOUS DISTRIBUTION OF FETAL HAEMOGLOBIN

Individuals with this disorder have no clinical or haematological abnormalities. There is heterogeneity of the fetal haemoglobin among the red cells but no detectable globin-chain imbalance.

CHAPTER 9

GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY

Glucose-6-phosphate dehydrogenase (G-6-PD) is an enzyme occurring in many tissues including red blood cells, white blood cells, platelets, liver, spleen, adrenal cortex, lens and saliva. However, it is in red cells that a deficiency of this enzyme has important clinical implications and it will be discussed further only in this context. Studies on red cell G-6-PD are difficult, as although the level of activity of the enzyme is high, the enzyme itself constitutes less than 0,01% of the protein of human red cells. By contrast, haemoglobin forms 96% of the red cell protein.

A. PHYSIOLOGY OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE

In the normal red cell, 90% of glycolysis occurs through the anaerobic Emden-Meyerhof pathway with the production of the high energy product adenosine triphosphate. Reduced nicatinamide adenine dinucleotide phosphate (NADPH), a product of this pathway, supplies the energy for the reduction of methaemoglobin. The remaining 10% of red cell glycolysis occurs through the aerobic hexosemonophosphate (HMP) shunt pathway. This pathway has the only two reactions available to a cell without mitochondria (as in a mature red cell) for producing NADPH. The first reaction which involves the oxidation of glucose-6-phosphate (G6P) to 6 phosphogluconate (6PG), is catalysed by G-6-PD. The second reaction catalized by 6-phosphogluconate dehydrogenase (6PGD), cannot occur in the absence of the first. Thus a deficiency of G-6-PD will result in a reduction of NADPH from both sources.

Glutathione is a tripeptide of glutamic acid, glycine and the sulphydryl-bearing amino acid, cysteine. The sulphydryl group of cysteine allows glutathione to exist in a reduced form (GSH) or an oxidised form (GSSG) when 2 molecules of glutathione are united through a disulphide bridge with the loss of 2 hydrogen molecules.

Glutathione reductase, an enzyme, and NADPH, the co-enzyme which supplies the electrons, are necessary for the reduction of oxidised glutathione. Glutathione, a co-factor of glyoxylase, apparently plays an important role in maintaining both normal oxidation-reduction conditions and the integrity of sulphydryl groups of proteins within the red cell. It is known that acute haemolysis in G-6-PD deficiency does not occur until GSH is nearly exhausted. It has been suggested that this haemolysis is associated with loss of membrane sulphydryl groups and the formation of haemoglobin denaturation products which appear as Heinz bodies (Jacob and Jandl, 1962).

B. HETEROGENEITY OF G-6-PD DEFICIENCY

The heterogeneity of glucose-6-phosphate dehydrogenase is well recognised. Numerous variants of G-6-PD have been reported which differ greatly in enzyme activity and other properties. By utilizing electrophoretic mobility, thermostability, catalytic properties, pH activity and the Michaelis constant (which is defined as the concentration of substrate that allows the enzyme to function at half-maximal velocity and is a measure of how low the substrate concentration must be without seriously impairing the function of an enzyme), more than 100 variants of G-6-PD have been detected. In this way, it has been found that individuals with the manifestations of G-6-PD deficiency may quantitatively have sufficient G-6-PD which is, however,

qualitatively at fault.

Individuals with G-6-PD deficiency can be placed into two broad groups - those having G-6-PDA, the negro variety, and those having the Mediterranean type, G-6-PD Mediterranean. The first difference noted between the two was that the level of enzymatic activity was decreased to a far greater extent in the Mediterranean variety. These divisions are not exclusive to population groups, and occasionally people of Mediterranean origin may have the mild form of G-6-PD deficiency (the Negro variety).

(1) NEGRO VARIETY - G-6-PD DEFICIENCY

This entity was first recognised in Negroes of the American Military forces, who on receiving prophylactic antimalarial therapy, developed a sensitivity reaction. Classically, 2 to 3 days after receiving primaquine (an 8-aminoquinoline), the patient's haematocrit would begin to fall and they would develop signs and symptoms of varying severity. Problems ranged from minor symptoms to more severe haemolytic attacks characterised by abdominal pain, haemoglobinuria, jaundice and anaemia. However, even if the drug therapy was continued, it was discovered that the course of the disease was self-limiting and that it was only the older cell population which was haemolysed (Butler, Dern and Alving, 1954). A new steady state is reached in which the remaining red cells are gradually destroyed as they become older.

Starch gel electrophoresis revealed 2 varieties of G-6-PD in non-deficient American Negroes. These were designated G-6-PDA and G-6-PDB, the latter is the same type as that which occurs in normal individuals of all race groups, while G-6-PDA is largely confined to Negroes. It

moves faster electrophoretically and in distinction to G-6-PDB, has been demonstrated by amino acid analysis to have aspartic acid replacing asparagine (Yoshida, 1967).

The variety of G-6-PD occurring in deficient American Negroes has the same electrophoretic mobility as G-6-PDA and hence has been designated G-6-PDA-. It was found that the enzymatic activity of G-6-PDB, A and A- is similar in reticulocytes but markedly different in older cell populations. Thus it has been suggested that G-6-PDA- may be analogous to some of the abnormal haemoglobins which, because of amino acid substitution, are less stable than normal haemoglobin molecules (Giblett and Eloise, 1969).

(2) MEDITERRANEAN VARIETY

G-6-PD deficiency of the Mediterranean type (G-6-PDB-) tends to occur largely in Southern European countries, the Middle East and countries of south east Asia (Allison, 1963; Szeinberg, 1963). Clinically, individuals in this group vary from being mildly affected on exposure to certain drugs, to those suffering from the more severe congenital non-spherocytic haemolytic disease (CNSHD). This latter group, besides suffering from acute haemolysis on exposure to the implicated medicines, also tend to have a chronic haemolytic disease with chronic anaemia, jaundice and possibly gallstones and splenomegaly. They have marked decrease in red cell viability and elevated reticulocyte levels. Differences between G-6-PD of the Mediterranean and Negro type include lower red cell G-6-PD activity in the Mediterranean variety, 2-7% of normal as opposed to 10-15% of normal in Negroes (Kirkman, Schettini and Pickard, 1964). Cells of all ages are destroyed on

ingestion of primaquine with the former but not in the latter; G-6-PD deficiency may be manifested in other tissues such as white cells, as opposed to solely red cells, and patients with G-6-PD deficiency of the Mediterranean variety may suffer from favism and neonatal jaundice, whereas in the Negro variety this is unlikely.

Favism, the acute haemolysis which occurs after ingestion of the bean, Vicia faba, as mentioned previously, occurs in patients with G-6-PD deficiency of the Mediterranean type. However, not all individuals with this variety of G-6-PD deficiency suffer from favism, which appears to have an additional genetic aspect (Stamatoyannopoulos, et al, 1966).

The electrophoretic mobility of G-6-PD in patients of Mediterranean origin is the same as those of normal Caucasians and Negroes, and hence has been designated G-6-PDB-. Through using specialized chromatography in a calcium phosphate gel column, G-6-PDB- has been separated from G-6-PDB (Yoshida, Stamatoyannopoulos and Motulsky, 1967). Doxiadis, et al (1964) have found on the island of Lesbos that the risk of severe jaundice of the newborn due to G-6-PD deficiency is increased in comparison to other areas in Greece. In addition the incidence of neonatal jaundice, not due to G-6-PD deficiency or ABO incompatibility, was significantly higher on Lesbos as opposed to other regions in Greece. This additional icterogenic factor has not been identified but it seems possible that it has a genetic basis.

POTENTIALLY HAEMOLYTIC DRUGS IN G-6-PD DEFICIENCY

ANTIMALARIALS

PRIMAQUINE
PAMAQUINE
PENTAQUINE
PLASMOQUINE
QUINACRIN (Atabrine)
QUININE
CHLOROQUINE

SULPHONAMIDES

SULPHANILAMIDE
SULPHAMETHOXYPYRIDAZINE (Kynes, Midical)
SALICYLAZOSULPHAPYRIDINE (Azulfidine)
SULPHISOXAZOLE (Gantrisin)

SULPHONES

SULPHOXONE (Diasone)
THIAZOLSULPHONE (Promizone)
DIAMINODIPHENYL SULPHONE (DDS)
SULPHAPYRIDINE
SULPHAPYRIMIDINE
SULPHATHIAZOLE

ANTIPYRETICS AND ANALGESICS

ACETYLSALICYLIC ACID
ACETANILID
ACETOPHENETIDIN (Phenacetin)
ANTIPYRINE
AMINOPYRINE

NITROFURANS

NITROFURANTOIN (Furadantin)
FURAZOLIDONE (Furoxone)
NITROFURAZONE (Furacin)

OTHER DRUGS

DIMERCAPROL (BAL)
METRYLENE BLUE
NAPHTHALENE (Moth Balls)
AMINOSALICYLIC ACID
PHENYLHYDRAZINE
ACETYLPHENYLHYDRAZINE (Pyrodin)
PROBENECID (Benemid)
VITAMIN K (Water-soluble analogues)
TRINITROTOLUENE
PARA-AMINO-SALICYLIC ACID
QUINIDINE
PARA-AMINO PHENOL
PARA-HYDROXYACETANILID
PARA-AMINOBENZOIC ACID
PRONESTYL
DIPNENYLHYDRAMINE
CHLORAMPHENICOL
ASCORBIC ACID

C. GENETIC ASPECTS OF G-6-PD DEFICIENCY

The pattern of inheritance of G-6-PD deficiency was not obvious when the intiial investigations into the disorder were being performed. The reason for this was that persons suffering from primaquine induced haemolysis were mostly men, namely soldiers and prison volunteers. Thus it was not until extensive family studies were undertaken that it became obvious that G-6-PD deficiency was inherited in an X-linked manner.

As discussed earlier, there is great genetic heterogeneity within G-6-PD deficiency. This heterogeneity is illustrated by varying clinical expression of the disorder, not only between, but also within various population groups; differing levels of activity of the enzymes between Caucasian and Negro G-6-PD deficient individuals and variations in electrophoretic migration. Differences in thermostability, the Michaelis constant and catalytic function further help in categorizing the nature of the enzyme.

In 1960, Motulsky noted that G-6-PD deficiency occurs in a wide belt of tropical Africa, the Mediterranean area, the Near East, India, south east Asia and the Phillipines. He further took cognisance of the fact that the geographic distribution of G-6-PD deficiency coincided with that of Plasmodium falciparum, the organism causing the most severe form of malaria, and sickle cell haemoglobin. Based on these findings, he made the reasonable suggestion that G-6-PD deficiency might provide some selective advantage against falciparum malaria. Allison (1960) came to similar conclusions, noting the high incidence of G-6-PD deficiency in East Africa where malaria is endemic.

Within local areas in Greece (Stamatoyannopoulos and Fessas, 1964),

Cyprus (Plato, Rucknagel and Gershowitz, 1964), and Sardinia (Siniscalco, et al, 1966) the correlation between the gene frequency of G-6-PD deficiency and the endemicity of falciparum malaria has been well demonstrated. The work done by Siniscalco, et al (1966) on Sardinia was particularly illustrative as malaria was endemic there until recently, while the population has been relatively stable. A significantly higher incidency of G-6-PD deficiency was found in the coastal areas where malaria was endemic in comparison to the malaria-free areas of higher altitude. Contributory evidence is the absence of G-6-PD deficiency in people occupying non-malarious regions of the world, and the demonstration that Plasmodium falciparum parasite rates and densities were lower in young African children with G-6-PD deficiency than in children with normal enzyme levels.

Conflicting data has been reported by Kidson and Gorman (1962) who noted the low incidency of G-6-PD deficiency in Malays and Indonesians, while the surrounding populations of Thailand, the Phillipines and coastal New Guinea have a high incidence. They therefore questioned the validity of the hypothesis that people with G-6-PD deficiency had a selective advantage over those with normal G-6-PD in malaria endemic areas. They then undertook population studies in New Britain and New Guinea, and found that various linguistic groups living under similar conditions of nutrition and parasitic infestation, and living close to one another, had significantly varying incidences of G-6-PD deficiency.

The most convincing evidence for the advantage of G-6-PD deficiency against falciparum malaria comes from Luzzatto, Usanga and Reddy (1969) who utilized the phenomenon of X chromosomal inactivation, to demonstrate that G-6-PD deficient red cells contain fewer malarial parasites than

normal cells. Due to X chromosomal inactivation, a female heterozygote for G-6-PD deficiency would have 2 cell lines - 1 G-6-PD deficient and 1 normal. These workers examined females who were heterozygous for G-6-PD deficiency, and who had been exposed to malaria in natural circumstances, and demonstrated significantly higher parasitic counts in the normal compared to the G-6-PD deficient cells.

G-6-PD has been extensively utilised for the demonstration of random X chromosomal inactivation in accordance with the Lyon hypothesis (Lyon, 1961). G-6-PD has been particularly valuable in this field as it can be measured quantitatively by electrophoretic techniques. Davidson, Mitowsky and Childs (1963) cultured fibroblasts from the skin of a female heterozygous for 2 electrophoretic types of G-6-PD and showed the expected mixture of two electrophoretic bands in the original culture. These workers then prepared a series of subcultures, each one having been derived from a single cell of the original cell population. Only 1 electrophoretic type of G-6-PD was found in each clone. This experiment demonstrated that only 1 of the 2 X chromosomes was functional, as would be expected from the Lyon hypothesis.

Linkage on the X Chromosome

Good evidence exists that the locus for G-6-PD deficiency is closely linked to those for protan and deutan colour blindness. The G-6-PD locus may be at a recombination fraction of only 0,03-0,04 from the deutan locus (Porter, Schulze and McKusick, 1962; Siniscalco, Filippi and Latte, 1964) and 0,07 from the protan locus (Motulsky, 1968).

In brief it seems that the region on the X chromosome containing the loci for G-6-PD haemophilia A and B, colour blindness and possibly X m

and the Hunter syndrome is distant from the region containing the loci for X g, X-linked icthyosis, occular albinism and possibly Fabry's disease (Kirkman, 1971).

CHAPTER 10

SICKLE CELL HAEMOGLOBINOPATHIES

A. HISTORICAL INTRODUCTION

Lehmann and Huntsman (1974) noted that in 1910 a Chicago physician reported peculiar elongated and "sickle-shaped" cells in the blood of a West Indian student. They also commented that in 1927 Hahn and Gillespie discovered that sickling only occurred at a low oxygen tension. At roughly the same time Neel and Beet independently managed to show that sickle cell anaemia is the homozygous state, while the sickle cell trait is the heterozygous state. Subsequently the discovery by Pauling, et al (1949) that haemoglobin S (HbS) had a differing isoelectric point from HbA, allowed Pauling to formulate the concept of molecular disease. By means of peptide mapping it was demonstrated that the chemical difference in haemoglobin S (HbS) is the substitution of valine for glutamic acid at the 6 position of the beta-chain (Hunt and Ingram, 1959).

B. PATHOGENESIS

The pathological and hence clinical features of sickle cell disease are dependent on the substitution of valine for glutamic acid in the beta-chain of the haemoglobin molecule. It has been suggested that the globin chain with the altered amino acid adheres to a complementary section on the alpha- or beta-chain of a neighbouring molecule (Hunt and Ingram 1959). Under conditions of deoxygenation crystals are formed which are similar in shape to sickled cells (Harris, 1950). As these crystals grow in length they form tactoids which disappear on reoxygenation (Perutz and Lehmann,1968). If this happens repeatedly,

irreversible sickling eventually occurs, with resultant cellular destruction.

The level of haemoglobin S (HbS) in the red cell is important in determining whether sickling will occur, and levels of less than 40% of the total haemoglobin are usually not associated with sickling (Weatherall and Clegg, 1972). Flow rate, and thus viscosity, are also important factors in initiating sickling. It takes 2 to 3 minutes for sickling to become fully established and with a further reduction in flow rate the oxygen tension will drop further, with resultant increase in sickling and viscosity, which in turn increases the stasis and decreases the flow rate. Thus a vicious circle is set up. In addition, a drop in pH which tends to occur with a sluggish circulation, enhances sickling.

C. SICKLE CELL ANAEMIA (homozygous state)

1. Clinical Features:

Infants are normal until beta-chain synthesis is fully established at about 4 to 6 months. Characteristic early findings include pallor, mild jaundice and splenomegaly and frontal bossing of the skull. Children are usually smaller than their normal peers but as their epiphyses fuse later than normal they develop an eunuchoidal habitus.

2. Course and Complications:

There is marked variability in the life span of patients with sickle cell anaemia, depending greatly on environment, though very few have a normal lifespan. In tropical Africa 50% of infants die in the first few years of life, while fewer than 10% reach adult-

hood (Weatherall and Clegg, 1972).

In childhood painful infarcts of the hands and feet may occur, while in older youths aseptic necrosis of the femoral head may develop and be misdiagnosed as Perthe's disease. Infarctions may also occur in the spleen, liver, lungs, intestine and central nervous system. Other complications include chronic leg ulcers, which occur in 50% of Jamaican patients (Lehmann and Huntsman, 1974) and salmonella infections, particularly septicaemia and osteomyelitis. Haemolytic and aplastic crises are life threatening.

3. Laboratory Findings

The haemoglobin level is usually between 5 and 9 grams per 100 ml. Packed cell volume lies in the 20 to 25% range and the reticulocyte count is markedly elevated in the 15 to 25% range. Peripheral blood smear shows some partially sickled cells with occasional target cells and nucleated red cells.

Paper electrophoresis reveals predominantly the slow moving haemoglobin S with a normal haemoglobin A_2 level and a variable haemoglobin F level which is usually in the 5 to 10% range.

4. Treatment and Prophylaxis

Management of a sickle cell infarctive crisis involves maintaining hydration, adequate analgesia, treating secondary infections and keeping the patient warm to avoid vascular stasis.

Magnesium sulphate given intravenously has been used with some success. Magnesium, a calcium antagonist in the clotting process,

partially delays the formation of thrombin. It also has a vasodilatory effect and discourages sludging of sickle cells.

Sickling prophylaxis is aimed at preventing factors which promote sickling from occurring. Keeping the blood pH as alkaline as possible by giving 2 to 3 grams of sodium bicarbonate in diluted doses during the day, is of use. This is of added importance during infections, especially of the respiratory tract which may result in acidosis.

Care is required during anaesthesia to prevent anoxia, dehydration and circulatory stasis by cooling. Likewise, pregnancy and labour must be supervised where possible at institutions where personnel are aware of the dangers facing the patient.

Due to decreased red cell survival time and increased haemolysis an anaemia is usually present. As there may be problems due to iron overload and resultant haemachromatosis, the anaemia is best treated conservatively with folic acid. Where studies indicate iron deficiency this may be given. Blood transfusion is indicated in the occurrence of crises of an aplastic or sequestration nature (where red cells are sequestrated in an internal organ) and where there is a risk of pulmonary oedema and cardiac failure.

D. SICKLE CELL TRAIT (Heterozygous for HbS)

Individuals with the sickle trait are usually clinically asymptomatic. However, under circumstances where there is decreased oxygen tension, sickling may occur. These include high altitude flying in an unpressurized aircraft, hurried, inadequate anaesthesia and, rarely,

severe pneumonia. Females may be discovered to have the sickle cell trait during pregnancy when they are found to have an unexplained anaemia. Haematological parameters and peripheral blood smear are normal in patients without complications. Paper electrophoresis reveals normal quantities of haemoglobins F and A_2, haemoglobin A and the slower moving haemoglobin S.

E. OTHER HAEMOGLOBINOPATHIES

Haemoglobin S may be found in association with other abnormal haemoglobins and thalassaemia. When HbS is found in association with some abnormal haemoglobin such as HbK, there may be no clinical or haematological abnormality, whereas with HbC a moderate haemolytic anaemia will be present.

Similarly, haemoglobin S occurring with the thalassaemia gene may give rise to a severe sickle cell anaemia or a completely symptom free state which is only discovered on routine haemoglobin electrophoresis.

CHAPTER 11

HEREDITARY SPHEROCYTOSIS

Hereditary spherocytosis (HS) is an hereditary haemolytic disorder characterized by a variable degree of anaemia, splenomegaly, spherocytosis and increased osmotic fragility of the red cells. Jaundice, often of a mild degree, which is unaccompanied by bile in the urine (acholuric) is an inconstant feature.

A. HISTORY

Vanlair and Masias (1871) were the first authors to report a patient with what was probably hereditary spherocytosis. They described an individual with anaemia, acholuric jaundice, splenomegaly and abnormally small erythrocytes and termed the disorder microcythaemia. Wilson and Stanley (1890, 1893) reported a kindred in which six members had a chronic condition characterized by splenomegaly, and a predisposition to jaundice. Minowski (1900) gave a detailed description of hereditary spherocytosis and drew attention to the fact that the osmotic fragility of the red cells is increased in the disorder. This observation is now used as an important diagnostic parameter for hereditary spherocytosis.

B. PREVALENCE

Hereditary spherocytosis is probably the commonest haemolytic anaemia amongst people of northern European origin. The prevalence is quoted as 2,2 per 10 000 and about 25% of the cases are apparently sporadic (McKusick, 1975). Though found in the African Negro, the condition is rare amongst this group (Kline and Wolman, 1957; Metz,

1959).

Hereditary spherocytosis has been diagnosed soon after birth (Truceo and Brown, 1967), and at ages ranging through to old age. Diagnosis is most often made between 10 and 45 years.

C. GENETICS

Hereditary spherocytosis is inherited in an autosomal dominant fashion. Phenotypic expression is often extremely variable and in the same family some members may suffer from a chronic haemolytic anaemia while others may be asymptomatic and only diagnosed in population surveys or family studies. Homozygosity has been suspected in a family where of 13 affected children 9 were physically or mentally defective (Bernard as quoted by Wintrobe, 1974).

D. CLINICAL MANIFESTATIONS

Patients may suffer from a chronic haemolytic anaemia of moderately severe extent or have very mild symptoms. These two extremes may occur in the same kindred. Clinical findings include anaemia with mild icterus which increases with fatigue, cold, emotion and pregnancy (Wintrobe, et al, 1974). Periodically, the jaundice and anaemia may worsen acutely with episodes of vomiting, fever and abdominal pain which may last from 5 to 14 days. This is said to be a haemolytic crisis; however, findings in one study suggest that such attacks may be acute aplastic crises (Owen, 1948).

The haemolysis results in a raised serum bilirubin level and increased pigment (gall) stone formation. For this reason, cholelithiasis is a common presenting symptom and this complication occurs in 43-85% of

people with hereditary spherocytosis (Wintrobe, et al, 1974). Splenomegaly is found in about 80% of affected individuals and the spleen may be massively enlarged. Dermatological complications include a chronic dermatosis and ankle pigmentation as a result of chronic leg ulcers.

E. RADIOLOGICAL FINDINGS

Thickening and striations of the frontal and parietal bones with the characteristic tower skull ("turmschädel") are the commonest radiographic findings. Skeletal changes of a less severe nature but similar to those found in sickle cell anaemia and thalassaemia may be present. Heterotopic bone deposition is occasionally found.

F. HAEMATOLOGICAL FINDINGS

The haemoglobin concentration is usually at about 10g per 100 ml, although during a crisis it may fall to very low levels.

The MCHC is characteristically high (37 to 39g per cent). Reticulocytes are usually increased in number and values usually range from 5 to 20%. In the peripheral blood smear cells with a typical spherical configuration (spherocytes) are present in large numbers.

G. LABORATORY FINDINGS

Osmotic fragility of red cells in hypotonic saline is increased. This may occur only as a "tail" at low concentrations or after incubation for 24 hours.

Spontaneous autohaemolysis of blood after incubation at $37^{o}C$ occurs to

a greater extent than in patients suffering from an auto-immune haemolytic anaemic or normal individuals. In patients with hereditary spherocytosis, this increased autohaemolysis is corrected by the addition of glucose (Selwyn and Dacie, 1954). The Coombs test is usually negative and serum bilirubin is increased.

H. DIAGNOSIS

The diagnosis of hereditary spherocytosis in its typical clinical and familial background is relatively easy. However, in its mild form it may pose a diagnostic problem and require relatively extensive laboratory investigation.

Initial diagnostic features are anaemia, splenomegaly, unconjugated bilirubinaemia, increased urobilinogen (not bilirubin) in the urine and stools and reticulocytosis.

Peripheral blood smear reveals spherocytes and osmotic fragility is increased. Autohaemolytic studies show increased autohaemolysis corrected by the addition of glucose.

Recognition of the hereditary nature of the disorder provides additional diagnostic support.

I. PROGNOSIS

The prognosis varies with the severity of the disorder. Mildly affected patients have a normal lifespan and even individuals with a chronic anaemia may reach an advanced age. Death may occur during a crisis and may be related to cardiac decompensation or biliary tract disease (Diamond, 1938).

J. TREATMENT

Iron therapy is contra-indicated due to the risk of iron overload and resultant haemochromatosis. Similarly, blood transfusions should not be given except in the case of a haemolytic or aplastic crisis.

Folic acid deficiency may complicate the disorder and in these circumstances folate therapy should be utilized.

The single most useful procedure in hereditary spherocytosis is splenectomy. After splenectomy, the haemoglobin rises, serum bilirubin decreases, reticulocytosis decreases and red cell survival time increases. The osmotic fragility remains high and the peripheral smear still reveals spherocytes. Splenectomy is especially advisable in patients who are continuously anaemic, icteric, having had an aplastic or haemolytic crisis or suffering from cholelithiasis. Cholecystectomy for gallstones complicating hereditary spherocytosis should not be performed without splenectomy. However, this procedure is contra-indicated in infancy due to the increased risk of infection, and should be delayed until about 5 years of age (Burman, 1958).

K. PATHOPHYSIOLOGY

The precise nature of the membrane defect leading to increased haemolysis in hereditary spherocytosis has not been fully elucidated but several important observations have been made.

The spherical configuration of the red cells is similar to that of normal red cells which have been incubated in hypotonic saline and which are about to burst. However, in phlebotomized patients with hereditary spherocytosis whose red cells were hypochromic and thin, the

red cell survival time was not increased (Crosby and Conrad, 1960).

The red blood cell requires a constant supply of glucose. If there is a lack of glucose in vitro, adenosine triphosphate (ATP) becomes depleted and the mechanism for maintaining membrane impermeability to cations is lost. In hereditary spherocytosis, there is an increased sodium flux into the red cells (Wiley, 1972) which stimulates the adenosine triphosphatase system, resulting in ATP breakdown to provide energy for pumping the sodium out of the cell (Mohler, 1965). Adenosine diphosphate (ADP) and phosphate (P) are released and glycolysis is stimulated. Thus the effects of decreased glucose are exaggerated in red cells of hereditary spherocytosis. However, the increased sodium flux does not explain the accelerated red cell destruction in vivo, as after splenectomy this defect persists although red cell survival is essentially normal (Wintrobe, et al, 1974).

It has been noted that the total lipid content of red cells in hereditary spherocytosis is decreased while the proportions of the various lipids are normal (Reed and Swisher, 1966). After splenectomy, this abnormality is not as obvious (De Gier, et al, 1964). The increased loss of lipid has been said to be due to accelerated metabolism necessary for the more rapid pumping of sodium. Decreased lipid content results in diminished pliability of the red cells which renders them more liable to sequestration in the spleen.

Although certain structural abnormalities of the red cell membrane have been demonstrated by various workers, the exact nature of these anomalies has not been fully elucidated. The chief problem is that not all researchers in this field have been able to reproduce the same

results.

Evidence for an abnormal red cell membrane protein has been presented by Jacob, Amsden and White, 1971, 1972). However, this work has been questioned recently by Engehard, et al (1974) who found the protein to be normal. Similarly, an excess of negative charges of the component proteins has been implicated by some workers (Engelhardt, 1976) and refuted by others (Boivin and Galand, 1976; Johnson, 1978).

Abnormalities in the interaction of lipid and protein membrane have been shown by Kuiper and Livne (1972) but not by others (Zail and van der Hoek, 1975a). Calcium concentrations have shown to be abnormal by Feig and Basilian (1974) but not Zail and van der Hoek (1976).

The most recent direction of study has been into the role of membrane-bound protein kinase, an enzyme which by virtue of its catalysis of the phosphorylation of several membrane polypeptides has been postulated to effect cell shape and deformability. A significant decrease in cyclic AMP-dependent protein kinase activity in hereditary spherocytosis erythrocyte membrane has been demonstrated by some (Greenquist and Shohet, 1976; Matsumoto, Yawata and Jacob, 1977) but not confirmed by others (Zail and van der Hoek, 1975b, Beutler, Guinto and Johnsson, 1976).

SECTION IV

METHODOLOGY

CHAPTER 12

DEMOGRAPHY

1. BACKGROUND INFORMATION

Historical information was obtained with the aid of the Greek Consulate, Church and community of Cape Town. Early history of the Greek population in Cape Town was obtained from the teacher of the Greek community, together with a book written in 1923 on Greek peoples in Southern Africa (Nickolaides, 1923). In addition, older members of the community were consulted as to their personal history and that of their peers and progenitors. Statistical data on the population size, its movements and distribution was given by the same sources.

ASCERTAINMENT OF RESPONDENTS

A. REQUIREMENTS FOR STUDY OF GENE FREQUENCY

A random sample of the Greek community of Cape Town was required for this part of the investigation. Blood samples of 250 individuals, i.e. 5% of the Greek community were to be analysed. It was essential that these individuals were not first degree relatives, i.e. not siblings or parent/children. A major stumbling block proved to be the reluctance of members of the Greek community to accept venepuncture. Reasons for this appeared to be fear of pain, superstition, inconvenience and indifference.

SOURCE OF SUBJECTS

1. A circular was sent under the auspices of the Greek community committee to the members of the Greek community, explaining the aims of the study, and requesting volunteers for testing. The response to the circular was poor. However, the circular enlightened the community as to the nature of the work and facilitated subsequent contact.

2. Permission was granted by the Executive Committee of the Board of the Faculty of Medicine of the University of Cape Town to use Greek volunteers from the student body in the survey. The response from the students was also indifferent.

3. The South African Defence Force was approached, and the Surgeon-General gave permission for Greek servicemen to be tested.

4. Inpatients at Groote Schuur Hospital, excluding patients being treated or investigated for thalassaemia, and other haematological dis-

orders were investigated. This study also allowed the author to ascertain if there was any special pattern of non-haematological genetic disease in Greek people in Cape Town.

5. The Greek community of Cape Town come together on Sundays to watch Greek film shows. The author therefore decided this meeting would be a suitable occasion for him to speak to reasonably large numbers of Greek people. As a result, the author explained the reasons and aims of the study at 2 film showings. The results of this exercise were satisfactory. Thereafter, a slide was projected at subsequent film showings to remind members of the survey.

6. The largest number of people tested were randomly approached. Individuals thus tested would recommend friends and acquaintances who would be willing to be sampled. This process proved to be extremely time-consuming but worthwhile in terms of the results obtained. Much time was spent in travelling to subjects' homes, private businesses or place of work.

7. The ecclesiastical authorities were approached concerning the possibility of their referring couples for premarital testing. This would not only have been for the duration of the survey but as a continuing service to the Greek community. The church expressed appreciation of the work being done for the community but found itself unable to give practical assistance.

B. PATIENTS WITH THALASSAEMIA MAJOR

Two patients of Greek origin, with thalassaemia major, domiciled in Cape Town, were traced through the Peninsula medical services. These individuals were subjected to a full clinical, radiological and

haematological examination. In addition, cognisance was taken of special investigations which had been performed as part of their hospital investigations and management. Where possible, the families of these patients were investigated clinically and haematologically.

CHAPTER 13

CLINICAL AND LABORATORY TECHNIQUES

During the first meeting with the investigation subjects, a personal history including age, marital status, number of children and place of origin was elicited. A brief medical history was taken, with special note of details of previous anaemia, jaundice, gallstones and leg ulcers. A 20 ml specimen of blood was obtained by venepuncture from the antecubital veins and then taken within 2 hours to the Cape Provincial Blood Grouping Laboratories, under direction of Dr M C Botha, where it was analysed.

In view of the difficulty in obtaining respondents and satisfactory completion rates the author concentrated on this aspect of the survey. In all, the field work involved one year's full time and effort.

The author became fully conversant with the laboratory techniques which were used. However, in view of the amount of time and effort involved in obtaining the blood specimens and the logistics of the survey, the greatest proportion of the highly specialized analyses was performed by qualified medical technologists.

The following standard laboratory procedures were undertaken on the blood specimens;

1. Peripheral blood smears
   (a) red cell morphology
2. Supra-vitally stained preparations
   (a) reticulocytes
   (b) haemoglobin H inclusion bodies

3. Blood grouping, including the ABO, rhesus and Duffy systems.
4. Red cell parameters -
   (a) haemoglobin levels (Hb)
   (b) packed cell volume (PCV)
   (c) red cell count (RCC)
   (d) mean corpuscular haemoglobin concentration (MCHC)
   (e) mean corpuscular volume (MCV)
   (f) mean corpuscular haemoglobin (MCH)
5. Osmotic fragility
6. Electrophoresis
   (a) abnormal haemoglobins (Lehman and Ager, 1960)
   (b) quantitation of haemoglobin A_2 (Black, et al, 1960)
7. Quantitation of haemoglobin F (Singer, et al, 1951)
8. Brilliant cresyl blue dye test (Motulsky and Campbell-Kraut, 1961)

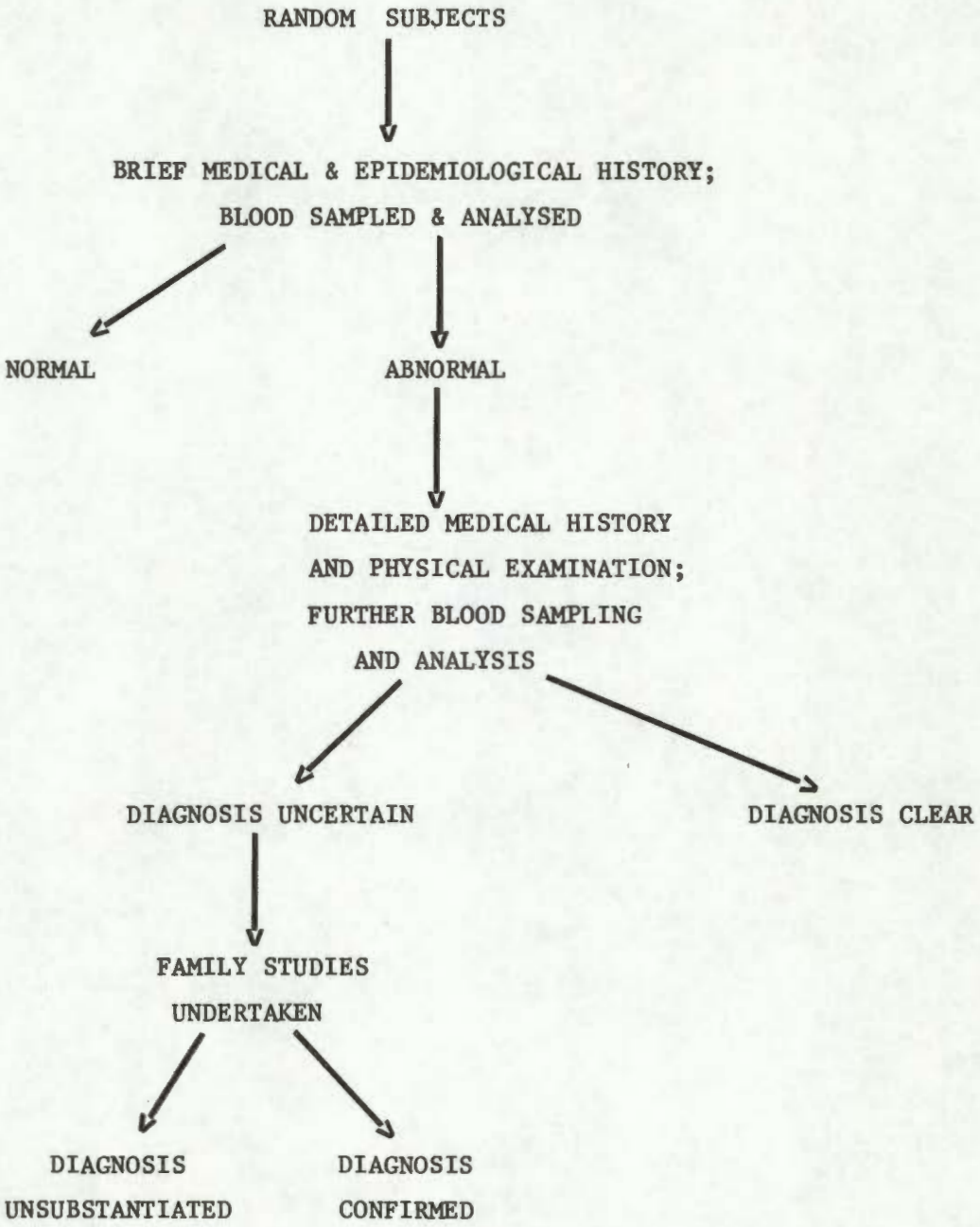
Laboratory investigations undertaken for special indications:

9. Methaemoglobin reduction test for G-6-PD deficiency (Bruwer, et al, 1960)
10. Solubility test for haemoglobin S (Itano, 1953)
11. Autohaemolysis studies if spherocytosis was suspected
12. Alpha/beta chain synthetic rates where alpha-thalassaemia carrier status was considered (Weatherall, et al, 1969).

Once individuals had been found to have a haematological abnormality, a more detailed history was obtained. Emphasis was laid on the family origins and medical problems, with special reference to symptoms or past findings of an anaemia. Thereafter, the author carried out a thorough medical examination, with particular emphasis on the haematological system, including the spleen. Where possible, members of the family were examined and tested. However, this proved impossible at times as many family members were still domiciled in Greece,

Fig. 13 - 1

INVESTIGATIVE SEQUENCE



Details of the laboratory methods for the procedures undertaken are given in Appendix A.

SECTION V

RESULTS

CHAPTER 14

DEMOGRAPHIC AND GENERAL RESULTS

The Greek population of Cape Town numbers about 5000 and of these 250 were randomly studied. Haematological investigations were carried out in this way in approximately 5% of primarily non-related individuals of this community.

A. AGE

The ages of the respondents ranged from 17 to 73 years in the following distribution (see Fig. 14 - 1).

10 - 20 years	13 respondents
20 - 30 years	57 respondents
30 - 40 years	67 respondents
40 - 50 years	61 respondents
50 - 60 years	31 respondents
60 -	21 respondents

B. SEX

Of the 250 individuals 150 (60%) were male and 100 (40%) were female.

C. OCCUPATION

Persons from a cross section of the Greek society were investigated and included labourers, shop assistants, artisans, students, businessmen, members of the diplomatic corps and professional people.

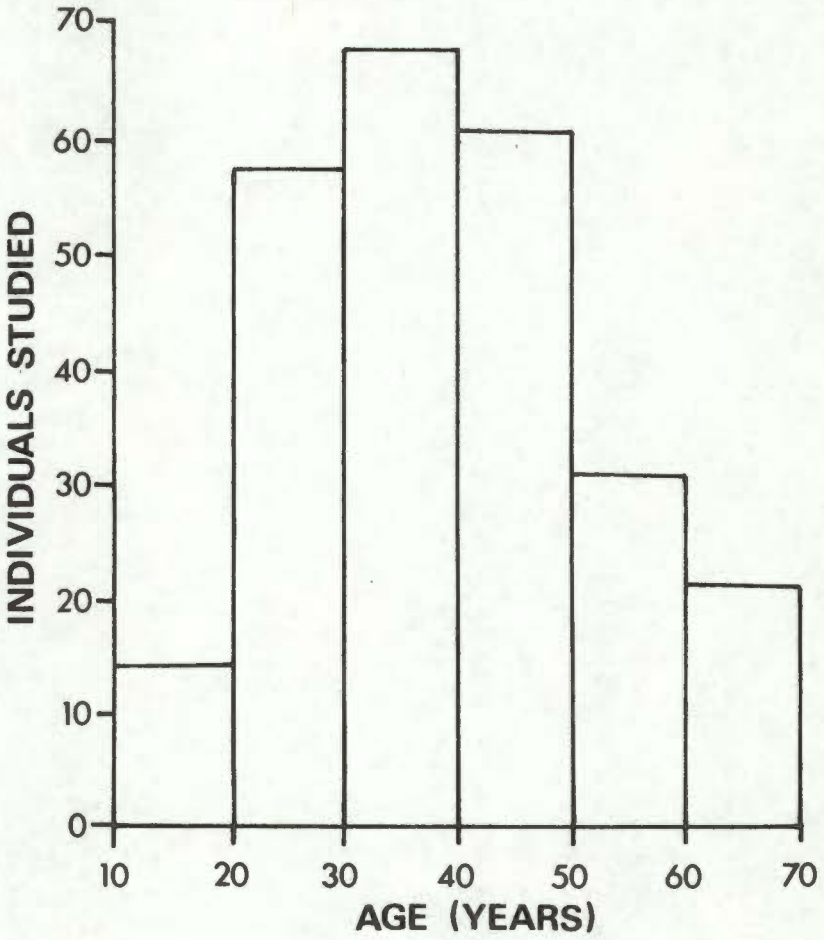


Fig. 14 - 1 Age distribution of respondents

D. DEMOGRAPHIC INFORMATION

The majority of the respondents originated from the Greek islands, especially the north-eastern Aegean islands. At least 27% had their antecedents in Lesbos. The reason for this distribution has already been discussed under the topic of Greeks in South Africa (Section 1, Chapter 4).

A detailed analysis of the origin of the subjects is shown below in Table 14 - 1 and illustrated in Fig. 14 - 2:-

TABLE 14 - 1

AGE DISTRIBUTION OF RESPONDENTS

	Numbers	Per cent
1. North-eastern Aegean Islands:	92	37
(a) Lesbos	67	27
(b) Lemnos	14	6
2. Central Greece:	39	15
3. Peloponnese:	37	15
4. Northern Greece:	12	5
5. Asia Minor:	12	5
6. Cyprus:	15	6
7. Other Greek Islands:	44	17
(a) South-eastern Aegean Islands	11	
(b) Ionian	18	
(c) Crete	10	
(d) Cyclades	5	

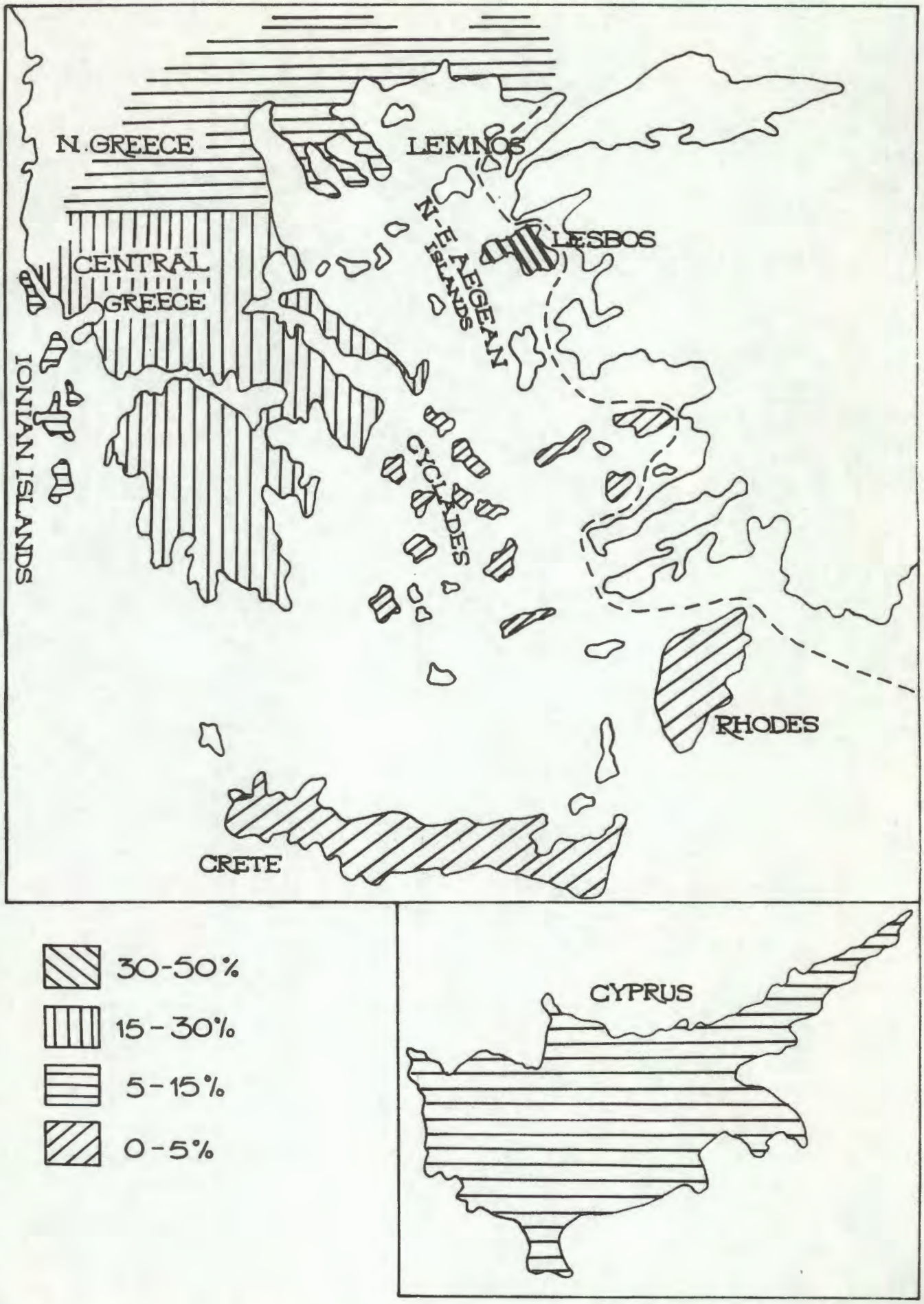


Fig. 14 - 2

DISTRIBUTION OF ORIGINS OF RESPONDENTS

E. OTHER GENETIC DISORDERS IN THE GREEK POPULATION OF CAPE TOWN

Whilst the haematological survey was being carried out, note was taken of the possible occurrence of other genetic conditions within the Greek population of Cape Town. One couple included in the survey had a child with Down's Syndrome. This child was the couple's first born and the mother was 18 years of age at the time of the birth. The mother subsequently gave birth to a normal child.

Diabetes and hypertension, both disorders with a genetic component, were found in the survey group but with an incidence probably in the range of that occurring in the White population as a whole in Cape Town.

No other genetic disorders were found in the Greek population of Cape Town.

CHAPTER 15

ALPHA-THALASSAEMIA

DIAGNOSTIC CRITERIA

A. Homozygous Alpha-Thalassaemia

The homozygous alpha-thalassaemia state results in hydrops fetalis and the diagnosis is straightforward where the clinician has a high index of suspicion. The gene for alpha-thalassaemia has a particularly high frequency in south-east Asia (Poortrakul, et al, 1970) and homozygous alpha-thalassaemia is considered in any hydropic birth where the parents have antecedents from this area. Confirmation of the diagnosis is by analysis of the cord (umbilical) blood.

B. Heterozygous Alpha-thalassaemia

The heterozygote may be asymptomatic or have stigmata of varying degrees characteristic of alpha-thalassaemia.

For laboratory confirmation of the diagnosis of the alpha-thalassaemia heterozygote the following are considered:

1. Red cell morphological abnormalities
2. Red cell parameter abnormalities, especially MCH and MCV
3. Red cell haemoglobin H inclusion bodies
4. Alpha/beta chain synthesis rate ratios
5. Haemoglobin Bart's in cord blood
6. Presence of haemoglobin H on electrophoresis

The clinical severity of the disorder depends on the number of abnormal genes, which in addition influences laboratory findings (see Chapter 7).

Hence, it may be reasonably easy to diagnose haemoglobin H disease in which 3 out of 4 genes are involved, but not the alpha-thalassaemia trait where only a single gene is implicated. The red cell morphology, MCV and MCH may be clearly abnormal in haemoglobin H disease but only be marginally abnormal in persons suffering from mild alpha-thalassaemia. Haemoglobin electrophoresis will show haemoglobin H in the former condition but nothing in the mild carrier state of alpha-thalassaemia.

A further indication that an individual may have heterozygous alpha-thalassaemia is the presence of typical red cell inclusion bodies. However, this finding is not diagnostic because as few as 2 red cells with inclusion bodies per 10 000 red cells can occur in heterozygous alpha-thalassaemia. Similarly, numerous inclusion bodies may be present in the absence of the disorder (Botha, 1977).

The easiest, and in fact sometimes the only time to diagnose the alpha-thalassaemia trait is at birth by demonstration of haemoglobin Bart's in the cord blood. In difficult diagnostic situations, family studies may be of use in deciding whether an individual carries alpha-thalassaemia. Finally, in equivocal cases the only certain method of diagnosing mild heterozygous alpha-thalassaemia is to measure alpha/beta chain synthesis rate ratios in order to demonstrate a decreased production of alpha chains.

INTRODUCTION

The difficulty in diagnosing alpha-thalassaemia has been discussed above and will be commented upon later.

During the survey no patients with hydrops fetalis (the result of homo-

zygous alpha-thalassaemia) were born and no history of a previous hydropic birth was obtained. Haemoglobin H disease, where 3 of the 4 genes are affected, and which is diagnosed by finding a typical H-band on electrophoresis was also not encountered.

However, in no less than 22 persons peripheral blood smear revealed typical haemoglobin H inclusion bodies. These ranged in number from 1/10 000 to 60/10 000. These red cell inclusion bodies are suggestive, but not indicative of heterozygous alpha-thalassaemia and therefore it was decided to undertake further studies in a proportion of these persons. As the relevant analyses to be done were extremely time consuming it was possible to investigate only a limited number of subjects in this survey. These selected for further investigation had a high proportion of inclusion bodies (more than 10/10 000) or those having red cell anomalies suggestive of the thalassaemia trait (decreased MCV and MCH) as well as any red cell inclusion bodies. On this basis a further 6 respondents were reviewed and alpha/beta chain ratios estimated to enable the diagnosis of heterozygous alpha-thalassaemia to be made.

On this basis 3 persons were diagnosed as having the alpha-thalassaemia trait with a resultant prevalence of 1,2 per cent and a gene frequency of 0,006 in the Greek community of Cape Town. The findings in these 3 individuals will now be summarized below.

1. Personal data

Details of age, sex, marital status, offspring, occupation and origin are summarized in Table 15 - 1.

TABLE 15 - 1

PERSONAL DATA OF SUBJECTS WITH THE ALPHA-THALASSAEMIA TRAIT

Case	Age	Sex	Marital status	Children	Occupation	Origin
1	31	M	M	2	Tax consultant	Cephalonia, Ithaca
2	41	M	M	2	Cafe owner	Lesbos
3	26	F	S	nil	Teacher	Lesbos

a) Age

Persons tested varied in age from 26 to 41 years. This is of no significance.

b) Sex

Two males as well as one female were found to carry alpha-thalassaemia. As the inheritance of alpha-thalassaemia is autosomal equal distribution between the sexes would be expected.

c) Marital status and offspring

The 2 males who carry alpha-thalassaemia were married while the female was single. The ability to find a marriage partner was not impaired by the disorder. Each marriage produced 2 children demonstrating that the fertility of the affected individuals was not diminished in any way.

d) Occupation

Occupation was not influenced by the disorder.

e) Origin

Two of the individuals with heterozygous alpha-thalassaemia originated from Lesbos, while the other subject's antecedents were traced to Cephalonia and Ithaca. This is of importance for although more persons from Lesbos were involved in the survey than from any other single place, the proportion from the rest of Greece as a whole was nevertheless higher. Extrapolating from this data it appears possible that Lesbos has a higher proportion of alpha-thalassaemia than the rest of Greece. However, the numbers are too low for any final conclusions to be drawn. Unfortunately at present there is no comparable data from Greece.

2. Red Cell Parameters

These findings are summarized in Table 15 - 2.

TABLE 15 - 2

RED CELL PARAMETERS OF RESPONDENTS WITH THE ALPHA-THALASSAEMIA TRAIT

CASE	Hb g per 100 ml	PCV %	MCHC %	RETICS	MCV fl	RCC mm^3	MCH pg
1	16,7	47	35,3	0,9	70	6,68	25
2	15,5	55	28,0	1,2	72	7,62	20
3	14,2	43	33,0	1,0	89	4,81	30

A. Haemoglobin

The haemoglobin in the 3 subjects varied from 14,2 to 16,7 g per 100 ml, with a mean of 15,5 g per 100 ml and a standard deviation

(SD) of 1,3. Thus the levels were within the normal range. It can be seen that although alpha-thalassaemia can result in an anaemia, the minimally affected individual who has 1 or 2 genes involved may not be anaemic at all.

B. Packed Cell Volume (PCV)

The PCV in the affected individuals ranged from 43 to 55 per cent with a mean of 48 and SD of 6,1. These values, as expected, are within normal limits.

C. MCHC

The mean MCHC was 32,2 per cent which is also in the normal range and of no significance.

D. Reticulocyte Count

The reticulocyte count in the 3 affected subjects varied from 0,9 to 1,2 per cent with a mean of 1,0 and a SD of 0,2. Persons with haemoglobin H disease, where there are 3 of the genes involved, and who are more severely affected clinically and haematological, usually have a reticulocytosis in the 5 per cent range (Weatherall and Clegg, 1972). However, persons with fewer genes involved may not have an abnormal reticulocyte count as is demonstrated here.

E. Mean cell volume (MCV)

The MCV, one of the most reliable indications of the thalassaemias, varied from 70 to 89 fl with a mean of 77 fl and SD of 10. Normal values range from 78 to 98 fl. Thus, only 2 of the 3 affected individuals have evidence of this important diagnostic indicator of the thalassaemias.

F. Red Cell Count (RCC)

The RCC ranged from 4,48 to 7,62 million per mm^3 with a mean of 6,37 and SD of 1,44. Higher than normal red cell counts are expected in the thalassaemias. This is the case in 2 of the 3 subjects.

G. Mean Cell Haemoglobin (MCH)

The MCH, a usually dependable indicator of the thalassaemias, ranged between 20 and 30 pg, with a mean of 25 and SD of 3. The normal range is 27 to 31 pg and thus, as with the MCV, 2 out of the 3 affected individuals have this characteristic abnormality.

3. Further Laboratory Data

Findings summarised in Table 15 - 3.

TABLE 15 - 3

LABORATORY DATA OF SUBJECTS WITH THE ALPHA-THALASSAEMIA TRAIT

Case	Hb pattern	Hbf %	HbA %	Osmotic fragility	HbH inclusion bodies	Alpha/beta chain ratios
1	AA	0,1	2,2	Slightly increased	25 / 10 000	0,85
2	AA	0,6	1,7	normal	4 / 10 000	0,83
3	AA	0,6	2,2	normal	60 / 10 000	0,58

A. Haemoglobin electrophoresis and quantitation

The haemoglobin pattern in the 3 subjects was AA. It is significant that no H-band was demonstrated on electrophoresis. Quanti-

tation disclosed that the level of haemoglobin F (Hbf) ranged between 0,1 and 0,6 per cent, with a mean of 0,4 and a SD of 0,3. Haemoglobin A_2 (HbA_2) ranged between 1,7 and 2,2 per cent with a mean of 2,0 and a SD of 0,3. These values are all within normal limits.

B. Osmotic fragility

In 2 of the 3 subjects, the osmotic fragility of the red blood cells was normal, whereas in 1 it was slightly increased. These findings are not typical of the thalassaemias where one expects there to be a decreased osmotic fragility. However, this picture is in keeping with individuals with 1 or 2 affected genes where the typical stigmata may be minimal or absent.

C. Inclusion Bodies

An important indicator of the alpha-thalassaemia carrier trait is the presence of the typical inclusion bodies found on incubating the blood with brilliant cresyl blue and which are due to precipitated beta-chains. These were found is all of the cases diagnosed as having heterozygous alpha-thalassaemia and varied from $\frac{4}{10\,000}$ to $\frac{60}{10\,000}$ with a mean of $\frac{30}{10\,000}$.

The presence of these peculiar inclusion bodies (see Fig. 15 - 1), although occurring characteristically in alpha-thalassaemia, may also occur in normal individuals and the diagnosis of the alpha-thalassaemia trait can thus not be made on this basis even in the presence of other typical abnormal red cell parameters. This will be discussed further in Chapter 25.

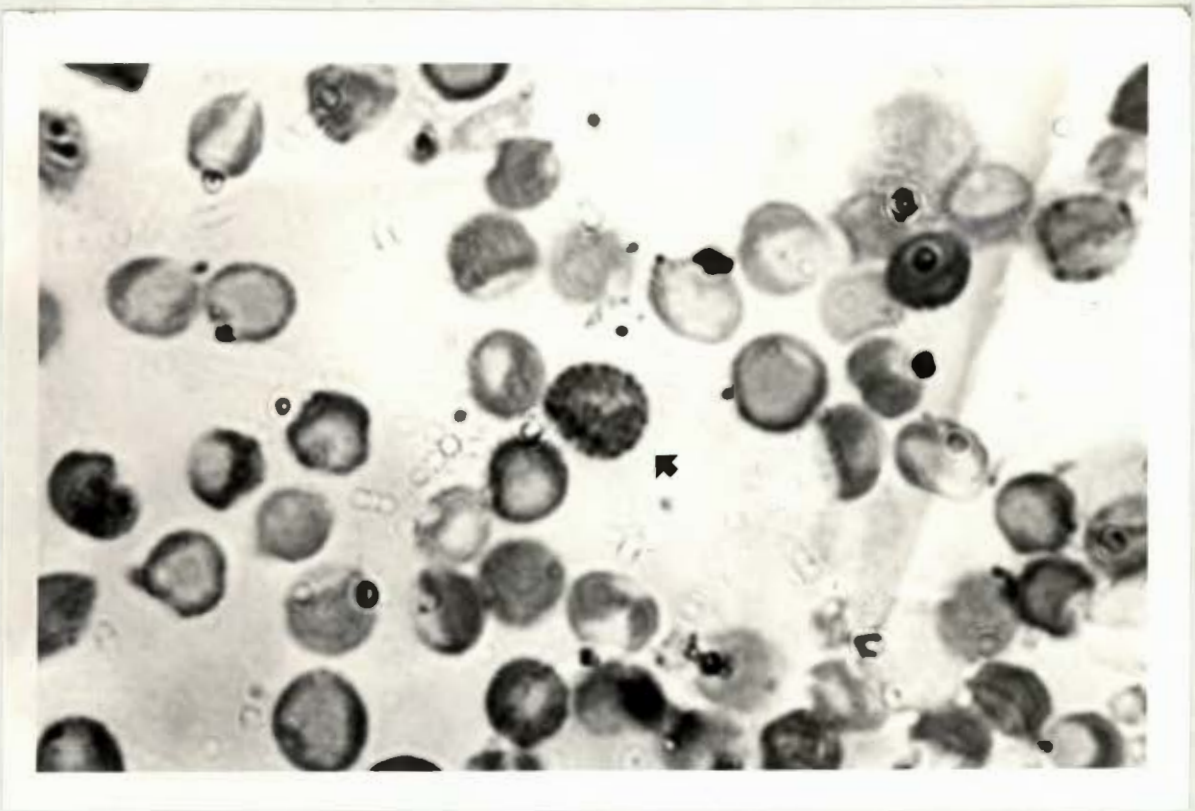


Fig. 15 - 1 Haemoglobin H inclusion body in the centre of the field of peripheral blood smear of a person carrying alpha-thalassaemia.

E. Alpha/Beta chain ratios

The most reliable method of diagnosing the alpha-thalassaemia carrier state is by studying the relative rates of synthesis of the alpha and beta chains in vitro. It was on this basis that heterozygous alpha-thalassaemia was finally diagnosed in the 3 subjects being discussed. All 3 individuals had defective rates of synthesis of the alpha chains when investigated by the method of Weatherall and Clegg (1969).

Normal values in this test are 0,91 to 1,18, the alpha-thalassaemia trait (where 1 or 2 genes are involved) 0,50 - 9,90 and haemoglobin H disease (3 out of 4 genes affected) 0,20 to 0,50.

In cases 1 and 2 where the values are 0,85 and 0,83 respectively definite but not gross impairment of alpha-chains synthesis is exhibited. Subject 3 manifests a greater reduction in chain production with a value of 0,58.

4. CLINICAL DATA

Clinical data is briefly summarized in Table 15 - 4.

TABLE 15 - 4

CLINICAL DATA OF RESPONDENTS WITH THE ALPHA-THALASSAEMIA TRAIT

Case	Symptoms	Signs	Affected kin
1	Nil	Nil	Not tested
2	Nil	Nil	Son
3	Anaemia noted in past	Slight Splenomegaly	Not tested

A. Symptoms

Cases 1 and 2, the males, had no symptoms referrable to an anaemia. The single female, No. 3, had previously had symptoms of an anaemia though not when seen by the author.

B. Signs

No signs of a haematological disorder were found in cases 1 and 2. Subject 3 was not anaemic but her spleen was just palpable below the left costal margin.

C. Affected kin

After respondent 2 had been investigated, it was discovered that his family had been previously studied and that his son had the alpha-thalassaemia trait.

It was not possible to investigate the families of subjects 1 and 3.

CHAPTER 16

BETA-THALASSAEMIA

DIAGNOSTIC CRITERIA

A. HOMOZYGOUS BETA-THALASSAEMIA

Homozygous beta-thalassaemia (thalassaemia major, Cooley's Anaemia), is relatively easy to recognize. The distinct clinical presentation together with the results of haemoglobin electrophoresis and family studies makes the diagnosis reasonably straightforward. However, individuals may have a clinical condition of intermediate severity and still have homozygous beta-thalassaemia diagnosed by laboratory means and family studies. The latter situation occurs most often in Negro patients.

A clinical picture which resembles thalassaemia major may occur when an individual has a combination of heterozygous beta-thalassaemia and an abnormal haemoglobin. This situation can be clarified by haemoglobin electrophoresis.

B. HETEROZYGOUS BETA-THALASSAEMIA

Parameters used in the diagnosis of heterozygous beta-thalassaemia include:

(1) haemoglobin A2 and F levels
(2) mean corpuscular volume (MCV)
(3) mean corpuscular haemoglobin (MCH)
(4) peripheral blood smear
(5) osmotic fragility
(6) family studies

The most important single diagnostic tool in this investigation of beta-thalassaemia was haemoglobin electrophoresis and the demonstration of a raised level of haemoglobin A2 and/or haemoglobin F was considered highly suggestive of heterozygous beta-thalassaemia. The normal values for haemoglobin A2 and F in the laboratory undertaking the tests were 1,5 to 3% for the former, and 0 to 1,7% for the latter. The diagnosis of heterozygous beta-thalassaemia was further substantiated by the demonstration of a decreased MCV and MCH with a moderately raised red cell count.

Weatherall and Clegg (1972) consider a decreased MCH to be the haematological finding which gives the best discrimination from normal. An abnormal blood smear was also taken into account. Typical abnormalities include target cells, microcytosis, anisopoikilocytosis and basophilic stippling. The blood picture may vary from almost normal to that resembling homozygous beta-thalassaemia with numerous target cells, marked hypochromia and microcytosis.

A decreased red cell osmotic fragility which is amplified after incubation for 24 hours is considered to be of diagnostic importance. However, in the author's laboratory, the normal distribution curve is probably shifted to the right. Thus, if a patient had a normal osmotic fragility but other parameters positive for thalassaemia, the diagnosis of heterozygous beta-thalassaemia was not invalidated. In equivocal cases with minor abnormalities, family studies may provide the necessary confirmatory evidence for the diagnosis of heterozygous beta-thalassaemia.

For the purpose of this study, the finding of a raised haemoglobin F and/or haemoglobin A2 together with 2 other abnormal parameters was con-

sidered sufficient to substantiate the diagnosis of heterozygous beta-thalassaemia. There is one exception to these criteria, namely, case 23, whose findings were all normal except for haemoglobin H type inclusion bodies in his peripheral blood. When alpha/beta chain synthesis ratios were undertaken it was found that he had heterozygous beta-thalassaemia. The tests were repeated and the findings substantiated.

INTRODUCTORY RESULTS

Twenty-three of the 250 subjects involved in this study were found to be heterozygous for beta-thalassaemia. This can be stated as a prevalence of 9,2% and a gene frequency of 0,046.

1. PERSONAL DATA

Details of age, sex, marital status, offspring, occupation and place of origin are shown in Table 16 - 1.

TABLE 16 - 1

PERSONAL DATA OF SUBJECTS WITH HETEROZYGOUS BETA-THALASSAEMIA

CASE	AGE	SEX	MARITAL STATUS	CHILDREN	OCCUPATION	ORIGIN
1	25	M	S	Nil	Doctor	Athens, Salonica
2	38	M	D	1	Electrician	Aigon
3	47	M	M	1	Teacher	Cyprus, Chios
4	51	M	M	2	Café owner	Pelasgia
5	27	F	M	2	Supermarket partner	Lesbos
6	44	M	M	1	Café owner	Lesbos
7	40	M	M	3	Shop owner	Lesbos
8	29	F	M	1	Dry cleaner	Cyprus
9	38	M	M	1	Businessman	Cyprus
10	20	F	S	Nil	Secretary	Tripolis
11	65	M	M	2	Retired	Cyprus
12	49	M	M	3	Café owner	Lesbos
13	27	M	M	Nil	Doctor	Kalamata
14	32	M	M	1	Electrician	Lesbos
15	52	M	M	1	Café owner	Patras, Chios
16	41	M	M	2	Café owner	Lesbos
17	33	F	M	2	Café owner	Lesbos
18	23	F	S	Nil	Clerk	Cyprus
19	58	M	M	-	Ship's captain	Athens
20	35	M	M	Nil	Businessman	Hagios Efstaios
21	46	M	M	3	Factory manager	Crete
22	48	F	M	3	Housewife	Galaxidion
23	26	M	M	2	Estate consultant	Cephalonia

a) AGE

The age of the beta-thalassaemia heterozygotes ranged from 20 to 65 years with a mean of 42 years. The age distribution (see Fig. 16 - 1) was as follows:

0 - 20 years	.....	Nil respondents
20 - 30 years	.....	7 "
30 - 40 years	.....	6 "
40 - 50 years	.....	6 "
50 - 60 years	.....	3 "
60 - 70 years	.....	1 respondent

In each of these broad age groups except the under 20's and over 60's the incidence is approximately 10%.

From the above figures it is evident that lifespan is not compromised by heterozygosity for beta-thalassaemia.

b) SEX

Of the 23 heterozygotes 17 were male and 6 were female. Thus, as there were 150 males in the population screened, the percentage of male beta-thalassaemia heterozygotes was 11,3%, and as there were 100 females in the group as a whole the percentage of beta-thalassaemia heterozygotes was 6%.

No obvious reason is evident for the difference in incidence between males and females.

c) MARITAL STATUS AND CHILDREN

The fact of having the beta-thalassaemia trait does not mitigate against obtaining a marital partner. Only 3 of the 23 respondents

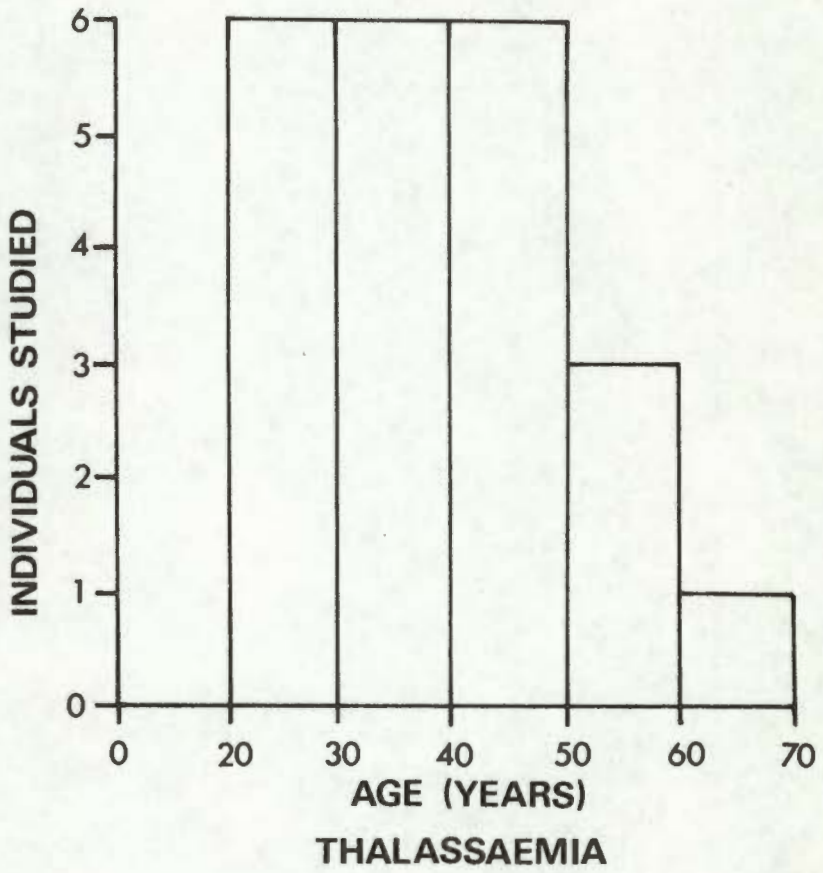


Fig. 16 - 1 Age distribution of subjects with heterozygous beta-thalassaemia

were single, and all 3 were in fact engaged to be married. Besides these 3, 2 others have no children. One has a decreased sperm count, while the other couple were still young and were deliberately postponing procreation. Thus, having beta-thalassaemia trait does not affect the ability to reproduce in any way.

d) OCCUPATION

The occupation of individuals with heterozygous beta-thalassaemia show no particular bias. Occupations vary from café owners and artisans to professional people. As previously mentioned, a large proportion of the respondents from the Cape Town Greek community are café owners.

e) ORIGIN

See Table 16 - 2 and Fig. 16 - 2.

TABLE 16 - 2

ORIGINS OF RESPONDENTS WITH HETEROZYGOUS BETA-THALASSAEMIA

PLACE	CASES	%
1. North East Aegean Islands	10	10,8%
Lesbos	7	10,5%
2. Central Greece	3	7,7%
3. Peleponnese	3	8,1%
4. Cyprus	4	30,0%
5. Crete	1	10,0%
6. Cephalonia	2	10,0%

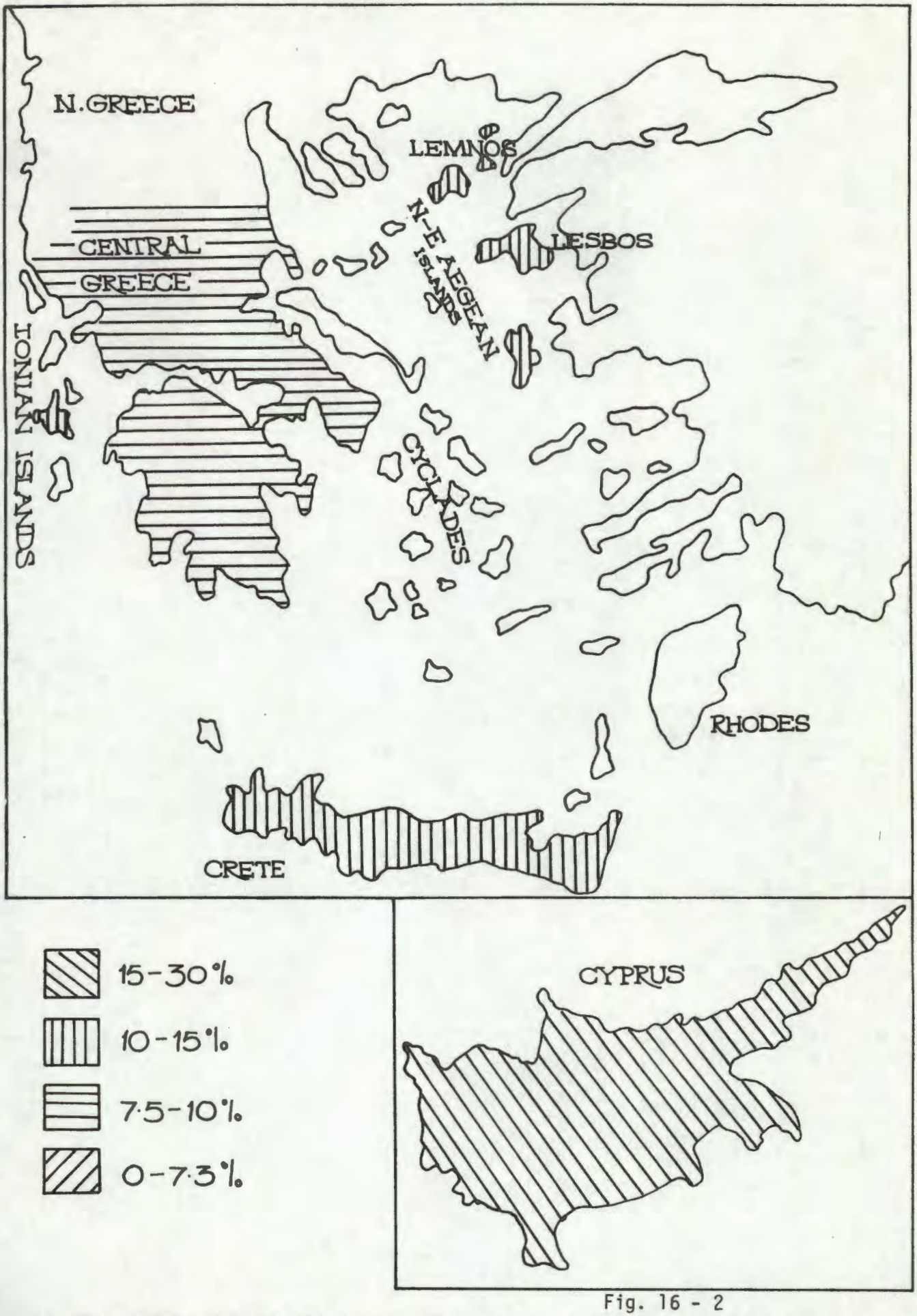


Fig. 16 - 2

DISTRIBUTION OF ORIGINS OF INDIVIDUALS WITH HETEROZYGOUS β-THALASSAEMIA

Seven out of the 23 heterozygotes originated from the following towns on the island of Lesbos: Plomarian, Yera and Madamados. These are all fertile, low-lying areas in which it is probable that malaria was endemic until fairly recently.

A further 3 individuals had their antecedents on the North Eastern Aegean islands; 1 from Agios Efstratios, and the other 2 from Chios. These are both low-lying areas.

From the above figures, it can be seen that the prevalence of beta-thalassaemia in individuals in Cape Town who originated from the North Eastern Aegean islands is slightly more than 10%, and 10,5% for persons from Lesbos.

Three individuals who originated from Central Greece, from the low-lying towns of Galaxidion, Athens and Pelasgia also have heterozygous beta-thalassaemia. A prevalence of 7,7% may be calculated. Three respondents coming from Tripolis, Kalamata and Aigion, have the thalassaemia trait. Tripolis is not low-lying as is the case of the other 2 towns. In addition, 1 of the parents of an individual with heterozygous beta-thalassaemia originates from Patras, in the Peleponnese. The prevalence in people from the Peleponnese is 8,1%. Four individuals, plus a parent of a further subject with heterozygous beta-thalassaemia had antecedents from 2 low-lying coastal towns in Cyprus, namely, Paphos and Kyrenia. The prevalence in individuals originating from Cyprus and at present living in Cape Town is 30%. Finally, 1 of the 10 individuals from Crete and 2 of the 5 from Cephalonia has the thalassaemia trait.

2. LABORATORY FINDINGS

Details of abnormal haemoglobin, percentage haemoglobin A_2 and F, osmotic fragility and peripheral blood smear are given in Table 16 - 3.

TABLE 16 - 3

LABORATORY FINDINGS IN RESPONDENTS WITH HETEROZYGOUS BETA-THALASSAEMIA

CASE	ELECTRO-PHORESIS	HbF %	HbA_2 %	OSMOTIC FRAGILITY	PERIPHERAL SMEAR TC	M	AP	OTHERS
1	AA	7,9	2,4	decreased	+	++	+	-
2	AA	1,5	4,8	normal	++	+	+	ovalocytes +
3	AA	0,7	4,6	normal	+	+	+	-
4	AA	2,5	2,4	normal	-	+	+	-
5	AA	0,9	4,2	normal	++	+	++	ovalocytes + tear drop cells +
6	AA	0,8	5,6	normal	+	+	+	-
7	AA	0,4	5,1	decreased	not tested			
8	AA	0,9	3,5	normal	-	+	+	-
9	AA	0,9	5,3	decreased	-	+	+	-
10	AA	2,2	5,3	normal	++	++	++	tear drop cells + ovalocytes +
11	AA	3,6	4,5	normal	++	+	+	-
12	AA	0,8	4,1	normal	-	+	+	-
13	AA	0,4	3,1	normal	-	++	+	-
14	AA	0,8	4,9	normal	+	+	+	-
15	AA	0,6	3,4	decreased	-	++	+	-
16	AA	1,3	3,9	normal	-	+	+	-
17	AA	4,1	5,7	decreased	+	++	+	basophilic stippling
18	AA	0,4	4,8	normal	not tested			
19	AA	0,2	4,6	decreased	++	+	+	-
20	AA	1,0	4,6	normal	++	+	+	-
21	AA	2,7	2,4	normal	-	-	+	-
22	AA	0,3	3,7	normal	+	+++	+	-
23	AA	0,1	2,6	normal	-	-	-	-

TC = Target cells
M = Microcytes
AP = Anisopoikilocytosis

a) HAEMOGLOBIN ELECTROPHORESIS

There were no haemoglobin structural variants occurring in association with heterozygous beta-thalassaemia in the 23 subjects. The haemoglobin pattern in every instance was AA.

In 3 of the 23 beta-thalassaemia heterozygotes the level of HbF was raised, but not that of Hb A_2. Normal values for HbF are up to 1,7%, and the values in the respondents varied from 2,5% to 7,9%, with a mean of 4,3%. In the 1 instance (subject 21) there were minor haematological abnormalities, creating a diagnostic difficulty, whereas in the other 2 cases (1 and 4), the diagnosis was relatively clearcut. In a further 3 respondents the value of haemoglobin F as well as that of haemoglobin A_2 was raised. These were subjects 10, 11 and 17, in which the value of HbF ranged from 2,2% to 4,1% with a mean of 3,3%. Hb A_2 values ranged from 4,5% to 5,7% with a mean of 5,1%. Diagnosis was clearcut in these cases.

In considering the above 2 groups together, the HbF values ranged from 2,5% to 7,9% with a mean of 3,8% and a standard deviation of 2,1. In 16 respondents haemoglobin A_2 alone was raised, with a normal value for haemoglobin F and in a further 3 subjects (Nos. 10, 11 and 17), who have been mentioned previously, the values for both haemoglobin A_2 and F were increased. In all these individuals abnormal red corpuscular parameters confirmed the diagnosis of heterozygous beta-thalassaemia. The mean haemoglobin A_2 level in the 19 cases with a raised A_2 level was 4,5% with a standard deviation of 0,75. The mean for Hb A_2 in these cases was 4,2% with a standard deviation of 1,0. A normal level for haemoglobin A_2 is 1,5 to 3,0%. Subject 23, an exception, had normal levels of both

Hb A_2 and HbF. He will be discussed at length later. The distribution of haemoglobins A_2 and F are shown in Figs. 16 - 3 and 16 - 4.

b) OSMOTIC FRAGILITY

Table 16 - 3 demonstrates that osmotic fragility studies, as undertaken in the laboratories involved in this investigation, is only an aid to diagnosis and not an absolute diagnostic parameter and that it is unsuitable as a screening procedure in population studies. In 6 of the 23 respondents with heterozygous beta-thalassaemia, the osmotic fragility was decreased initially, or, after 24 hours incubation, whilst in 17 cases it was normal. A decreased osmotic fragility curve is demonstrated in Fig. 16 - 5.

c) PERIPHERAL SMEARS

All of the 21 peripheral blood smears examined exhibited abnormalities to a greater or lesser extent, ranging from anisopoikilocytosis to smears revealing many target cells, microcytes, ovalocytes,basophilic stippling and tear drop cells (as found in thalassaemia major). Subject No. 21, who had the most minor abnormalities of other haematological parameters, also had only minor changes consisting only of anisopoikilosytosis. However, in the rest of the respondents there was no clear correlation between clinical severity, magnitude of haematological abnormalities and changes evident in peripheral blood smear. A typical target cell is shown in Fig. 16 - 6.

3. RED CELL PARAMETERS

Table 16 - 4 gives details of haemoglobin, packed cell volume,

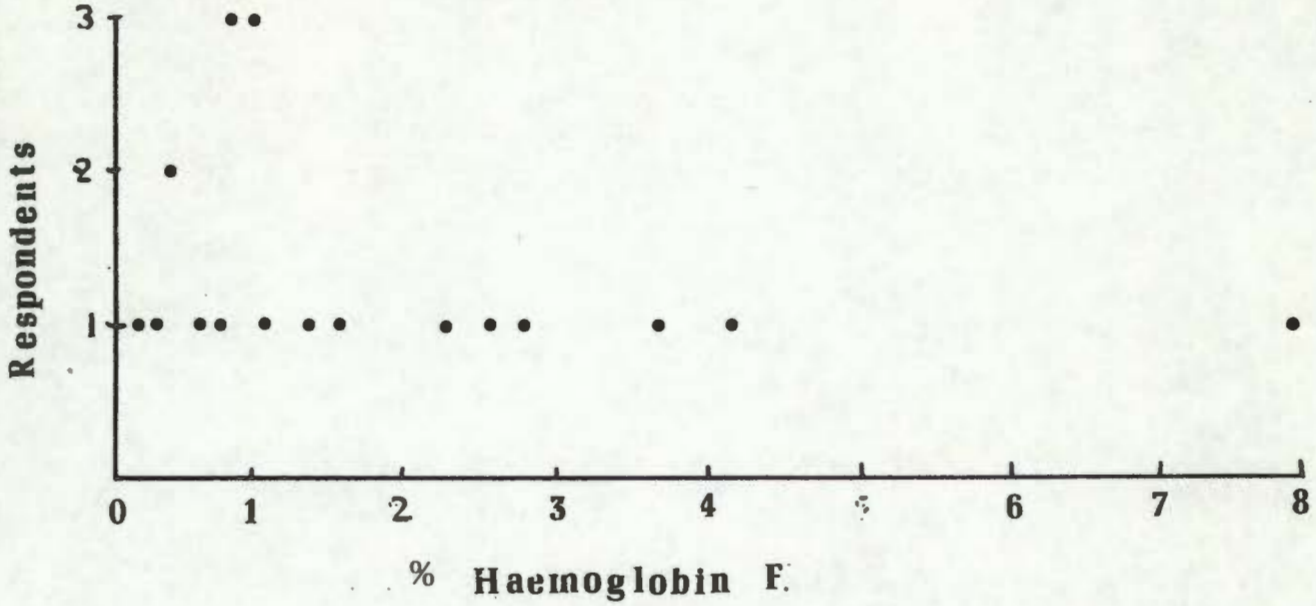


Fig. 16 - 3 Range of haemoglobin F in subjects with heterozygous beta-thalassaemia

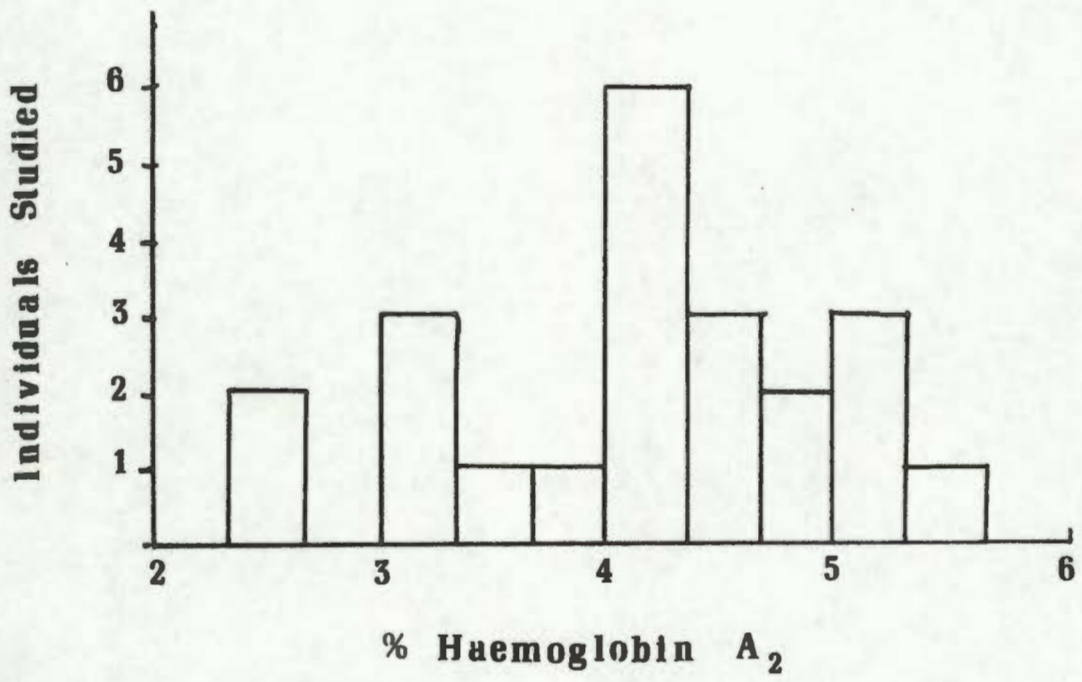


Fig. 16 - 4 Range of haemoglobin A_2 in respondents with heterozygous beta-thalassaemia

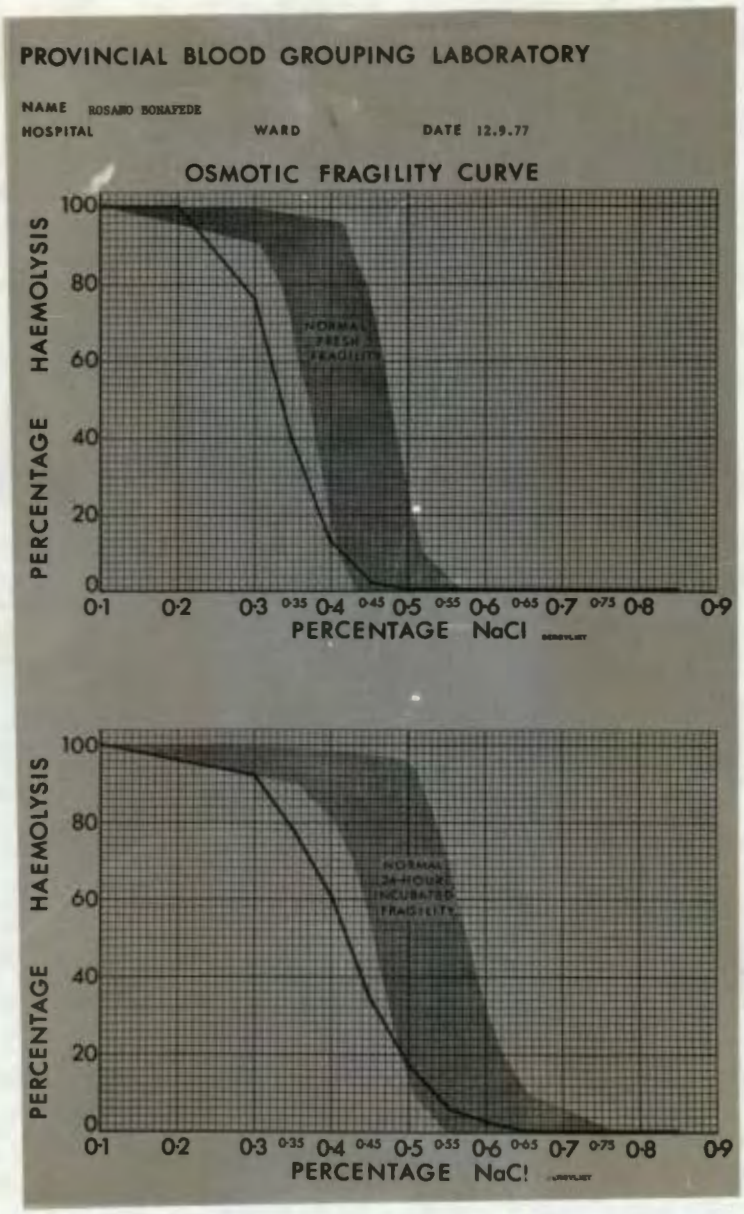


Fig. 16 - 5 Osmotic fragility curve demonstrating decreased fragility in a patient with heterozygous beta-thalassaemia.

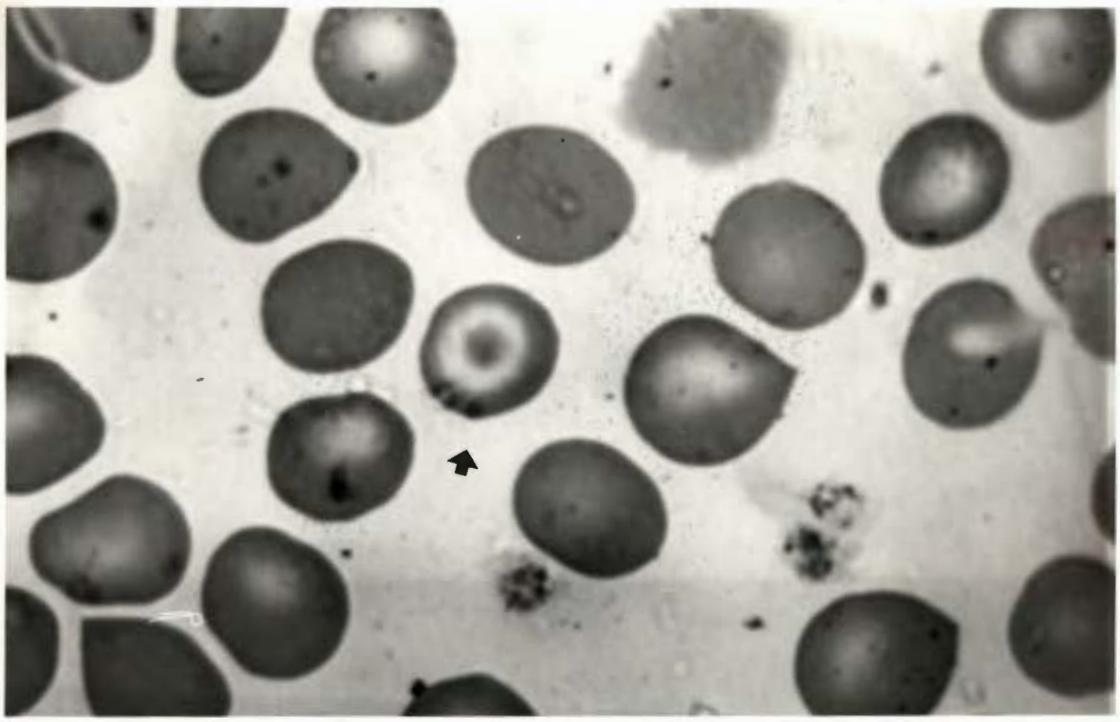


Fig. 16 - 6 Peripheral blood smear in beta-thalassaemia heterozygote. Characteristic target cell is in the centre of the field.

mean corpuscular haemoglobin concentration, mean corpuscular volume, mean cell haemoglobin, red blood cell count and reticulocyte count. Subject No. 11 had G-6-PD deficiency and stage IV carcinoma of the bladder, in addition to heterozygous beta-thalassaemia. Some of the red cell abnormalities could therefore be attributed to his neoplasm and for this reason he is not included in the statistics of red cell parameters which are given in the next section.

TABLE 16 - 4

RED CELL PARAMETERS IN SUBJECTS WITH HETEROZYGOUS BETA-THALASSAEMIA

CASE	Hb g/100 ml	PCV %	MCHC %	MCV fl	MCH pg	RBC mill/mm^3	RETICULOCYTE COUNT %
1	13,0	45	27	71	21	6,34	0,6
2	11,7	37	31,5	76	23	4,89	1,0
3	14,0	45	31	70	28	3,41	0,7
4	12,3	38	32	65	21	5,84	0,6
5	11,7	38	31	66	21	5,61	1,8
6	12,8	43	29,5	68	20	6,32	0,9
7	13,0	43	30	-	-	-	1,2
8	11,8	38	31	70	22	5,42	0,3
9	13,6	44	29,5	73	21	6,25	1,1
10	11,5	39	29	63	19	6,19	1,9
11	3,1	35	25,5	74	7	4,72	2,4
12	12,8	44	28,3	72	21	4,12	4,7
13	15,1	45	34	64	21	7,05	2,4
14	13,7	46	30	69	20	6,69	2,2
15	12,1	38	32	62	20	6,20	2,9
16	14,4	37	30	65	20	5,61	3,0
17	10,9	37	29	67	20	5,53	2,6
18	12.7	42	30	79	24	5,30	2,0
19	10,6	35	30	78	24	4,46	2,1
20	13,4	43	31	67	21	6,38	2,0
21	16,1	50	32	82	26	6,16	2,2
22	11,2	37	37	58	20	6,33	1,8
23	16,5	46	36	84	30	5,46	0,4

a) HAEMOGLOBIN

Normal haemoglobin levels in males vary from 14-18% and, for females from 12-16%. The haemoglobin values in the 6 females having heterozygous beta-thalassaemia, are generally lower than those of the males with the same disorder, and vary from 10,9 g per 100 ml-12,7 g per 100 ml with a mean of 11,6 g per 100 ml and a standard deviation of 0,6. The haemoglobin values in the 16 male subjects varies from 10,6 g per 100 ml-16,5 g per 100 ml with a mean of 13,2 g per 100 ml and a standard deviation of 1,4. Case No. 19, a male with a haemoglobin of 10,6 g per 100 ml was recovering from an acute pancreatitis at the time that he was investigated, and it is probable that he had some degree of anaemia as a result. Case No. 21, with a haemoglobin of 16,1 g per 100 ml, is at the other end of the spectrum. His other haematological abnormalities are of minor nature, consisting essentially of a decreased MCH and aniso-poikilocytosis on peripheral blood smear. Excluding subject No. 19, on the basis of his dual pathology, the mean haemoglobin for the group as a whole is 13,4 g per 100 ml with a standard deviation of 1,2. These statistics demonstrate the usually, but not always, minor degree of anaemia found in patients with thalassaemia minor.

b) PCV

The normal values for males varies from 40-54% and for females from 36-47%. The values in the female varied from 37-42% with a mean of 38,5%, with the majority of them being within the normal limits. The values of the males, excluding case Nos. 11 and 19, who had concurrent pathology, ranged from 37-50% with a mean of 42,7% and a standard deviation of 4,46. The males fell within normal limits, with the exception of subject 4.

c) MCHC

The normal values for MCHC are 34 ± 2%. The MCHC ranged from 27-34% in the 21 cases in the series. The mean was 30,4% with a standard deviation of 1,5. Four of the cases, Nos. 4, 13, 15 and 22 had values within normal limits, while the greater majority had values which were slightly lower than normal.

d) MCV

The MCV in normal persons varies from 78-98 fl (i.e. 88 ± 10 fl). In the 21 heterozygous beta-thalassaemia subjects studied, the MCV varied from 58-84 fl (see Fig. 16 - 7) with a mean of 69,5 fl and a standard deviation of 6,0. Respondent No. 23, whose stigmata are of a minor nature, has the highest MCV (84 fl), whilst another 3 cases, Nos. 18, 19 and 21, had MCV values which were within normal limits. The remaining 17 cases all had abnormally low MCV levels. A decreased MCV is typical of thalassaemia minor.

e) MCH

Normal values for MCH vary from 27-31 pg (29 ± 2 pg). In the 21 cases under consideration the MCH ranged between 19 and 30 pg (see Fig. 16 - 8), thus all except Nos. 3 and 23 were abnormally low. The mean for the 21 cases was 21,6 pg with a standard deviation of 2,1. Case 21 had a MCH of 26 pg at the lower end of the normal range, while subject 23 had a normal level of 30 pg. A low MCH is characteristic of the disorder.

f) RED CELL COUNT

The mean overall red cell count for 20 cases (those excluded being

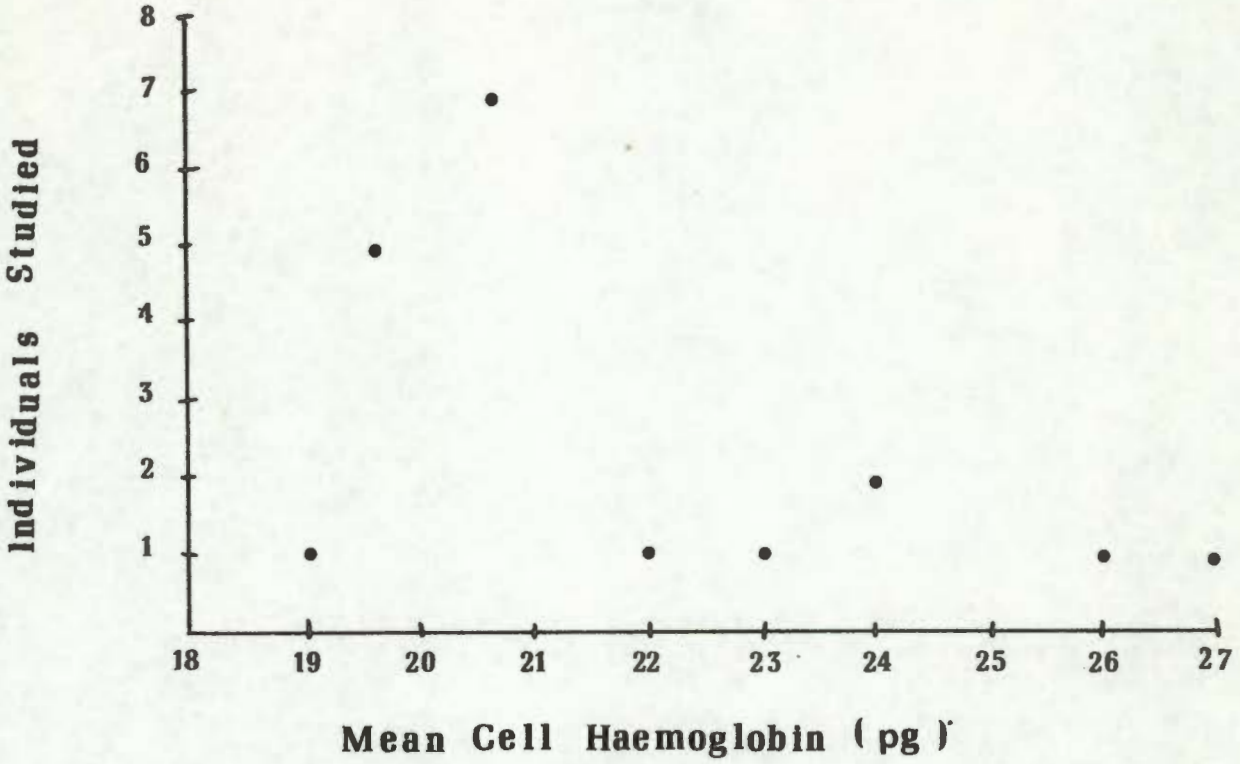


Fig. 16 - 7 Range of mean cell haemoglobin in subjects with heterozygous beta-thalassaemia

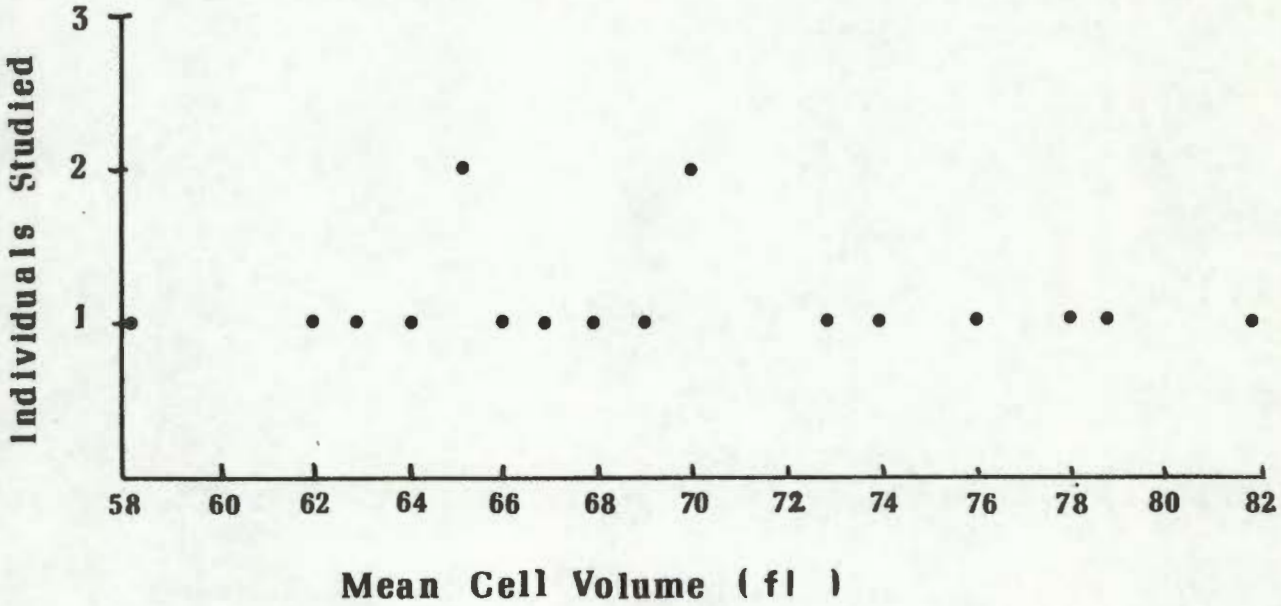


Fig. 16 - 8 Range of mean cell volume in respondents with heterozygous beta-thalassaemia

Nos. 11 and 21, who had concurrent significant illness, and No. 7 on which there is limited available data), was 5,88 million/mm^3 with a standard deviation of 0,79. The normal values in the general population are, males 5,4 $\pm$ 0,8 million/mm^3, and females 4,8 $\pm$ 0,6 million/mm^3. In the male cases under consideration, the mean was 5,94 million/mm^3 with a standard deviation of 0,92 and in the female cases, the mean was 5,74 million/mm^3 with a standard deviation of 0,43. Thus, the values for the men tend to occur within the high limits of normal or are mildly increased, whilst those for the women are moderately increased. In the heterozygous beta-thalassaemia it is usual for the red cell count to be raised.

g) RETICULOCYTE COUNT

The normal value for reticulocyte count is less than 2%. In the subjects with heterozygous beta-thalassaemia the reticulocyte varied from 0,4-4,7% with a mean of 1,81% and a standard deviation of 1,03.

4. CLINICAL DATA (See Table 16 - 5)

TABLE 16 - 5

CLINICAL DATA IN RESPONDENTS WITH HETEROZYGOUS BETA-THALASSAEMIA

CASE	SYMPTOMS	SIGNS	CONCOMITANT ILLNESS
1	nil	-	nil
2	nil	nil	Prolapsed inter-vertebral disc
3	Tiredness	nil	nil
4	Headaches	nil	nil
5	Dizziness, malaise	nil	nil
6	nil	nil	Acromegaly
7	-	-	G-6-PD deficiency
8	nil	nil	nil
9	nil	nil	nil
10	Fatigue, malaise	nil	nil
11	nil	nil	Ca bladder, G-6-PD deficiency
12	nil	nil	nil
13	nil	-	nil
14	nil	-	nil
15	nil	nil	nil
16	nil	nil	nil
17	Periodic weakness, malaise, fatigue	anaemic, 2 cm spleno-megaly	nil
18	nil	1 cm spleno-megaly	nil
19	nil	nil	Pancreatitis
20	nil	nil	Rheumatic fever
21	Fatigue	nil	nil
22	nil	-	nil
23	nil	nil	nil

a) SYMPTOMS

The majority of the 23 persons with heterozygous beta-thalassaemia were virtually asymptomatic. Minor symptoms which could possibly be due to anaemia were found in 3 females, and 3 males. On questioning the men more fully, it is probable that their symptoms were, in fact, due to stress and pressure from work. In the case of the women, their symptoms were probably due to a mild anaemia. However, a relatively large proportion of normal women within the Greek population had similar complaints and in view of this little significance is placed on this finding.

b) SIGNS

Signs due to the thalassaemia trait were elicited in only 2 individuals. These consisted of splenomegaly in both subjects, and in 1 there was in addition clinical detectable anaemia.

c) CONCOMITANT ILLNESS

Concomitant illness, both past and present, was evident in 6 of the 23 cases. Case 11, who had stage IV carcinoma of the bladder and G-6-PD deficiency, and case 19, who was recovering from acute pancreatitis, had some laboratory results which were affected by their major illnesses. There was, however, sufficient evidence to enable the diagnosis of heterozygous beta-thalassaemia to be established.

The occurrence of heterozygous beta-thalassaemia in association with G-6-PD deficiency is noteworthy. It is well recognised that both thalassaemia and G-6-PD deficiency occur with high frequencies in the Greek population, but no direct relationship between the 2

disorders is recognised. Thus, in the 250 persons screened we could expect the 2 disorders to occur concurrently

$$\frac{92}{100} \times \frac{6}{100} \times \frac{250}{1} = 1{,}38 \text{ cases}$$

(This calculation is based on the knowledge of the relative incidence of the 2 disorders in Cape Town.)

As the numbers studied in the series as a whole are relatively low, the discovery of 2 persons with both disorders out of 250 respondents, is in accordance with this figure.

Other illnesses and disorders encountered in persons with the thalassaemia trait have no practical or statistical significance, and therefore do not warrant further comment.

CHAPTER 17

GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY

DIAGNOSTIC CRITERIA

The diagnosis of G-6-PD deficiency is straightforward and essentially a laboratory matter, although a history of haemolytic episodes following the ingestion of fava beans or certain implicated medicines might arouse suspicion that an individual may be G-6-PD deficient.

Laboratory tests performed in this survey to diagnose G-6-PD deficiency were:-

(i) The brilliant cresyl blue test of Motulsky and Campbell-Kraut (1961)

(ii) The methaemoglobin reduction test (Brewer, et al, 1960)

In the abnormal brilliant cresyl blue test the dye colour change occurs after 40 minutes in a female and more than 44 minutes in a male (see Fig. 17 - 1). In G-6-PD deficient males the result is usually grossly abnormal and the diagnosis obvious, whereas in heterozygous females the test may only be marginally abnormal. In this situation the more sensitive methaemoglobin reduction test was performed. An abnormal result is when more than 5% of the methaemoglobin persists.

INTRODUCTORY RESULTS

Fifteen subjects of the 250 involved in this survey were found to have G-6-PD deficiency. These were 10 men and 5 women. Thus, the proportion of individuals suffering from G-6-PD deficiency is 6% amongst the

Fig. 17 - 1 Colour change completed in test tube on left in Motulsky test for G-6-PD deficiency

250 members in the Greek population of Cape Town. The prevalence for the males is 6,7% (i.e. 10 out of 150) and that for the females 5% (i.e. 5 out of 100). The discrepancy between males and females, though not large, may be explicable due to the fact that as G-6-PD deficiency is an X-linked inherited disorder, it may be difficult to diagnose in hemizygous females whose disorder may be asymptomatic and can therefore be missed during a simple screening procedure. Because of this discrepancy, the gene frequency will be calculated from the male prevalence and is 0,067.

1. PERSONAL DATA

Details of age, sex, marital status, offspring, occupation and place of origin in the 15 subjects with G-6-PD deficiency are given in Table 17 - 1.

TABLE 17 - 1

PERSONAL DATA OF SUBJECTS WITH G-6-PD DEFICIENCY

CASE	AGE	SEX	MARITAL STATUS	CHILDREN	OCCUPATION	ORIGIN
1	26	M	S	nil	Baker	Lemnos
2	50	F	M	3	Housewife	-
3	36	M	M	1	Ass. Consul	Arta
4	24	F	M	2	Housewife	Simis
5	29	F	M	1	Café owner	Lesbos
6	27	M	M	1	Café owner	Lemnos
7	41	F	M	3	Café owner	Lemnos
8	20	M	S	nil	Medical student	Lesbos
9	50	M	M	2	Factory manager	Evia
10	45	M	M	1	Artisan	Smyrne
11	46	F	M	2	Housewife	Cephalonia
12	60	M	M	3	Businessman	Imroz
13	32	M	S	nil	Restauranteur	Cephalonia
14	65	M	M	2	Retired	Cyprus
15	40	M	M	3	Shop owner	Lesbos

a) AGE

Persons diagnosed as having G-6-PD deficiency ranged in age from 20 to 65 years with a mean of 40 years. The age distribution of the respondents is shown in Fig. 17 - 2 and Table 17 - 2.

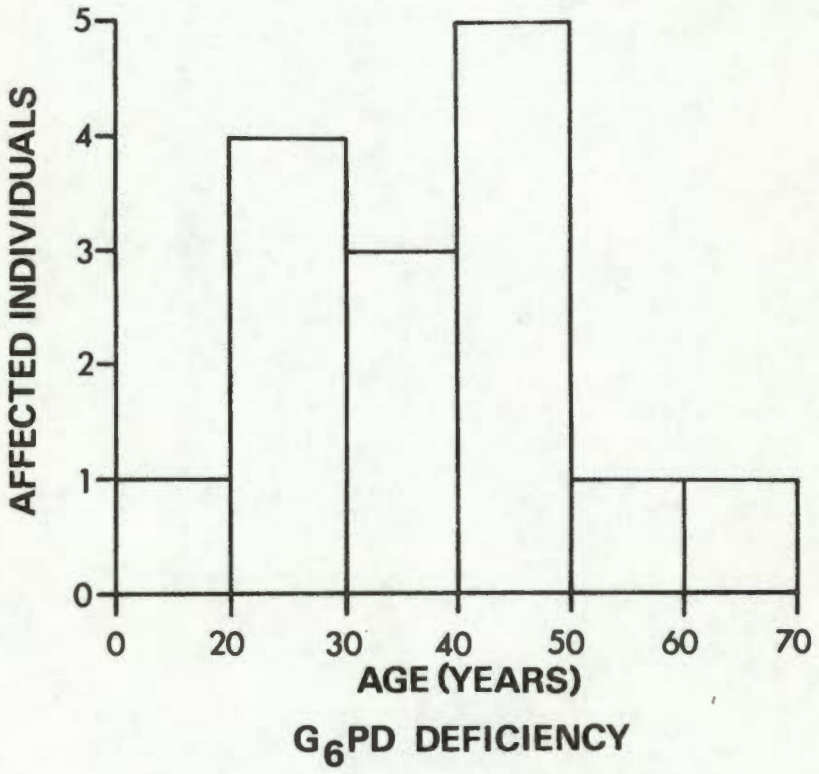


Fig. 17 - 2 Age distribution of subjects with G-6-PD deficiency

TABLE 17 - 2

AGE DISTRIBUTION OF SUBJECTS WITH G-6-PD DEFICIENCY

AGE	NO. OF CASES	% OF AGE GROUP WITH THE DISORDER
0 - 20	1	7,6
20 - 30	4	6,7
30 - 40	3	4,3
40 - 50	5	8,3
50 - 60	1	3,3
60 +	1	5,0

b) MARITAL STATUS AND CHILDREN

As all but 3 of the 15 individuals with G-6-PD deficiency were married and had families of normal size, it is evident that fitness to reproduce is not affected by the disorder.

c) OCCUPATION

As with thalassaemia, G-6-PD deficiency does not influence financial status or social class. Occupations vary from artisans and café owners to members of the diplomatic corps, in subjects covered in this survey.

d) ORIGIN

The places of origin of the G-6-PD subjects, except subject 2 whose origins are not known, is shown in Fig. 17 - 3 and Table 17 - 3.

TABLE 17 - 3

ORIGIN OF SUBJECTS WITH G-6-PD DEFICIENCY

PLACE	CASES	% OF SUBJECTS WITH G-6-PD DEFICIENCY AT EACH LOCALITY
1) North east Aegean Islands	7	7,5
a) Lesbos	3	4,3
b) Lemnos	3	20
2) South east Aegean Islands	1	9
3) Ionian (West) Islands	2	11,8
4) Asia Minor	1	9,9
5) Central Greece	1	2,5
6) Cyprus	1	6,6
7) Peloponnese	1	2,7

The majority of individuals in this survey with G-6-PD deficiency came from low lying areas where malaria was moderately endemic until recent times. This includes the North Aegean, South Aegean and Ionian islands which have a relatively high prevalence, ranging from 7,5% to 11,8%, whilst the Peloponnese and Central Greece have relatively low prevalence of 2,7% and 2,5% respectively. The 4,3% prevalence of G-6-PD deficiency found in respondents originating from Lesbos compares well with 4,6% and 3,9% found in surveys undertaken on the islands of Lesbos by Doxiadis, et al (1964). Stamatoyannopoulos and Fessas (1964), investigating G-6-PD deficiency in 5 areas in Greece, noted a prevalence of 0,86-6,22% on Corfu in the Ionian islands. These investigations demonstrated that the

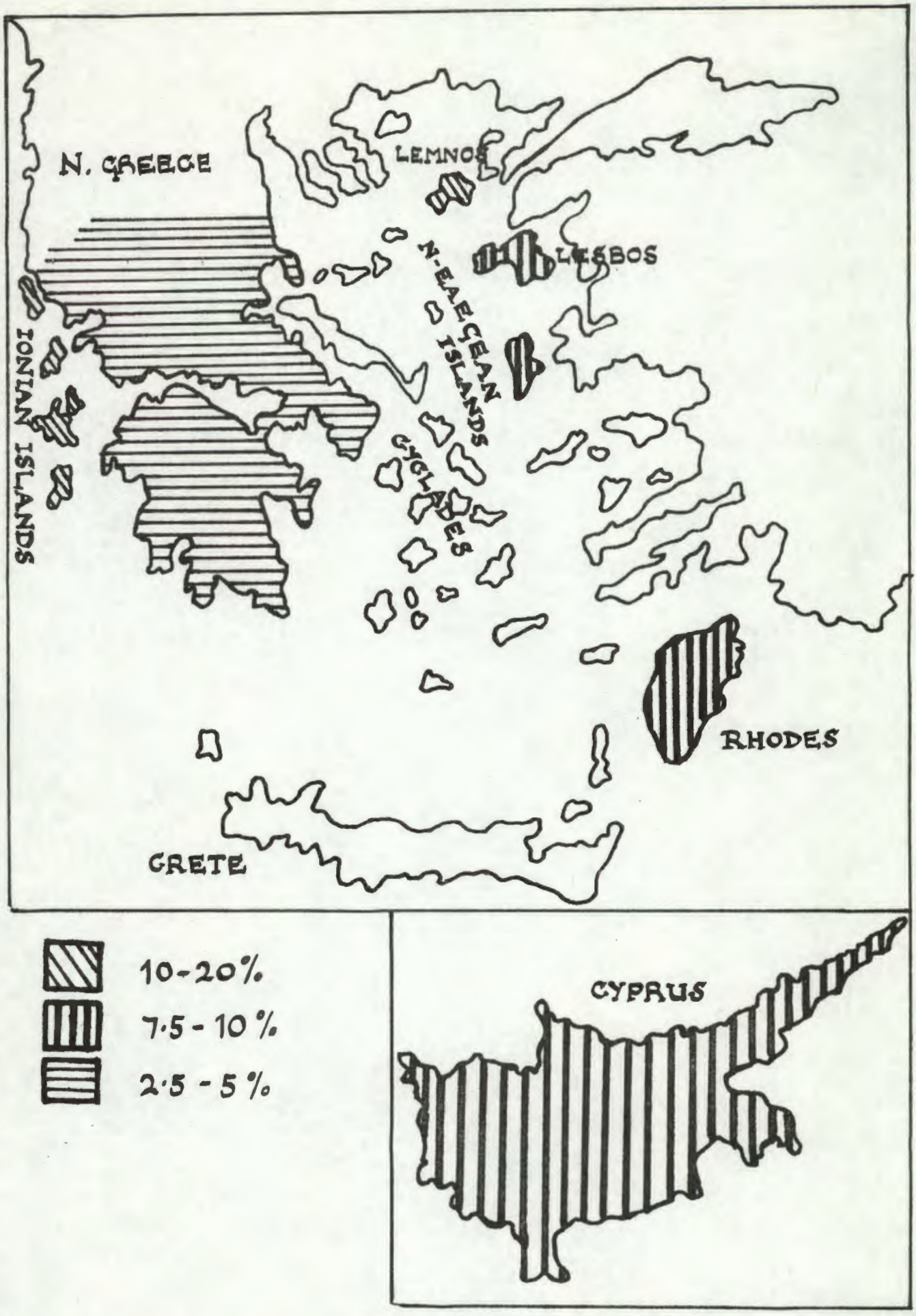


Fig. 17 - 3

DISTRIBUTION OF ORIGINS OF INDIVIDUALS WITH G6PD DEFICIENCY

prevalence of G-6-PD deficiency is higher in areas where malaria was endemic recently and lower where it was not. These findings were similar in the Arta area where the prevalence ranged from 2,8% to 16,4% in parallel with past malarial endemnicity.

2. HAEMATOLOGICAL PARAMETERS

Details of haemoglobin, packed cell volume, mean corpuscular haemoglobin concentration, mean corpuscular volume, mean corpuscular haemoglobin, red cell count and reticulocyte count are given in Table 17 - 4.

Subjects 2 and 14 will not be considered in this analysis as at the time of testing they were suffering from concurrent illnesses, which were the main contributory factors to their low haemoglobins and other abnormal laboratory findings. Respondent 2 was in chronic renal failure, and case 14 had terminal stage IV carcinoma of the bladder.

TABLE 17 - 4

RED CELL PARAMETERS OF RESPONDENTS WITH G-6-PD DEFICIENCY

CASE	g per 100 ml Hb	% PCV	% MCHC	fl MCV	pg MCH	MILLIONS/MM^3 RBC	% RETICULOCYTES
1	13,0	39	33	88	29	4,45	0,4
2	6,6	24	27,5	103	28	2,3	0,3
3	15,5	48	32	96	31	5,0	2,8
4	12,4	42	29,5	93	28	4,54	1,8
5	12,7	46	32	92	29	4,33	3,0
6	14,7	45	33	94	31	4,77	2,8
7	12,2	39	31	90	28	4,35	2,6
8	13,8	45	31	92	28	4,87	1,4
9	14,7	46	32	91	29	5,05	1,5
10	15,5	34	35	87	31	5,07	0,6
11	11,3	38	30	93	29	4,05	1,2
12	16,1	46	35	86	30	5,29	1,4
13	14,3	41	35	92	32	4,48	3,5
14	3,1	35	25,5	74	7	4,72	2,4
15	13,0	43	30	-	-	-	1,2

a) HAEMOGLOBIN

The haemoglobin in the case of the females varied from 11,3%-12,7 g per 100 ml with a mean of 12,2 g per 100 ml and a standard deviation of 0,6. The males haemoglobin values ranged from 13,0 g per 100 ml-16,1 g per 100 ml with a mean of 14,5 g per

100 ml and a standard deviation of 1,1. It is evident that the haemoglobin levels in both men and women tended to be in the lower normal limits rather than in the higher normal limits. This suggests that they were chronically haemolysing their red blood cells.

b) PACKED CELL VOLUME (PCV)

The PCV (excluding cases 2 and 14) was normal in all but 1 of the subjects where it was marginally decreased - this was case 10 where the PCV was 34%. The reason for this low value is not apparent.

c) MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION (MCHC)

The MCHC was slightly decreased in 4 of the cases; however, the abnormality is not significant and does not warrant further comment.

d) MEAN CORPUSCULAR VOLUME (MCV)

The MCV is normal in all cases except in subjects 2 and 14. In subject 2 the MCV was 103 fl and thus macrocytic. This patient had chronic renal failure. Case No. 14 is microcytic having an MCV of 74 fl, which could be due to the patient's concomitant carcinoma of the bladder, or his β-heterozygous thalassaemia. A normal MCV is expected in this condition unless it is complicated by iron deficiency anaemia.

e) MEAN CORPUSCULAR HAEMOGLOBIN (MCH)

The MCH in all cases was normal.

f) RED CELL COUNT (RCC)

The RCC was normal in all cases except No. 2 where there was a complicating illness.

g) RETICULOCYTE COUNT

The reticulocyte count varied from 0,4%-3,0% excluding case numbers 2, 14 and 13. The latter subject was found to have hereditary spherocytosis in addition to G-6-PD deficiency, and as both could result in a reticulocytosis, it was decided to exclude these findings from the analysis. The overall mean reticulocyte count was 2,0% with a standard deviation of 0,9. The mean reticulocyte count for the females was 2,2% with a standard deviation of 0,8, whilst the mean for the males was 1,5% with a standard deviation of 0,8. As G-6-PD deficiency is inherited in an X-linked manner, with the feamles carrying the disease, while the males suffer from the disorder, it is to be expected that the men would have a higher reticulocyte count than the women. However, this was not so in the survey cases. Similarly, males would be expected to haemolyse at a higher rate than the females, with a resultant higher compensatory reticulocyte count. There was no obvious reason for this anomaly. If haemolysis was occurring it would be expected that the reticulocyte count would be increased.

3. DIAGNOSTIC PROCEDURES

These results are summarized in Table 17 - 5.

TABLE 17 - 5

DIAGNOSTIC PROCEDURES IN SUBJECTS WITH G-6-PD DEFICIENCY

CASE	MOTULSKY (MINUTES)	METHAEMOGLOBIN REDUCTION	OSMOTIC FRAGILITY
1	240	-	Normal
2	43	20,1%	Normal
3	300	-	Normal
4	50	16,7%	Normal
5	48	78%	Normal
6	240	-	Normal
7	52	-	Increased
8	120	-	Normal
9	59	-	Normal
10	65	-	Normal
11	60	-	Normal
12	180	-	Increased
13	300	-	Increased
14	133	-	Normal
15	300	-	Decreased

The brilliant cresyl blue dye screening test of Motulsky and Campbell-Kraut was used in screening the population investigated. This test was adequate in diagnosing males with the disorder, but in certain heterozygous females the results of these tests were equivocal and a more sensitive test was required. For these reasons, the methaemoglobin reduction test was performed on the females who had a slightly

increased brilliant cresyl blue dye test.

Eight of the males with G-6-PD deficiency had grossly elevated brilliant cresyl blue tests of greater than 120 minutes. The remaining 2 cases (Nos. 9 and 10) had values of 59 and 65 minutes respectively. These results fall more into the pattern of the Negro variety of G-6-PD deficiency. However, as has been previously mentioned, there is great heterogeneity within G-6-PD deficiency and many variants (more than 100) of G-6-PD deficiency have been identified.

A normal brilliant cresyl blue dye test in females is considered less than 40 minutes. The females carrying G-6-PD deficiency included in this survey had values of the above test ranging from 43-60 minutes. A methaemoglobin reduction test is considered abnormal when over 5%. In the females tested the results varied from 16,7% to 78%. Subject 7, with a brilliant cresyl blue dye test of 52 did not have a methaemoglobin test performed as her daughter had already been proven to have G-6-PD deficiency. Respondent 11 had a very abnormal brilliant cresyl blue test (60 minutes) and for this reason the methaemoglobin reduction test was considered unnecessary.

Osmotic fragility studies are not diagnostic tests for G-6-PD deficiency and are usually normal. Three of the respondents found to have G-6-PD deficiency had an increased osmotic fragility, while 1 had a decreased osmotic fragility. The decreased osmotic fragility in case 15 was due to concomitant heterozygous beta-thalassaemia, while case 13 had an increased osmotic fragility as a result of concomitant hereditary spherocytosis.

4. CLINICAL MANIFESTATIONS

These results are summarized in Table 17 - 6.

TABLE 17 - 6

CLINICAL MANIFESTATIONS OF RESPONDENTS WITH G-6-PD DEFICIENCY

CASE	SYMPTOMS	SIGNS	CONCOMITANT DISEASE
1	Dizziness following fava bean ingestion	Hepatosplenomegaly	Nil
2	Weakness	Nil	Chronic renal failure
3	Abdominal pain and weakness following fava bean and Disprin ingestion	Splenomegaly	Nil
4	Nil	Nil	Nil
5	Fatigue, malaise	Nil	Nil
6	Nil	Nil	Nil
7	Abdominal pain, malaise	Nil	Sinusitis/?Spherocytosis
8	Nil	Splenomegaly	Nil
9	Vague abdominal pain	Nil	Allergic to Carbacaine and Belladonna
10	Nil	Nil	Duodenal ulcer
11	Nil	Nil	Nil
12	Nil	Splenomegaly	Previous cholecystectomy for stones
13	Abdominal pain	Nil	Spherocytosis
14	Nil	Lower abdominal mass	CA bladder thalassaemia
15	Previous abdominal pain, anaemia following ingestion of fava beans	Nil	Previous cholecystectomy

a) SYMPTOMS

Of the 5 women with G-6-PD deficiency, 2 (cases 5 and 7) had symptoms in the past attributable to an anaemia which would tend to be exacerbated by G-6-PD deficiency. Case No. 2 had symptoms of weakness and lassitude which were probably due to her underlying chronic renal failure.

Three of the males (cases 1, 3 and 15) have suffered attacks of favism. In addition, case 3 is the only individual who has had a definite haemolytic crisis following the ingestion of drugs, in his instance acetyl salicylic acid. Case 9, who is allergic to carbacaine and belladonna, probably suffered from acute allergic reactions to these drugs and not haemolytic reactions. Cases 9 and 13 had vague symptoms of abdominal pain.

b) SIGNS

Four of the men with G-6-PD deficiency had clinically enlarged spleens. In 2 of them (cases 8 and 12) the spleen was just palpable, but in case 3 it was firm and extended 3 cm below the left costal margin. In addition, case 1 had a liver palpable 1-2 cm below the right costal margin. Case 14 had a large, firm, irregular lower abdominal mass which was due to his carcinoma of the bladder and was not related to his G-6-PD deficiency. In subjects undergoing chronic haemolysis splenomegaly is known to occur and therefore eliciting this sign clinically is expected in a proportion of cases.

c) CONCOMITANT DISEASES

The occurrence of G-6-PD deficiency in association with thalassaemia

is to be expected due to the high frequency of each in peoples of Greek origin. As calculated earlier in Chapter 16, 1, 38 individuals would be expected to have the 2 disorders concomitantly.

The discovery of 1 case (13) and possibly a second (7) of G-6-PD deficiency occurring in association with spherocytosis is higher than expected in Greeks in Cape Town. Statistically, one would expect 0,07-0,192 cases. This is calculated with the knowledge of the prevalence of G-6-PD deficiency and hereditary spherocytosis in the population being studied. The occurrence of 2 is probably attributable to chance.

Cases 12 and 15 who have previously had cholecytectomies for cholelithiasis, probably had this illness on the basis of their G-6-PD deficiency. Subject 12's osmotic fragility studies were increased but his autohaemolytic studies were normal, indicating that he was haemolysing at an increased rate due to some exogenous cause. As he had been taking disprin for headaches over a long period it is reasonable to suggest that he would not have an acute haemolytic episode but that he would haemolyse his older red cell population at a steady rate. This chronic increased rate of haemolysis could well explain his cholelithiasis and splenomegaly.

The occurrence of carcinoma of the bladder, chronic renal failure and duodenal ulceration were found to occur in the Greek population of Cape Town at a frequency similar to the rest of the population and it is probably chance that they have occurred in persons with G-6-PD deficiency.

CHAPTER 18

HEREDITARY SPHEROCYTOSIS (HS)

Diagncstic Criteria

Patients with HS may present with the clinical findings of anaemia, jaundice and splenomegaly.

Laboratory findings important in the diagnosis of HS include:-

the demonstration of
(1) anaemia
(2) bilirubinaemia
(3) spherocytes on the peripheral blood smear (see Fig. 18 - 1)
(4) an increased osmotic fragility of the red blood cells
(5) increased autohaemolytic studies corrected by the addition of glucose

A diagnosis of HS was made in this survey when a subject had an increased osmotic fragility, raised autohaemolytic studies corrected by the addition of glucose, splenomegaly or spherocytes on the peripheral blood smear and a dominant pattern of inheritance was demonstrated. This last feature was insisted upon as the haematological abnormalities were minimal in the majority of the respondents considered in this section. It is, however, recognized that the mutation rate for the gene may be as high as 25%.

INTRODUCTION

A small but significant proportion of individuals were found to have an increased osmotic fragility which suggested a haemolytic disorder. As hereditary spherocytosis would be high on the differential diagnostic

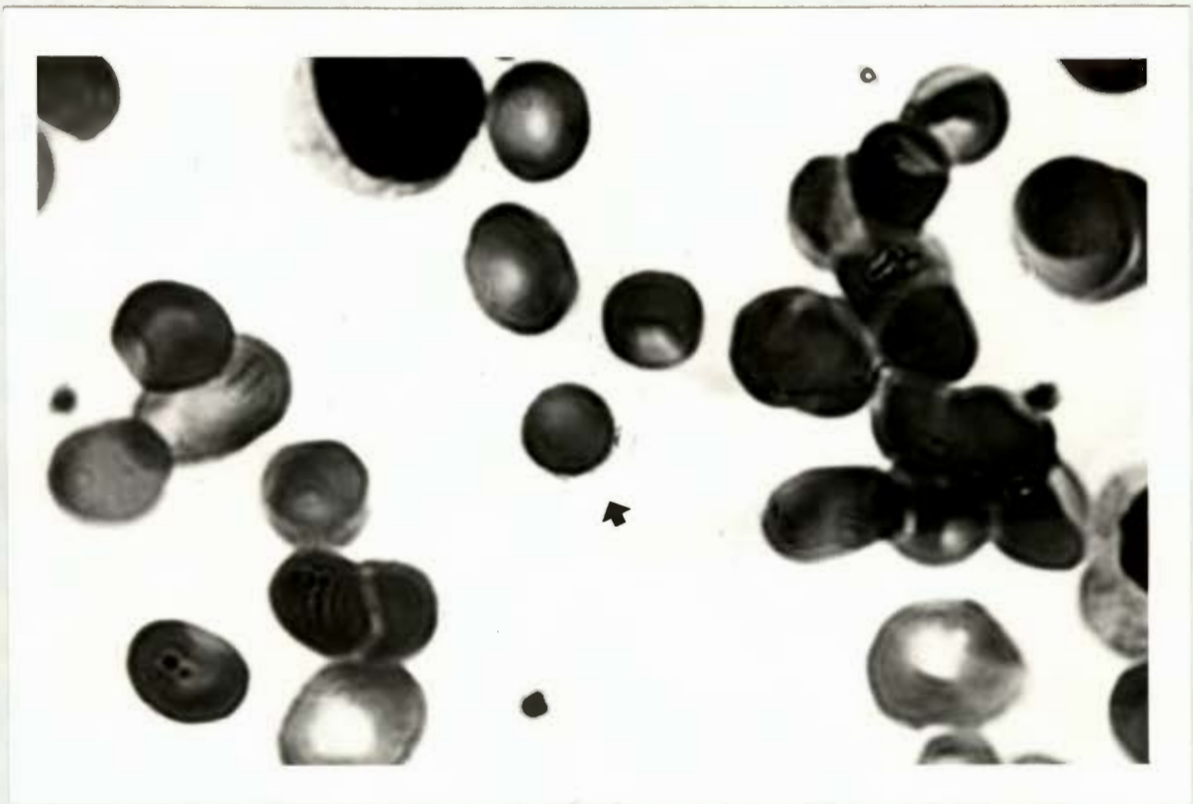


Fig. 18 - 1 Peripheral blood smear in hereditary spherocytosis. Typical spherocyte in centre of field.

list, further relevant investigations were instituted. In addition a full clinical history was obtained with special reference to a history of anaemia. jaundice, gallstones and affected kin. Further blood samples were obtained for a Coombs test and autohaemolytic studies and where possible other members of the family were examined physically and haematologically. Eight patients fell into this category, and of these a diagnosis of hereditary spherocytosis was made in 3. The other 5 respondents have a mild haemolytic disorder which possibly represents a minimal expression of the spherocytic gene and which would require comprehensive family studies for diagnostic confirmation.

INTRODUCTORY RESULTS

The patients whose findings are summarized in Tables 18 - 1, 18 - 2, and 18 - 3 and who are discussed below are those in whom a definitive diagnosis of hereditary spherocytosis was made. Those who may have HS but in whom there was not sufficient contributing evidence to confirm the diagnosis are also included. A positive diagnosis was made in 3 cases, namely numbers 1, 4 and 7. However, it is quite probable that some, if not all, of the others included in the following tables have HS.

In summary, therefore, the minimal prevalence of HS is 1,2% with a gene frequency of 0,006. However, the prevalence may be as high as 3,2% with a gene frequency of 0,016 in the Greek population of Cape Town.

The findings summarized in the various tables are now discussed in a manner similar to that for thalassaemia and G-6-PD deficiency.

1. PERSONAL DATA

These findings appear in Table 18 - 1.

TABLE 18 - 1

PERSONAL DATA OF SUBJECTS WITH HEREDITARY SPHEROCYTOSIS

CASE	AGE	SEX	MARITAL STATUS	CHILDREN	OCCUPATION	ORIGIN	BIRTH-PLACE
1	43	M	M	3	Company representative	Cassos	South Africa
2	33	M	M	2	Café owner	Neapolis	Neapolis
3	41	F	M	3	Café owner	Lemnos	Lemnos
4	41	F	M	3	Café owner	Lesbos	Lesbos
5	62	M	M	nil	Retired	Sotira	Sotira
6	60	F	M	2	Housewife	Athens	Athens
7	32	M	S	nil	Restauranteur	Kalamata Cephalonia	South Africa
8	53	F	M	2	Housewife	Crete	Crete

A. AGE

The age of the subjects with proven and possible HS varies from 32 to 62 and is of no special significance except to illustrate that the lifespan of these patients will probably not be compromised by the condition.

B. SEX

Of the 8 cases, 4 are male and 4 female. As HS is an autosomal dominantly inherited disorder, it is to be expected that there would be equal distribution between the sexes. This is in con-

trast to G-6-PD deficiency, which is inherited in an X-linked manner and where there is thus a preponderance of males suffering from the condition.

C. MARITAL STATUS AND CHILDREN

Seven of the 8 individuals are married, and of these, 6 have families of normal sizes. This is as expected, as there is no reason that their fitness to reproduce should in any way be affected.

D. OCCUPATION

There is nothing of importance to note under this heading.

E. ORIGIN

The place of origin of these subjects is of a uniform nature. There is no increased tendency for anyone to come from any particular place, area or situation with particular geographic characteristics. This is in contrast to the situation found in thalassaemia and G-6-PD deficiency where there appeared to be an increased prevalence in persons originating from certain islands and from the islands in general, when compared to the mainland.

2. RED CELL PARAMETERS

These findings are summarized in Table 18 - 2.

TABLE 18 - 2

RED CELL PARAMETERS OF SUBJECTS WITH HEREDITARY SPHEROCYTOSIS

CASE	Hb g per 100 ml	PCV %	MCHC %	RETICS	MCV fl	RCC mm^3
1	15,4	44	35	0,4	83	5,30
2	16,0	50	32	0,7	83	6,00
3	12,1	39	31	2,6	90	4,35
4	12.9	43	30	1,6	88	4,86
5	14,9	47	31,5	1,8	87	5,30
6	11,9	38	31	3,3	85	4,46
7	14,3	41	35	3,5	92	4,48
8	14,8	44	34	2,2	91	4,82

A. HAEMOGLOBIN

The mean haemoglobin of this group was 14,1 g/100 ml with a standard deviation (SD) of 1,5. When further subdivided, the mean for the males was 15,2 g/100 ml, with a SD of 0,72, while that for the females was 13,0 g/100 ml with a SD of 1,3. These results are all within normal limits. Since hereditary spherocytosis results in a haemolytic anaemia, it would be reasonable to expect that those with the abnormal gene would tend to have a haemoglobin level below normal. However, it is recognised that although the haemoglobin level is usually between 9 and 12 g/100 ml, an anaemia may not be present (Wintrobe, 1974). Evaluating the results as a whole, and especially in the 3 in whom the diagnosis of HS was confirmed,

it appears that the expression of the HS gene in these persons is indeed mild. This is also added evidence for the heterogeneity of the HS gene.

B. PCV

The mean PCV for the group as a whole was 43% with an SD of 4,0, while in the males it was 45,5% with an SD of 3,9, and in the males 41,0% with an SD of 2,9. These levels are, as expected, within normal limits.

C. MCHC

The mean MCHC in the above subjects was 32,4 g/100 ml with an SD of 2,0. The normal range is 34 $\pm$ 2%. A characteristic, but not invariable finding in HS, is an increased MCHC usually in the range 37 - 39 (Wintrobe, 1974). Cases 1 and 7 who do have HS have upper limit of normal levels of MCHC. The value in both is 35% whereas that in the other 6 subjects is within the lower normal range.

D. RETICULOCYTES

The reticulocyte level varied greatly in the cases from 0,4 to 3,5%. As a result the mean was 2% with an SD of 1,1. This pattern was also found in the 3 cases diagnosed as having HS where the respective values were 0,4, 1,6 and 3,5%. A usual feature of the disorder is an increased reticulocyte count. This feature too is not constant with levels varying from extremely low to extremely high values. Usual values are from 5 to 15%.

E. MEAN CELL VOLUME

The mean MCV in the cases under discussion is 87 fl with an SD of 3,5 and all occur within normal limits. These findings are common. However, the MCV may be as low as 62 or as high as 125 fl (Wintrobe, 1974). The cases in which one expects macrocytosis are those with a severe anaemia and marked reticulocytosis.

F. RED CELL COUNT

The red cell count ranged from 4,46 to 6,00 million per mm^3 with a mean of 5,0 and SD of 0,6. These findings are normal and of no noteworthiness.

3. DIAGNOSTIC CONSIDERATIONS

Clinical, as well as laboratory findings, useful in the diagnosis of hereditary spherocytosis and which have not already been discussed are presented in this section.

The findings are summarized in Table 18 - 3.

TABLE 18 - 3

DIAGNOSTIC CONSIDERATIONS IN RESPONDENTS WITH HEREDITARY SPHEROCYTOSIS

CASE	MEDICAL HISTORY	SIGNS	OSMOTIC FRAGILITY	PERIPHERAL BLOOD SMEAR	AUTODAEMOLYSIS WITH GLUCOSE	AFFECTED KIN	DIRECT COOMBS	PYRUVATE KINASE (UNITS)
1	nil	nil	increased	normal	4% corrected to 1,1%	son	not performed	1,55
2	nil	nil	increased	normal	5,1% corrected to 0,4%	not available	negative	1,34
3	Abdominal pain. G-6-PD deficiency	nil	increased	normal	6,4% corrected to 0,4%	nil	negative	1,27
4	Previous chole-cystectomy	3 cm spleno-megaly	increased	few sphero-cytes	16,3% corrected to 0,6%	daughter	negative	-
5	Previous chole-cystectomy	nil	increased	normal	5,7% corrected to 1,7%	not available	negative	1,36
6	Vasculitis	nil	increased	normal	4,6% corrected to 0,6%	not available	negative	-
7	Abdominal pain. G-6-PD deficiency	nil	increased	normal	14,9% corrected to 1,8%	father, sister	negative	-
8	Previous anaemia	nil	increased	normal	7,5% corrected to 0,2%	nil	negative	1,59

A. MEDICAL HISTORY

The most important diagnostic feature offered in the cases studied, was the fact that cases 4 and 5 had had a cholecystectomy for gallstones. HS is classified as a haemolytic anaemia and in affected individuals the haemolysis which occurs may be rapid or slow and insignificant. In the former group significant bilirubinaemia is found, which may be clinically apparent and result in pigment stones being formed in the gall bladder. These may then become infected and result in an attack of cholecystitis.

Case No. 4, who has HS, required surgical intervention for her cholecystitis while a young woman. However, as a diagnosis of HS was not made at the time, she did not have a splenectomy as is recommended.

Case No. 5 had gall bladder surgery done at the age of 61 years. However, there is insufficient evidence in his case to make a definite diagnosis of hereditary spherocytosis.

B. SIGNS

The clinical signs particularly sought for in the patients with possible HS were anaemia, jaundice, chronic leg ulcers and splenomegaly. The above are usually found in persons suffering from a symptomatic form of the condition while in individuals with a well compensated form of the disorder no clinical signs may be apparent. In only one (No. 4) of the 8 cases was there a definite finding of a firm spleen palpable 3 cm below the left costal margin. In a further 2 cases, Nos. 1 and 7, it was equivocal whether they had minor degrees of clinical detectable

splenomegaly. Cases No. 4 and 7, who are discussed at greater length later, had the most complete picture of HS.

C. OSMOTIC FRAGILITY

The single most important laboratory test for HS is the demonstration that the red blood corpuscles have increased fragility in hypotonic saline solutions. In all the 8 cases under consideration, there was a detectable and marked increase in osmotic fragility. A further 2 cases were found to have a raised osmotic fragility, but on further investigation it was decided that they definitely did not have HS. However, the cause for this abnormality was not determined.

It was not expected at the outset of the investigation that there would be a significant proportion of individuals with hereditary spherocytosis. The first indication that this might be so occurred when it was found that there was a relatively large number of cases with raised osmotic fragility studies. These persons and their families, where possible, were then further studied. An abnormal osmotic fragility curve typical of HS is shown in Fig. 18 - 2.

D. AUTOHAEMOLYTIC STUDIES

The laboratory that conducted the tests for the author, routinely performed autohaemolytic studies as a final diagnostic tool for hereditary spherocytosis. In this test, on the addition of certain reagents, it is demonstrated that significant autohaemolysis occurs. However, on the addition of glucose to the patient's serum this can be prevented from occurring and this is said to be typical for HS. It also

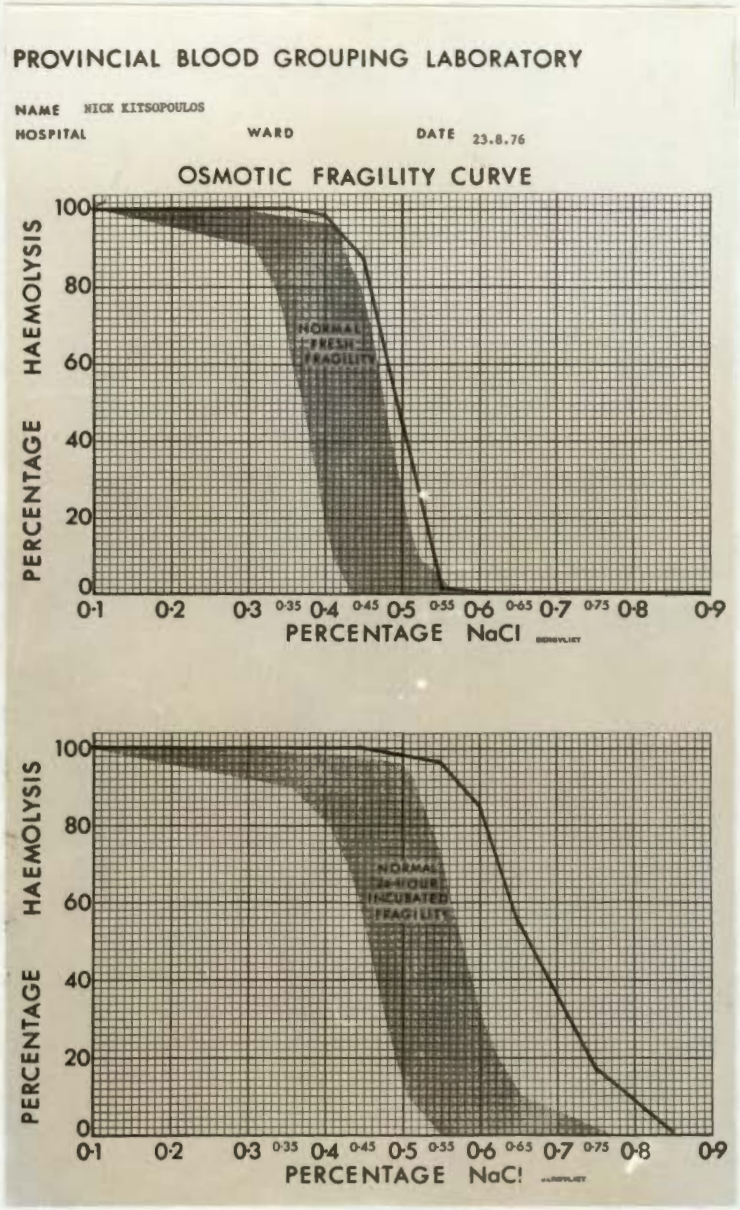


Fig. 18 - 2 Osmotic fragility curve demonstrating increased fragility more marked after 24 hours incubation in a patient with hereditary spherocytosis.

occurs in thalassaemia (Know-Macauly, et al, 1972) but the osmotic fragility is then decreased. Autohaemolysis of 4% and greater is considered to be significant and correction on the addition of glucose to 2% is said to be diagnostic of HS.

In all the cases presented, abnormal autohaemolytic studies were demonstrated and on this basis, together with raised osmotic fragilities, they were included here. Autohaemolysis ranged from 4% to 16,3% with a mean of 11,7% and a SD of 6,7. When corrected with glucose, the range was 0,2 to 1,8% with a mean of 1,2 and an SD of 0,6.

The range in the 3 cases with confirmed HS was also 4 to 16,3% with a mean of 11,7% and an SD of 6,7. On correction with glucose the mean was 1,2% and SD 0,6.

It is apparent from these results that there is a correlation between clinical severity and abnormality of the autohaemolytic studies.

E. AFFECTED KIN

To confirm the diagnosis of hereditary spherocytosis, the author considered it necessary to demonstrate the typical dominant inheritance of the condition. This was possible in cases 1, 4 and 7. In case 1, the father, one daughter and one son were investigated and the son and father were found to have the disorder. The son and daughter of case 3 were tested and found not to have HS. In case 8, two daughters were studied and found to be normal. In case 4, only one of three children was tested and found to have the disorder. The

mother felt that her other two children were too young and thus was unwilling to have them tested. However, it is important that they be investigated later as the presence of the condition may have an important bearing on future disease processes and also may cause problems per se. After a diagnosis of hereditary spherocytosis was made in case No. 7, the author, while perusing old laboratory records, discovered the rest of his family had been extensively investigated for a haemolytic anaemia in the past when his sister had a problematical anaemia. During these studies, it was found that both his father and sister had HS. It was not possible to study the family of subject 2, and in the case of respondent 6, he had no living blood relatives. The 5 subjects whose families were investigated and found to be normal and whose families were not studied, were not diagnosed as having HS, as the typical pattern of inheritance could not be proven. However, it is nevertheless quite probable that some, if not all of these 5 subjects do have a mild form of HS.

F. DIRECT COOMBS TEST

A direct Coombs test was performed on all except 1 of the 8 cases, to exclude immune causes of haemolysis. The result was negative in all 7 individuals.

G. PYRUVATE KINASE ESTIMATION

The 5 respondents not diagnosed as having HS had their pyruvate kinase levels estimated to ascertain whether a deficiency of this enzyme was the cause of their mild haemolytic tendency. The results in all 5 subjects was within normal limits which

therefore excluded pyruvate kinase deficiency as a cause of their other abnormal laboratory findings.

SUMMARY

From the above results, it would be reasonable to state that 3 of the respondents have hereditary spherocytosis. This indicates that the minimum prevalence of hereditary spherocytosis amongst persons of Greek origin in Cape Town is 1,2% with a gene frequency of 0,005%.

The remaining 5 respondents have minimal clinical abnormalities. They also have increased osmotic fragility and abnormal autohaemolytic studies which are highly suggestive of hereditary spherocytosis. However, without adequate family studies and because of the minor degree of stigmata characteristic of the disease, no firm conclusion can be reached, though it seems possible that these persons have a mild expression of the hereditary spherocytosis gene. Inclusion of these persons would result in a prevalence of 3,2% with a gene frequency of 0,016.

Therefore, in summary, the minimal gene frequency for hereditary spherocytosis in the Greek community of Cape Town is 0,006% but it may be as high as 0,016%.

CHAPTER 19

MISCELLANEOUS INHERITED HAEMATOLOGICAL DISORDERS

A. SICKLE CELL TRAIT

Diagnostic Criteria

For the diagnosis of the sickle cell trait the demonstration of the "S" band on both paper and agar electrophoresis was considered necessary (see Fig. 19 - 1). Thereafter, decreased solubility of the "S" chain was required to be demonstrated by Itano's test.

One of the 250 respondents included in the survey was found on laboratory testing to have the sickle cell trait (i.e. HbS). He was a cafe owner aged 44 years who was born at Nafplion in the Peloponnese, where his family had their origins. From this data the prevalence of the sickle cell trait is 0,4% with a gene frequency of 0,002 in the Greek population of Cape Town.

He had neither symptoms nor signs of an anaemia and had never suffered any complications to suggest that sickling of his red blood cells had ever occurred. In addition, he had no chronic leg ulcers. From his past medical history it was ascertained that he had diabetes mellitus and a mildly elevated serum cholesterol.

Results of laboratory investigations

Hb:	16,0 g per 100 ml
PCV:	47%
MCHC:	34%
MCV:	96 fl
MCH:	33 pg
Red cell count	4,89 mill/mm^3

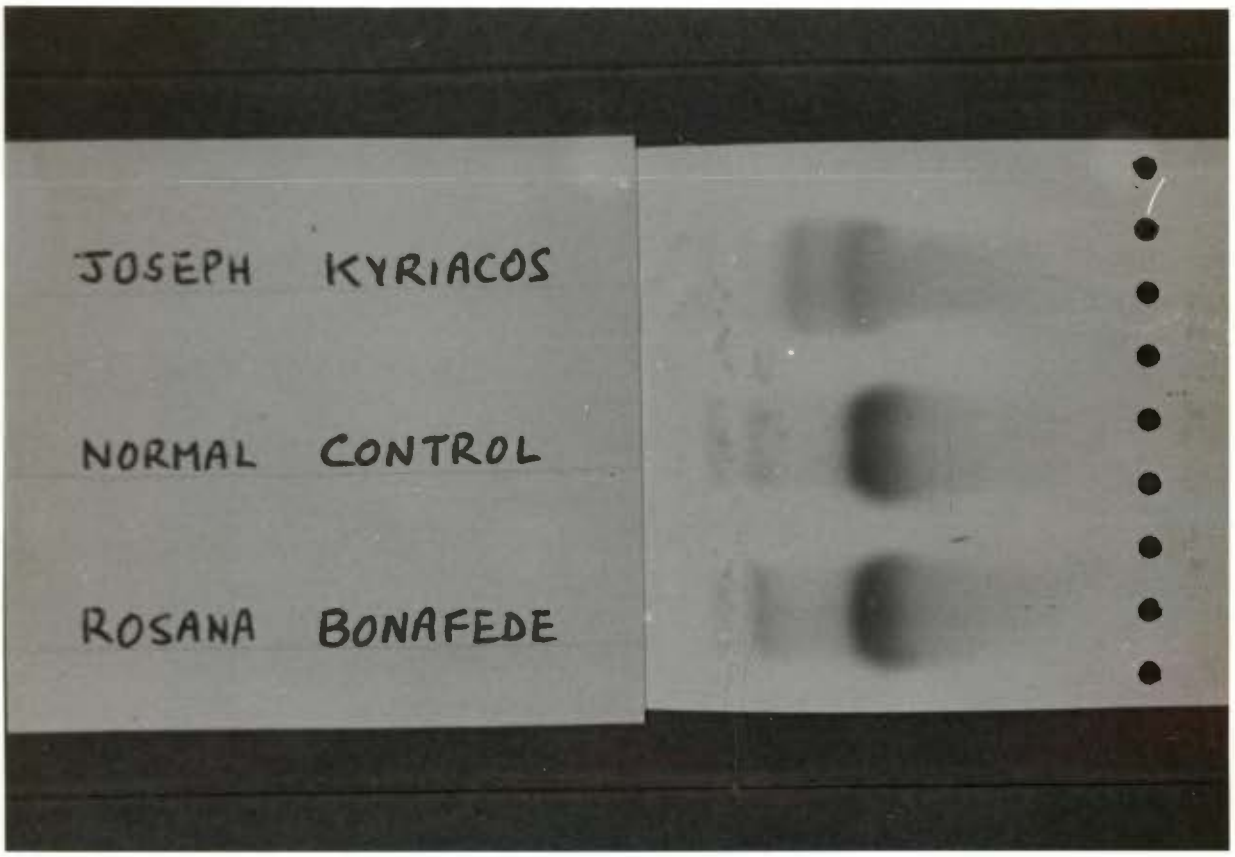


Fig. 19 - 1 Haemoglobin electrophoresis. Patient at top with "S" band, middle is normal control and patient at bottom has raised haemoglobin A_2.

Reticulocyte count:	1,6%
Osmotic fragility:	normal
Motulsky:	30 minutes
Haemoglobin pattern:	AS
Haemoglobin F:	0,8%
Haemoglobin A_2:	1,7%

In summary his laboratory findings reveal that he was not anaemic, his red cell parameters were normal and did not manifest the features typical of the thalassaemia syndrome. Haemoglobin A_2 and F were within normal limits. The only abnormality was the demonstration of the "S" band typical of the sickle cell traits. In addition this "S" chain was demonstrated to have decreased solubility when Itano's test was applied.

B. HAEMOGLOBIN LEPORE

One of the respondents who volunteered for the survey was found to have an abnormal haemoglobin which had previously been diagnosed in Greece as being haemoglobin Lepore.

The subject was a 28-year-old male doctor who had antecedents in St. George, Central Greece. He had never had any symptoms or signs referrable to the haemopoietic system and had been incidently diagnosed as having haemoglobin Lepore while being routinely screened in the Greek army during national service. Physical examination revealed no abnormality.

Results of laboratory investigations

Hb:	12,2 g per 100 ml
PCV:	38%
MCHC:	32%

MCV:	69 fl
MCH:	22 pg
Red cell count:	5,47 mill/mm^3
Reticulocyte count:	2,5%
Osmotic fragility:	normal
Motulsky:	30 minutes
Haemoglobin pattern:	A Lepore
Haemoglobin F:	9,5%
Haemoglobin A_2:	2,1%

COMMENT

This individual has the characteristic findings of haemoglobin Lepore. He was asymptomatic, as is typical, and had no physical signs. However, patients with the disorder may have minor physical signs. His blood picture too was characteristic of this disorder, with findings similar to the thalassaemia trait, i.e. MCV 69 fl, MCH 22 pg and Hb 12,2 g per 100 ml. His haemoglobin electrophoresis had a band which moved slowly in a position similar to haemoglobin S which could not be characterized in the laboratory undertaking the investigations, but which had previously been delineated in Greece. His haemoglobin F was 9,5% and haemoglobin A_2 2,1%. The latter finding is normal while the former characteristically increased. These results are also typical for haemoglobin Lepore.

SECTION VI

CASE REPORTS AND PEDIGREE STUDIES

INTRODUCTION

This section provides:

1. Detailed clinical and laboratory reports on the 2 patients previously known to have thalassaemia major. (No separate details on subjects with the thalassaemic trait have been included as these respondents were found to be essentially normal clinically with minor haematological abnormalities.)

2. The medical history and clinical findings together with laboratory results in certain respondents with G-6-PD deficiency.

3. The clinical and laboratory data of the 3 patients with hereditary spherocytosis, as well as the details of the equivocal cases who were not positively diagnosed as having HS.

4. Pedigree studies showing the typical inheritance of the major disorders in the survey.

CHAPTER 20

THALASSAEMIA MAJOR

1. V.P.

A. HISTORY

The patient was born in Cape Town on 10th July, 1966, and both his parents originated from Lesbos. He was irritable from birth, and pale when unwell. At 2 years of age he developed acute gastroenteritis requiring admission to the Children's hospital. It was then noted that he was anaemic with a haemoglobin of 6,8 g per 100 ml and had a 1 cm splenomegaly of unknown origin. Investigations indicated a diagnosis of thalassaemia major which was confirmed by family studies. Complications hereafter include numerous incompatible blood reactions, careous teeth and chronic leg ulcers. In 1973, he presented with haematemesis, when a diagnosis of infectious hepatitis was made. Psychological problems manifesting as enuresis and aggressive behaviour required referral to the psychiatric department.

B. EXAMINATION

The patient was anaemic with facies typical of thalassaemia major. He had prominent malar eminences, a depressed nasal bridge and protuberant upper teeth as a result of maxillary hyperplasia, all resulting in the typical "mongoloid" facies (Fig. 20 - 1). Examination of his cardiovascular system disclosed a haemic murmur over his praecardium, while abdominal examination revealed a 7 cm hepatomegaly and 13,5 cm splenomegaly (Fig. 20 - 3). He also had evidence of chronic leg ulcers.

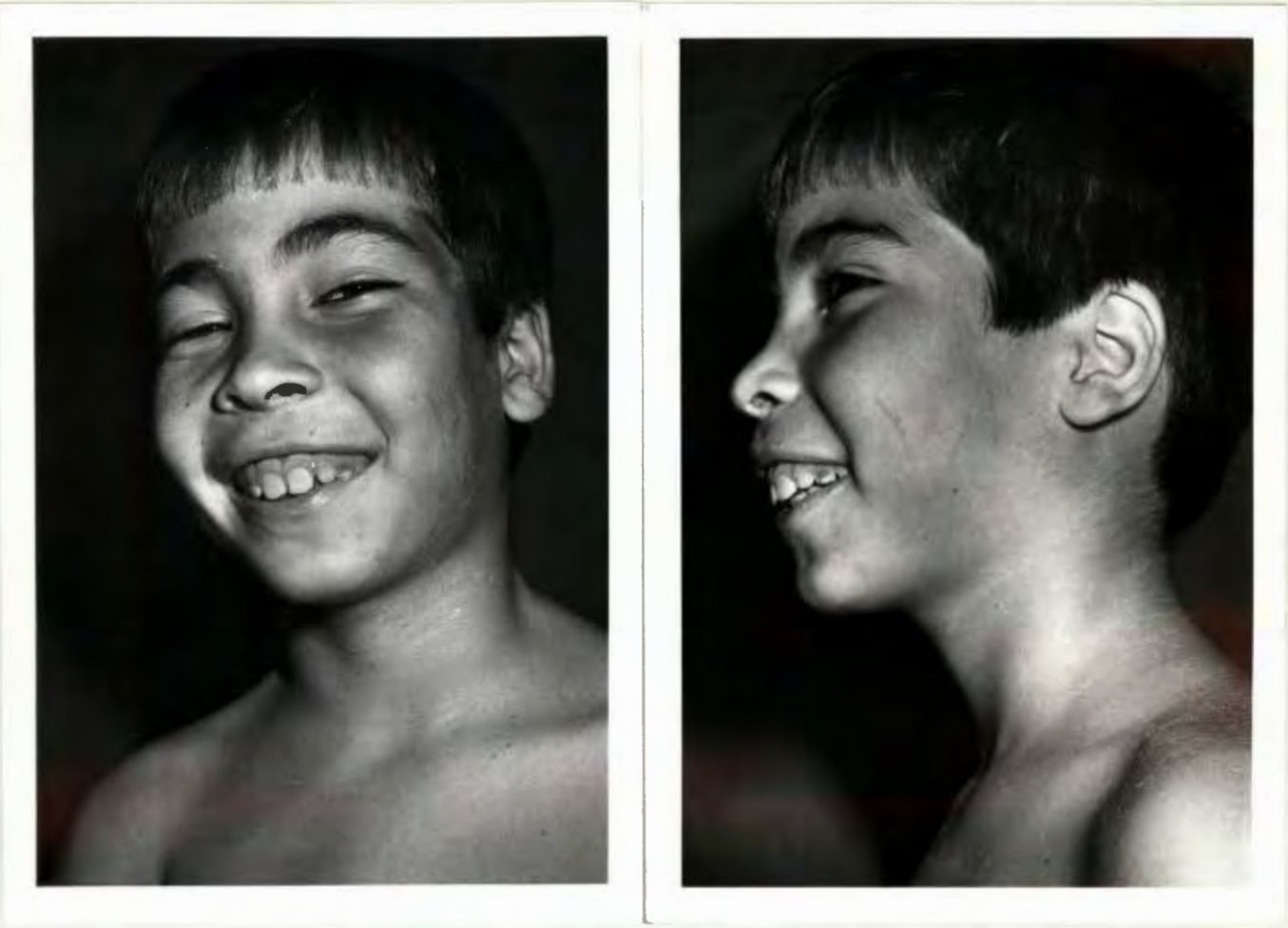


Fig. 20 - 1 The thalassaemic facies. Views showing the deformity of the maxilla and protrusion of the teeth, flattening of the nasal bridge, and wide-set eyes.

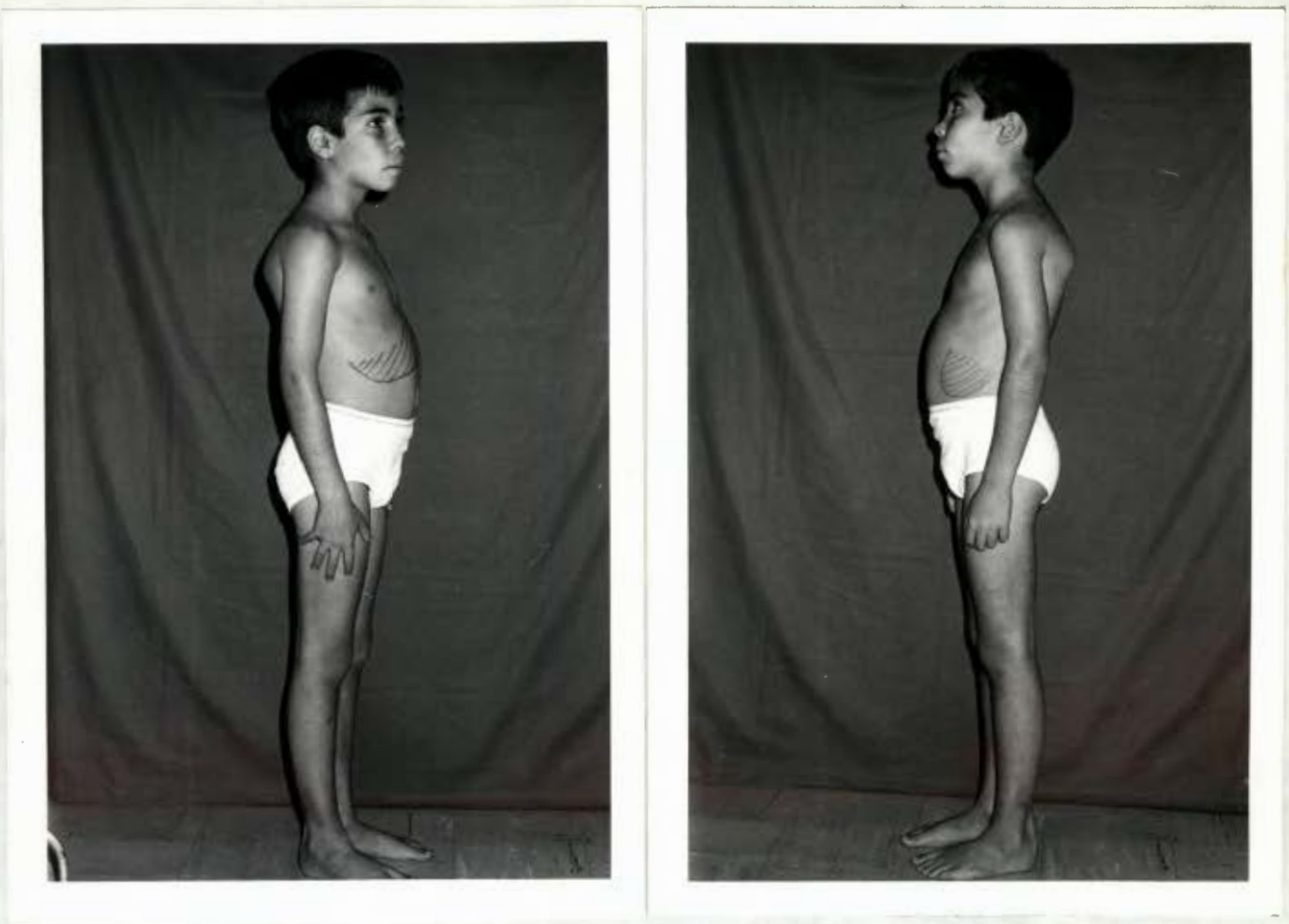


Fig. 20 - 2 Patient 1. Views demonstrating the thalassaemia facies and the patient's hepatosplenomegaly.

Fig. 20 - 3 Views showing the hepatosplenomegaly characteristic of thalassaemia major.

Radiological studies of the chest displayed an increased transverse diameter of heart with gross decalcification of the thoracic cage. The typical "hair-on-end" appearance was present in the calvarium, while the femur, lumbar spine, feet and hands showed osteoporosis with broad trabeculation and some defect in modelling (Figs. 20 - 4, 20 - 5, 20 - 6).

C. LABORATORY INVESTIGATIONS

At 2 years of age, when thalassaemia major was diagnosed, he had haemoglobin of 6,8 g/100 ml and peripheral blood smear demonstrating microcytosis, hypochromia with anisocytosis, polychromasia and bizarre forms. Haemoglobin analysis revealed a fetal haemoglobin of 89,5% and an inconclusive level of haemoglobin A_2.

At 11 years of age his red cell parameters were as follows:

Hb	8,8 g/100 ml
PCV	30%
MCHC	36%
MCV	78 fl
Retic. count	2,5%
Red cell count	3,11 million per cu. mm.
Peripheral smear	Target cells +, microcytosis +, anisopoikilocytosis and basophilic stippling present

Haemoglobin analysis:	HbF	31%
	HbA_2	3,0%

Biochemistry	Units
Total bilirubin	2,6
Conjugated bilirubin	0,8

Fig. 20 - 4 Roentgenological findings in patient 1. Typical "hair-on-end" appearance of the skull.

Fig. 20 - 5 Radiological appearance of vertebrae of patient 1 demonstrating rarefaction of the bones.

Fig. 20 - 6 Radiological appearance in thalassaemia major. Hands and feet show rarefaction with early lace-like appearance of small bones.

Total protein	6,9
Albumin	4,3
SGPT	31
Serum ferritin	6 880 ug/L

Iron studies revealed that percentage saturation in 1973 varied from 50-90%. In 1974 it was 54%; in 1975, 76,4%, and in 1976, 34%.

Australian antigen was reported as being positive in 1973, but negative on several subsequent occasions. Serum immunoglobulins were normal.

D. FAMILY STUDIES

Mother	HbF	4,1%
	HbA_2	5,9%
Father	HbF	1,3%
	HbA_2	3,9%

Red cell parameters and peripheral blood smear were typical of the thalassaemia trait in both parents.

E. TREATMENT

Since the diagnosis of thalassaemia major has been made he has been treated with intravenous packed cells aimed at keeping his haemoglobin at about 10 g per 100 ml. His monthly blood requirements have been 2 units of blood given when his haemoglobin has dropped to 6-8 g/100 ml. This has then brought his haemoglobin up to about 12 g/100 ml. In addition, with each unit (500 ml) of blood he receives 5 g of desferrioxamine and 100 mg of vitamin C

intravenously, and vitamin C 50 mg and folate 1 mg daily per mouth.

2. P.C.

A. HISTORY

P.C., a male, born in 1952, was found to be anaemic and required a blood transfusion when approximately a year old, during an influenza-like illness. As an infant and child he intermittently required blood transfusions, and at the age of 6 years a firm diagnosis of "Cooley's anaemia" was made. Over the past 5 years he has suffered the following complications of his disease:

i) Recurrent viral pericarditis
ii) Yersinia enterocolitica septicaemia
iii) Uric acid nephropathy which required dialysis
iv) Effusion of left hip joint which resulted in sciatic nerve compression
v) Post-transfusional hepatitis
vi) Pleural effusion and atelectasis due possibly to a pulmonary embolus (though never proven)
vii) Arthritis of the left ankle
viii) Bone pain

B. EXAMINATION

On examination in 1976 he was anaemic, slightly jaundiced and had increased pigmentation in his skin creases. Paucity of secondary sexual characteristics resulted in an inappropriate youthful appearance. In addition, he had a mild mongoloid facies due to midfacial hyperplasia (Fig. 20 - 7).

A soft, pansystolic murmur of grade 2/6 intensity was audible over

Fig. 20 - 7 Patient 2 aged 24 years. View demonstrating inappropriate youthful appearance, mild thalassaemia facies, increased facial skin pigmentation and massive hepatosplenomegaly.

the praecordium but no further abnormality was demonstrated in the cardiovascular system. Abdominal examination revealed a 10 cm hepatomegaly and massive splenomegaly extending into the left iliac fossa (Fig. 20 - 8). His testes were hypoplastic but he had no further signs of endocrine failure.

C. RADIOLOGICAL FINDINGS

The bones were generally demineralized and osteoporotic, with broad traveculations and some defective modelling. These changes were particularly evident in the lumbar spine, femur and pelvis, while his hands and feet were more mildly affected (Figs. 20 - 9 and 20 - 10). Radiographs of the skull revealed that the outer table of the calvarium was thin with trabeculations lying at right angles to the inner table (Fig. 20 - 11). The skeleton was immature as evidenced by the fact that the bone age was that of an individual of 16 years when he was 22 years of age.

D. LABORATORY FINDINGS

Haematological

Hb	8,9 g per 100 ml
PCV	27%
MCHC	33%
MCV	82 fl
Reticulocyte count	2%
Red cell count	3,30 million per cu. mm.

Haemoglobin Analysis

HbA_2	1,0%
HbF	4,1%

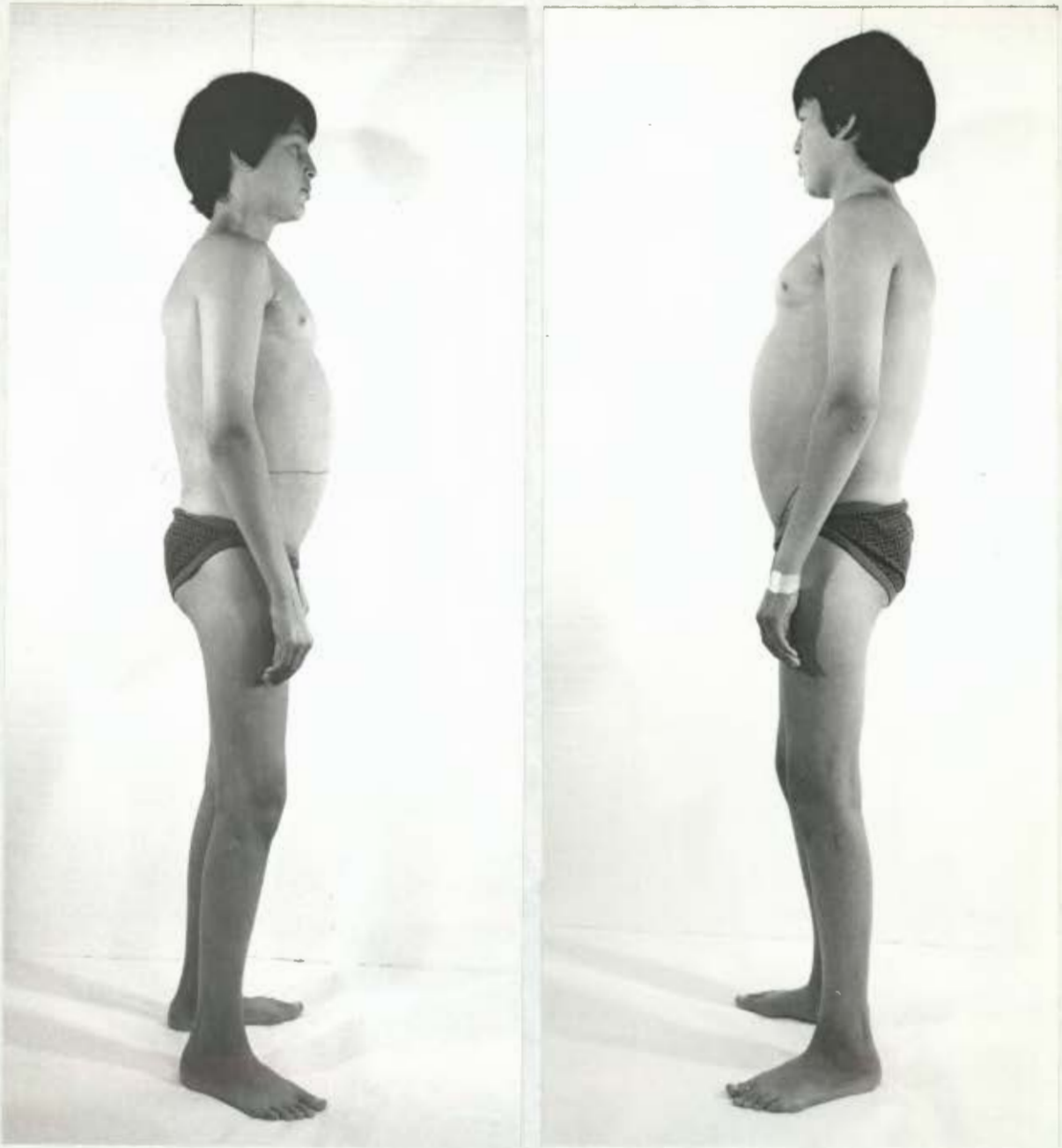


Fig. 20 - 8 Patient 2. Lateral views showing protuberant abdomen as a result of massive hepatosplenomegaly.

Fig. 20 - 9 Roentgenological findings in patient 2. Gross demineralization and rarefaction of femur, lumbar vertebrae and pelvis.

Fig. 20 - 10 Patient 2. View demonstrating rarefaction of small bones of hands and feet.

Fig. 20 - 11 Radiological findings of patient 2.
Top: Skull shows thinning of outer table, widening of diploic space and cross striations making up the characteristic "hair-on-end" appearance.

Bottom: Pelvis and femora, demonstrating rarefaction and some defective bone modelling.

Biochemistry	Patient	Normal
Calcium	8,9	9,0-11,0
Uric acid	7,4	2,0- 8,0
Total bilirubin	2,3	0,1- 1,0
Alkaline phosphatase	101	30-85

Endocrine stress test indicated that his hypogonadism was probably due to pituitary hypofunction.

E. TREATMENT

The hypertransfusion regimen is followed in the patient. At intervals of approximately 2 weeks when his haemoglobin has dropped to about 8-9 g per 100 ml he receives 2-3 units (500 cc) of blood, which increases his haemoglobin to approximately 12 g per 100 ml. In addition, he receives the iron chelating agent desferrioxamine over 6 hours intravenously 2-3 times per week; and ascorbic acid 250 g 3 times per day. He also requires allopurinol 100 mg 3 times per day to maintain a normal serum uric acid level. Since being studied his therapy has been slightly altered. He now receives transfusions of frozen washed cells and desferrioxamine by constant intravenous infusion.

CHAPTER 21

G-6-PD DEFICIENCY

1. I.R.

A. HISTORY

I.R., a 36-year-old male, initially offered no history suggestive of G-6-PD deficiency. However, on taking a more detailed history it became apparent that following the ingestion of fava beans or of aspirin he had had episodes of feeling ill, abdominal pain and dizziness. On examination he was neither anaemic nor jaundiced and the only significant feature was a spleen palpable 2 cm below the left costal margin with no hepatomegaly.

B. SPECIAL INVESTIGATIONS

Hb	15,5 g per 100 ml
PCV	48%
MCHC	32%
Reticulocyte count	2,8%
MCV	96 fl
Motulsky	5 hours
Osmotic fragility	normal
Blood group	O positive

In summary the only stigmata are a grossly abnormal Motulsky test together with a mildly elevated reticulocyte count.

C. MANAGEMENT

He was given a list of potentially haemolytic drugs to avoid and recommended to wear a Medic-Alert disc indicating that he is

G-6-PD deficient.

2. I.V.

A. HISTORY

I.V., a female, was born in 1911 and originated from the island of Lesbos.

At the age of 61 years she presented with an attack of rigors, vomiting, backache and urine becoming dark following the ingestion of 8 aspirin tablets over 2 days for a cold. In the past she had had similar episodes and had been anaemic when pregnant.

Hospital admission was necessary during this episode and she was found to have signs of anaemia and jaundice; no signs of chronic liver disease or hepatosplenomegaly were present. During this admission laboratory investigations revealed the following and a diagnosis of G-6-PD deficiency was made.

Urine	trace of urobilin	
Blood	Hb	8,1 g per 100 ml
	MCV	87 fl
	MCHC	34%
	Reticulocyte count	4,6%
Chemistry	Total bilirubin	2,6
	Conjugated bilirubin	0,5 units
	LDH	708
	Remaining liver function tests	Normal
	Motulsky	35 minutes
	Methaemoglobin reductase test	11,1%
	Intravenous pyelogram and cholecystogram both normal	

Although her Motulsky test was normal, the methaemoglobin reductase test enabled a diagnosis of G-6-PD deficiency to be made. In addition, her son was found to have the disorder.

When seen by the author in 1976 she was neither jaundiced nor anaemic and was in good health.

CHAPTER 22

HEREDITARY SPHEROCYTOSIS

The cases presented here are both those in which a diagnosis was definitely made, as well as those in which a final diagnosis could not be made with certainty. The reasons are given in each instance.

CASE 1

Case 1 (see Chapter 18) was noted on routine testing to have a raised osmotic fragility. On obtaining a more detailed history it was ascertained that his son had been found to have a splenomegaly of unknown origin 2 years previously. Autohaemolytic studies revealed a value of 4% (the upper limit of normal) with no additives, corrected to 1,1% with glucose in Case 1 and a value of 7,8% corrected to 0,3% in the case of his son. Peripheral blood smear showed slight anisocytosis.

On the basis of the splenomegaly, increased osmotic fragility, the enhanced autohaemolysis and the hereditary nature of the disorder, a diagnosis of hereditary spherocytosis (HS) was made. The variable expression of the dominantly inherited gene is well demonstrated in this particular kindred.

CASE 4

Case 4 gave a history of having had a cholecystectomy for gallstones, and on examination she was found to have a 3 cm firm splenomegaly. Osmotic fragility was increased and autohaemolytic studies revealed a value of 16,3% with no additives, corrected to 0,6% with the addition

of glucose. A few spherocytes were seen on peripheral blood smear. On performing limited family studies, her 14-year-old daughter was found to have an autohaemolysis value of 10,6% corrected to 0,4% on addition of glucose.

A diagnosis of hereditary spherocytosis was made on the basis of the patient's cholecystectomy, splenomegaly, raised osmotic fragility, abnormal autohaemolytic studies, and the demonstration of the typical hereditary pattern of the disorder.

CASE 7

Case 7 gave a history of vague abdominal pain which was not suggestive of any specific pathological process. Physical examination revealed no abnormal signs but haematological examination revealed an abnormal brilliant cresyl blue dye reaction (for G-6-PD deficiency) and an increased osmotic fragility. As his osmotic fragility was increased to a greater extent than would be expected from G-6-PD deficiency alone, autohaemolytic studies were undertaken and the results (14,9% with no additives, and 1,8% with glucose) indicated hereditary spherocytosis. In addition, he had a moderate number of spherocytes on his peripheral blood smear and his reticulocyte count was 3,5%.

Later, it was discovered that his family had been investigated in the past for an inherited anaemia and that both his father and sister had been shown to have hereditary spherocytosis, while his deceased mother had carried G-6-PD deficiency.

EQUIVOCAL CASES

The remaining cases - 2, 3, 5, 6 and 8 (see Chapter 18) provide a far

more difficult diagnostic problem.

Respondents No. 2 and 8 gave no suggestive history and on clinical examination there were no findings to suggest a diagnosis of HS. Case 2 had no abnormal haematological parameters while case 8 had a marginally raised reticulocyte count of 2,2% (peripheral blood smear in both cases was normal). The only significant abnormalities were that of osmotic fragilities of the red corpuscles of both were increased and the results of autohaemolytic studies were abnormal. Case 2 had a value of 5,1% corrected to 0,4% on glucose addition and case 8 had a value of 7,5% corrected to 0,2% by addition of glucose. Limited family studies in case 8 were non-contributory and a firm diagnosis of hereditary spherocytosis could not be substantiated.

Case 3, who gave a history of vague abdominal pain, was found to carry G-6-PD deficiency in addition to having an increased osmotic fragility. Because of this latter finding, autohaemolytic studies were undertaken and these showed result of 6,4% corrected to 0,4% on addition of glucose. Peripheral blood smear revealed occasional polychromatophilic red cells while the reticulocyte count was 2,6%. The patient's daughter was found to carry G-6-PD deficiency but her osmotic fragility was normal.

Respondent 5, who had had a cholecystectomy in the past for gallstones, had an increased osmotic fragility and autohaemolysis of 5,7% corrected to 1,7% with glucose. No clinical abnormalities were found and peripheral blood smear was normal. He had a brother who was normal but no other relatives on whom family studies could be undertaken.

The only available record on case 6 stated that she had an increased osmotic fragility and an autohaemolysis of 4,6% corrected to 0,6% on the addition of glucose. Her reticulocyte count was 3,3%.

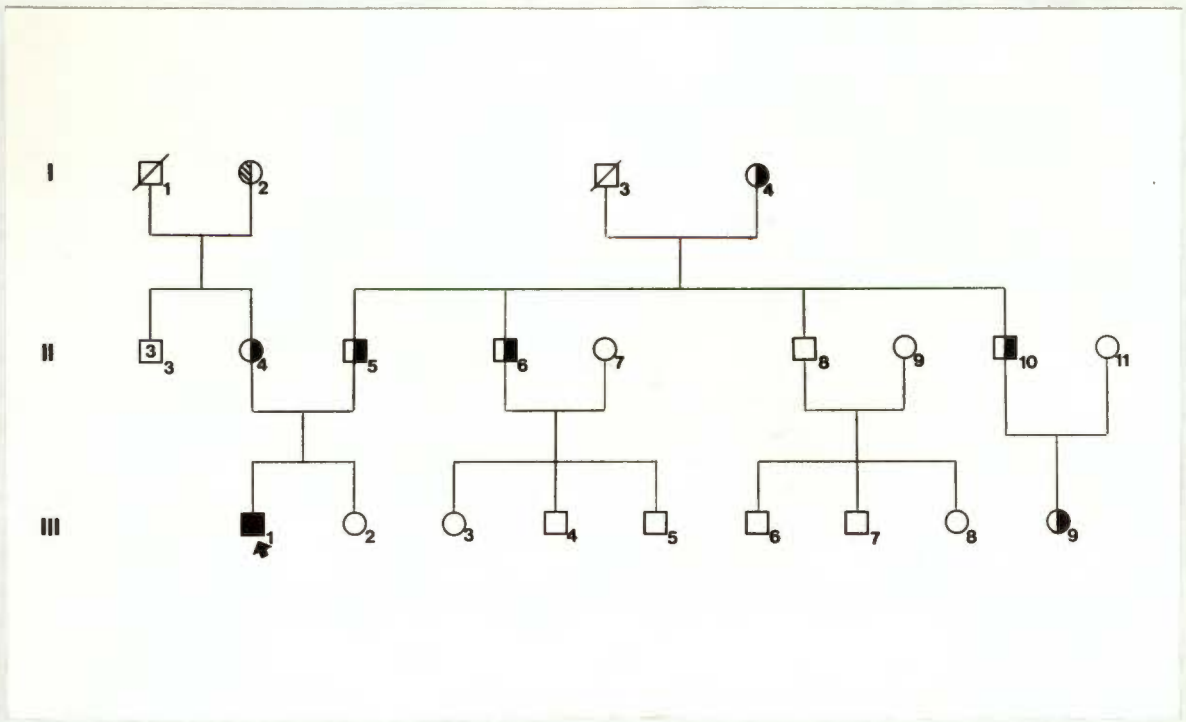
CHAPTER 23

PEDIGREE STUDIES

FAMILY 1

Family 1 (Fig. 23 - 1) illustrates the inheritance of thalassaemia, which is a recessive disorder, although the heterozygotes sometimes have symptoms and signs of varying severity. The proband (111 i) has thalassaemia major and is therefore homozygous for the disorder as he has received a thalassaemia gene from each of his parents. He has the clinically severe disorder and required frequent blood transfusions, while his parents are mildly affected. His mother has symptoms of a mild anaemia and his father is asymptomatic.

The proband has 3 uncles, 11-6, 11-8 and 11-10, 2 of whom, 11-6 and 11-10, have heterozygous beta-thalassaemia. One of them, 11-10, has passed on the thalassaemia trait to his daughter 111-9. The siblings of the proband's mother 11-1, 11-2 and 11-3 are not in South Africa and it is not known whether or not they have the thalassaemia trait. The proband's maternal grandmother 1-2, was tested and found not to have the thalassaemia trait, but in fact to carry G-6-PD deficiency, which is irrelevant in this context. It is therefore reasonable to assume that the mother of the proband received the gene for thalassaemia from her deceased father, 1-1, who was never tested for thalassaemia. The paternal grandmother, 1-4, had been tested and found to have passed the beta thalassaemia gene on to her son.



Normal male

Normal female

Homozygous male

Heterozygous male

Heterozygous female

Proband

Female with G-6-PD deficiency

Deceased male

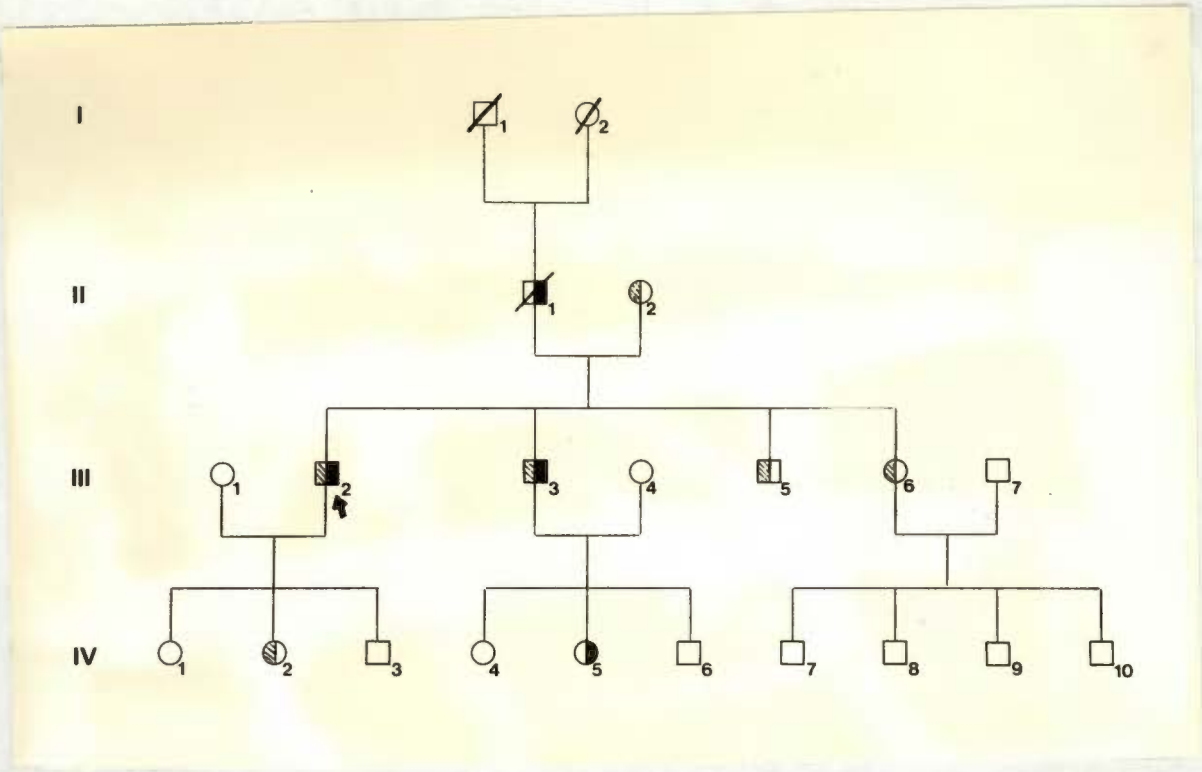
Fig. 23 - 1 Pedigree of family 1

FAMILY 2

Family 2 (Fig. 23 - 3) (which is related to family 1) illustrates the inheritance and association of heterozygous beta-thalassaemia and G-6-PD deficiency in the same family. The proband 111-2, who had had an attack of favism when a child in Greece, was found to have both the thalassaemia trait and G-6-PD deficiency. The acute haemolytic attack he suffered following the ingestion of fava beans was so severe that he required a blood transfusion.

The proband's mother, 11-2, also suffered a severe, acute haemolytic attack following the ingestion of acetyl salicylic acid, which suggests that this family have a particularly severe form of G-6-PD deficiency. However, G-6-PD deficiency had not been a problem in other members of the family. The diagnosis of the thalassaemic trait was made on comprehensive haematological investigations for a haemolytic anaemia and had, in fact, not been a problem in its own right. The occurrence of both disorders in the same person does not significantly increase the problems associated with each, though as each condition does predispose an affected individual to an anaemia, an anaemia due to one may be slightly exacerbated by the other.

The pedigree also illustrates that thalassaemia and G-6-PD deficiency are inherited independently. Subjects 111-2 and 111-3, who both have G-6-PD deficiency and heterozygous beta-thalassaemia, received the gene for each disorder from each parent and subsequently only passed one of the abnormal genes on to their offspring. Subject 111-2 passed a G-6-PD deficiency gene on to his daughter, while 111-3 passed a beta-thalassaemia gene on to his daughter. As the patterns of inheritance for these disorders are different, thalassaemia being recessive and G-6-PD deficiency being X-linked, this is the expected picture.



Normal male

Normal female

Male with G-6-PD deficiency and heterozygous beta-thalassaemia

Female G-6-PD heterozygote

Male with G-6-PD deficiency

Female beta-thalassaemia heterozygote

Deceased male

Deceased female

Proband

Fig. 23 - 2 Pedigree of family 2

FAMILY 3

Pedigree 3 (Fig. 23 - 3), the extended pedigree of families 1 and 2, who have common grandparents, and demonstrates a large family which has homozygous beta-thalassaemia, heterozygous beta-thalassaemia and G-6-PD deficiency. The family originates from the north-east Aegean island of Lesbos where the incidence for heterozygous beta-thalassaemia is approximately 10% (Fessas, 1976) and that for G-6-PD deficiency approximately 4% (Doxiadis, et al, 1964). It is therefore to be expected that there must be large families with both these disorders. As families 1 and 2 have been discussed in reasonable detail, this has not been repeated for family 3.

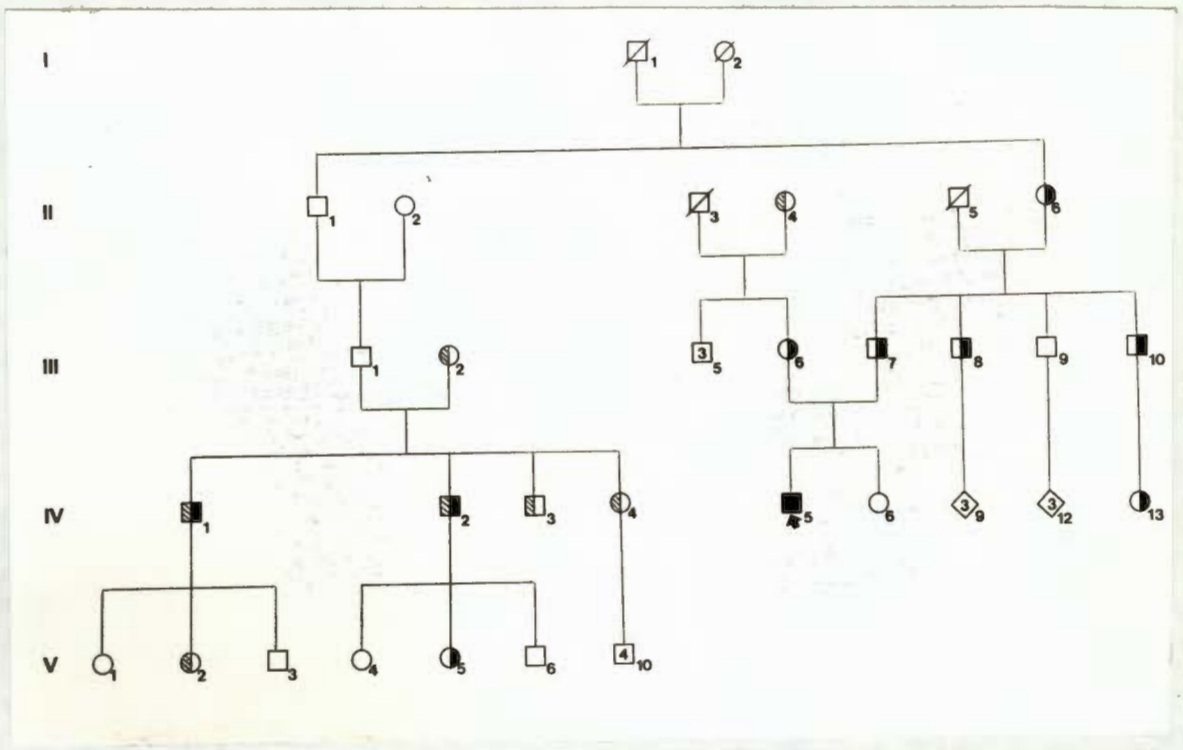


Fig. 23 - 3 Pedigree of family 3

FAMILY 4

Family 4 (Fig. 23 - 4) illustrated the inheritance of G-6-PD deficiency. The family was initially investigated as the parents of the proband, who was 6 months of age, were worried about an anaemia which he had developed during an influenza-like illness. The proband (111-1) had a grossly abnormal brilliant cresyl blue test indicating that he had G-6-PD deficiency. The brilliant cresyl blue test of his mother (11-2), and maternal grandmother (1-4), were moderately increased and the met-haemoglobin reductase test of his mother was grossly abnormal.

The X-linked pattern of inheritance of G-6-PD deficiency is well illustrated in this kindred. On investigating the proband's father (11-1), he was found to also have G-6-PD deficiency although he had always been asymptomatic. His own parents (1-1 and 1-2) were not tested but as G-6-PD deficiency is an X-linked disorder it is reasonable to assume that he had inherited his G-6-PD deficiency from his mother.

It is of interest to consider the possible genotypic expression in children of the proband's parents as the father has this X-linked disorder and the mother is heterozygous for it. There are 4 possibilities:

i) that the offspring be a homozygous girl with full expression of the disorder;
ii) a male with the disorder;
iii) a heterozygous female carrying the disorder, or
iv) a normal male.

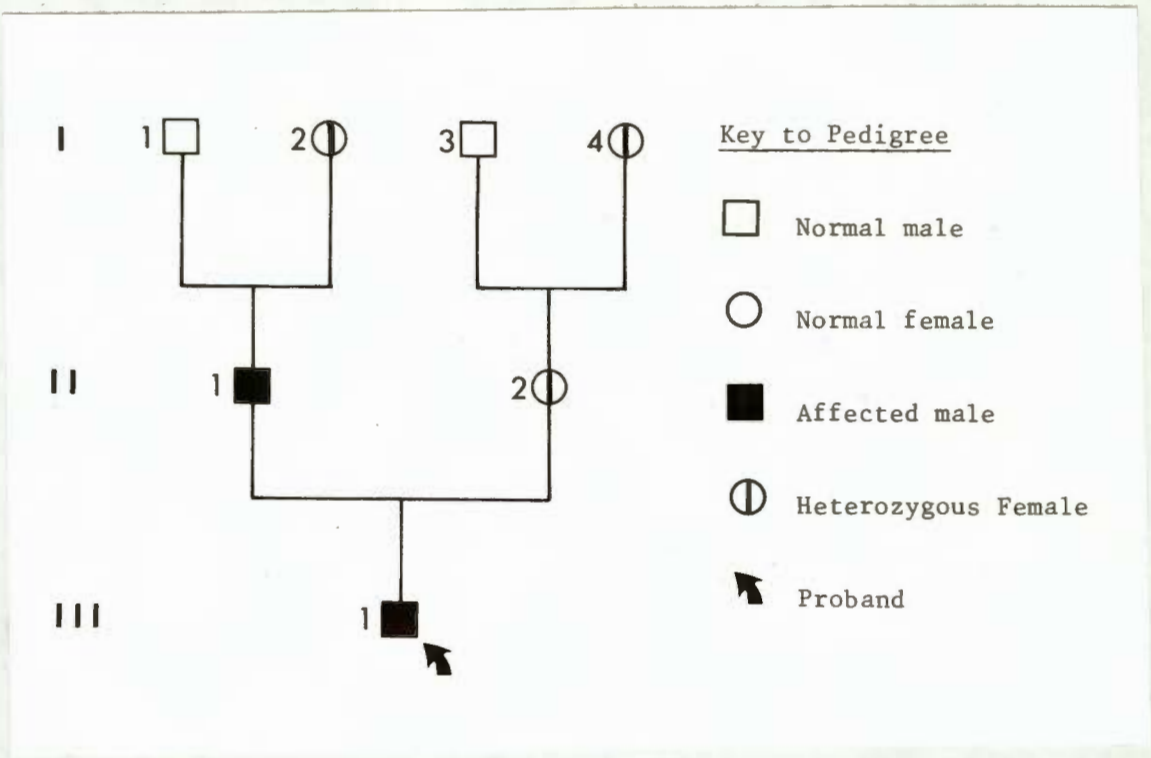


Fig. 23 - 4 Pedigree of family 4

FAMILY 5

Heterozygous beta-thalassaemia and G-6-PD deficiency occur together in family 5 (Fig. 23 - 5). The proband (111-1), a medical doctor, has heterozygous beta-thalassaemia and is asymptomatic. His father (11-1) was investigated for a haemolytic anaemia and it was discovered that he had both G-6-PD deficiency and the thalassaemic trait. On undertaking family studies it was ascertained that he received both abnormal genes from his mother (1-2) and passed both to his daughter (111-3). This situation contrasts with family 2 where the individuals with both genes received them from separate parents and passed only 1 abnormal gene on to the next generation. The wife (11-2) of 11-1 was also found to have heterozygous thalassaemia and the possibility therefore arose of an individual with thalassaemia major being born into this family. The brother of the proband (111-2) died as a young man in a motor vehicle accident, and although he was not tested, it is extremely unlikely that he had homozygous beta-thalassaemia, although it is possible that he had heterozygous beta-thalassaemia and/or G-6-PD deficiency. 111-3, the sister of the proband, who carries both G-6-PD deficiency and heterozygous beta-thalassaemia, suffers periodically from a symptomatic anaemia.

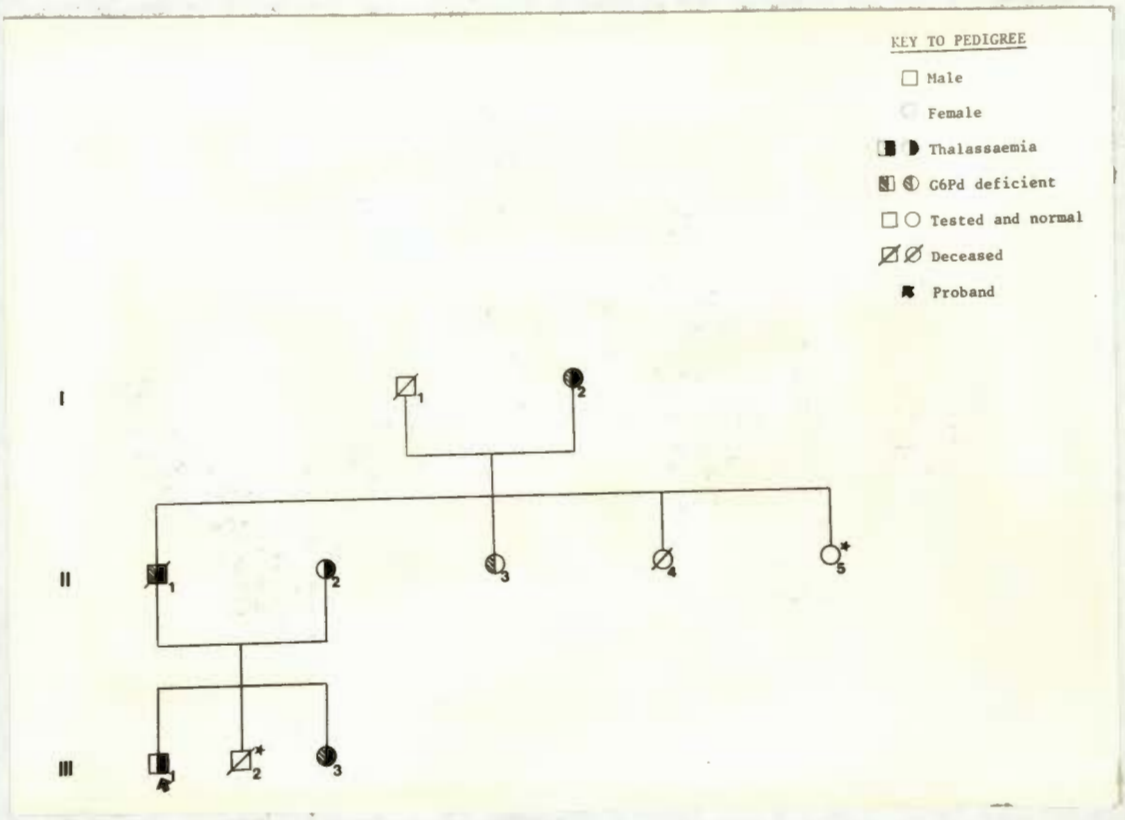


Fig. 23 - 5 Pedigree of family 5

FAMILY 6

Hereditary spherocytosis and G-6-PD deficiency occur together in family 6 (Fig. 26 - 6). The proband, 11-3, a male aged 32 years of age who, apart from complaining of vague abdominal symptoms, has always been in extremely good health. During the survey he was found to have both G-6-PD deficiency and HS, neither of which had caused him any problems. On perusing laboratory records it became evident that his family had previously been investigated extensively for an inherited anaemia, as his sister (11-2) had suffered from a significant anaemia. She was found to have hereditary spherocytosis, which she had inherited from her father (1-1). Both of her children (111-1 and 111-2) have been tested and found to be normal. It was also discovered that his mother (1-2), now deceased from a heart ailment, had heterozygous G-6-PD deficiency. Hereditary spherocytosis is inherited in a dominant pattern, whereas G-6-PD deficiency is X-linked and therefore the occurrence of the 2 in 1 family is pure chance. As both disorders result in a haemolytic anaemia, it is possible that the proband will suffer complications attributable to this and splenectomy may have to be considered in the future.

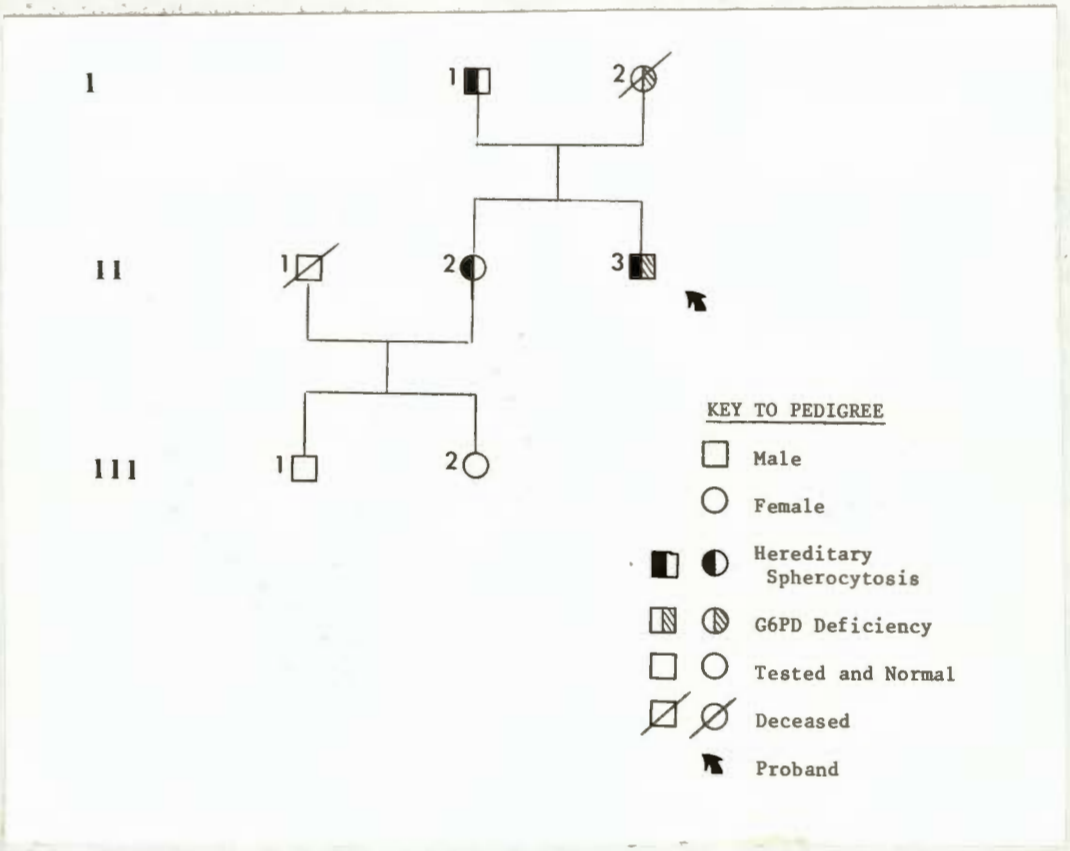


Fig. 23 - 6 Pedigree of family 6

SECTION VII

DISCUSSION AND CONCLUSION

In this section subjects relevant to the survey and disorders found in it which have not been fully elucidated earlier are discussed.

CHAPTER 24

SURVEY ORGANISATION AND GENERAL COMMENTS

INITIAL ORGANISATION

Once the basic aims of the survey had been formulated, the initial organisation proved to be remarkably straightforward. Probably the most severe problem was the characteristic Greek tendency to procrastinate! The Greek authorities, particularly the diplomatic corps, community office bearers and local teachers were appreciative of the aims of the study and its potential benefit for Greek individuals and the community as a whole. As a result demographic information was willingly and plentifully given and introduction was facilitated to the Greek community at all social levels.

The establishment of the laboratory facet of the investigation was fortunately without any significant problems. Dr. M.C. Botha, head of the Cape Provincial Blood Grouping Laboratories, co-operated fully, placing his facilities at the author's disposal. The Blood Grouping Laboratories routinely perform the tests which were required for the purposes of the study and thus no time had to be spent in the prior evaluation and reassessment of techniques.

BLOOD SAMPLING

The first snag, and one which continued for some time, was to persuade potential respondents to donate blood samples. Their reluctance was based upon initial fear, wariness and suspicion of the aims on the survey, especially on the part of the less well-educated individuals. However, the more intelligent and better educated subjects were

generally approachable and co-operative, probably because they were better able to understand the aims of this study.

With the passage of time it became apparent that the survey was being discussed extensively amongst members of the community, and individuals became more conversant with the aims of the study and increasingly willing to participate. It nevertheless took approximately 6 months to overcome this initial reluctance and at this stage the greater part of the author's time was spent in seeking respondents.

A further rate limiting factor was that the laboratory could only handle 5 blood specimens per day. Furthermore, the blood specimens had to be fresh, as certain of the tests had to be performed within 6 hours of venepuncture. The practical implications were that the blood could only be taken on week-days and during the mornings. For this reason advantage could not be taken of the fact that the Greek community met in significant numbers regularly on Sundays.

As many members of the community had small businesses which they could not leave during the week, and as blood could not be sampled during the week-end, much of the author's time was spent travelling to visit individuals in their homes or work places in order to sample their blood and obtain a family history.

Once the tests revealed that a respondent had an inherited haematological disorder, further time would then have to be spent by the author visiting these individuals to obtain a more complete clinical history, perform a comprehensive physical examination and, where possible, to study the relatives.

The author estimates that he undertook more than 1500 kilometres of local travel during the project for these reasons.

RANDOM SAMPLING OF SUBJECTS

After the survey was completed, the question arose as to whether or not the sample was truly random. On statistical analysis (by a qualified statistician) on the distribution of the Duffy blood group in the respondents, it appeared that this might not have been adequately random. However, it was subsequently demonstrated that the Duffy anti-serum used was at fault and on proceeding to analyse the ABC blood group system it was demonstrated that the sample was indeed random.

The Greek community of Cape Town is relatively small and, as has been previously noted, members tend to come mostly from one small island. Therefore, one would expect there to be some bias in the sample when analysed by the above means. To ensure that the survey population was as random as possible, no first degree relatives were included in the survey. Nevertheless, as the Greek community is a relatively close-knit one and the tendency is not only to marry persons of the same island but even of the same town, it is to be expected that there is a degree of in-breeding within this community and, therefore, by definition the sample could not be truly random.

LABORATORY TECHNIQUE

The tests performed on the blood were highly sophisticated and as such required specialized equipment and personnel. These criteria were met by the Cape Provincial Blood Grouping Laboratories who routinely performed these tests. The personnel were medical technicians who had

been especially trained in this field and were extremely competent. For these reasons there were no problems w th experimental techniques and normal values for the laboratory were well established. Nevertheless, in a minority of borderline cases clear interpretation of results was difficult. In the same way there were initially slight problems with the apparatus and techniques involved in estimating alpha/beta chain ratios in the diagnosis of the alpha-thalassaemia heterozygous state, a diagnosis which can be extremely difficult to establish.

Although the greatest bulk of the experiments were performed by experienced laboratory technicians, the author familiarized himself with the techniques involved and became fully conversant with all aspects of the equipment and execution of the analyses. More time could not be spent on this aspect of the study as so much of the author's time had to be employed in obtaining the subjects and their blood samples.

FAMILY STUDIES

In certain of the patients, detailed family studies are extremely useful in substantiating or negating a diagncsis. Situations where this is particularly relevant is in the diagnosis of alpha-thalassaemia carriers, hereditary spherocytosis, borderline cases of beta-thalassaemia and in certain females heterozygous for G-6-PD deficiency. In alpha-thalassaemia heterozygotes where only one or two of the genes are involved, the stigmata may be minimal. In this situation family studies are extremely important and the abnormality may only show up in the cord blood of a neonate.

The pattern of inheritance of the hereditary spherocytosis should by definition be demonstrated in making the diagnosis. In equivocal cases

of beta-thalassaemia heterozygotes where abnormalities are only of a minor degree, family studies may provide the diagnostic answer. In G-6-PD deficiency which is inherited as an X-linked trait, signs of the disease in female carriers may vary from nothing to the full blown disorder. The reason for this is clarified by the Lyon hypothesis which suggests that random inactivation of the X-chromosome occurs. Thus in female carriers of G-6-PD deficiency a greater or lesser number of cells which are G-6-PD deficient may be inactivated and if normal cells that are inactivated the individual may have the stigmata of the disorder and vice versa (Lyon, 1961, 1962). In the former situation it may again only be family studies which would clarify the situation and allow a diagnosis to be made by demonstrating that either the son or father of the subject have the condition or that the mother carries it.

At certain periods during the investigation one of the difficulties that arose was that it was impossible to undertake adequate family studies. The reason for this appeared to be that, especially in the cases of the younger immigrants, they had few if any relatives in Cape Town. In particularly difficult diagnostic situations, the fact that respondents had only a limited number of relatives in Cape Town continued to be a problem of some consequences.

GENETIC CONTROL AND COUNSELLING

BETA-THALASSAEMIA

The theoretical data as well as the cases that have been presented in this study amply demonstrate the severity of thalassaemia major. The

treatment of the condition is limited, does not cure, only partially alleviates and contributes its own problems and side effects. It is clear for these reasons that prevention is most important. This is best achieved through genetic control which will now be discussed at greater length.

For genetic control one needs to be aware of the condition itself, its severity, the mode of inheritance and whether antenatal screening and diagnosis are possible. The severity of the condition has been discussed and demonstrated at length earlier. The pattern of inheritance, as mentioned before, is autosomal recessive although the heterozygotes may manifest the symptoms and signs of the disorder to a greater or lesser extent. However, the heterozygotes are usually asymptomatic.

In Cape Town (and South Africa) at present, antenatal screening is not possible in certain centres elsewhere. Therefore when counselling a couple who are both heterozygotes one would have to recommend that they do not procreate at present. However, during the past 3 years antenatal diagnosis has been made possible. In order to achieve this, fetal blood sampling needs to be performed. This may be done either by blind puncturing of the placenta after its ultrasonic localization and then collecting red blood cells mixed with amniotic fluid, or by doing fetoscopy and then by aspirating blood from the large superficial placental blood vessels. This blood which has a mixture of maternal and fetal red blood cells is then analysed. Mixtures containing as little as 5% of fetal red blood are sufficient (Fairweather, et al, 1978). The fetal cells are then examined for presence or absence of beta-chain production.

Fetal blood sampling has a high mortality rate and is only indicated in 'at risk couples' who agree to the termination of the pregnancy should the fetus have thalassaemia major. This procedure is now being done in certain large European and American hospitals.

ALPHA-THALASSAEMIA

Alpha-thalassaemia in the homozygous state results in a hydrops fetalis and stillbirth, whereas haemoglobin H disease, the next severest form of the disorder, usually causes a moderate anaemia. The social and economic implications, therefore, differ from homozygous beta-thallassaemia. However, it is not reasonable for a female to carry an affected pregnancy to term if it can be prevented.

Antenatal diagnosis is now possible by analysing copy desoxyribonucleic acid (cDNA) prepared from cultured fibroblasts and demonstrating the number of alpha-globin genes which are missing (Wong, et al, 1978). Amniocentesis, which is within the capacity of most obstetric units, is all that is required to obtain fibroblasts from the amniotic fluid. However, the DNA analysis can only be done in highly specialized units. Wong, et al (1978) have also demonstrated that the amniotic fluid can be transported over long distances for the accurate analysis to be made.

Therefore, if an at risk pregnancy presented we could offer antenatal diagnosis after arranging transport of the amniotic fluid to the relevant laboratory. Amniocentesis would be undertaken at 15 weeks gestation. Thereafter, fibroblast culture and DNA analysis would require approximately 5 weeks. In the case of a fetus with homozygous alpha-thalassaemia, termination of the pregnancy would therefore have

to be undertaken at about 21 weeks gestation.

In summary, genetic counselling of persons carrying either of the thalassaemias involves informing them of their usually very mild disorder and of the possible risks for their offspring. When both husband and wife are carriers of the condition, it is important that they be informed of the seriousness of the disorder in its homozygotic forms and thereafter giving information as regards the possibility of antenatal diagnosis and the risks attached to these procedures. Finally, parents of a severely affected child should be informed of the severity of the disorder, progress in treatment and prognosis. They need also to be told of the risks attached to future pregnancies and of the status of antenatal diagnosis.

Groote Schuur Hospital recently acquired a fetoscope and it is hoped that in the near future fetoscopy and fetal blood sampling will be possible and that antenatal diagnosis of thalassaemia be offered at this institution. At the time of writing, as far as is known, no other institutions in South Africa have a fetoscope or are able to offer this service. Therefore at present the best that we can offer to parents with 'at risk' pregnancy is referral to an overseas centre which has the necessary expertise and facilities to diagnose thalassaemia major.

GLUCOSE 6-PHOSPHATE DEHYDROGENASE DEFICIENCY

G-6-PD deficiency is not a disorder of the same severity as thalassaemia major and therefore different genetic counselling and control is required. Nevertheless, knowledge of the disorder within the population at risk is necessary. It is important that the medical profession be aware of the communities with a high prevalence so that a rapid diagnosis

can be made. This is of particular concern in the case of pregnant females who normally have the tendency to anaemia antenatally and which is exacerbated by the condition, and for neonates who may present with a severe bilirubinaemia. Thus, although G-6-PD deficiency is important in the perinatal period, it is never necessary to consider termination of pregnancy in the disorder.

G-6-PD deficiency may also result in a specific illness at any time in persons having or carrying the disease. For these reasons it is important that the physician be aware of the population at risk so that rapid diagnosis be made and adequate management given. Thereafter it is necessary that patients with the disturbance be informed of the medicines and foodstuffs to be avoided so as to prevent the haemolytic complications from occurring. It is also recommended that persons with the condition wear a medic-alert disc and that relatives of affected individuals be tested to ascertain whether or not they are carrying the disorder. The above steps were carried out on affected subjects in the survey.

HEREDITARY SPHEROCYTOSIS

As with G-6-PD deficiency it is usually not necessary to consider termination of a pregnancy in a family in which there is HS. However, it is important that the diagnosis be confirmed in an affected individual and that relatives be tested to ascertain who does indeed have the condition.

HS is inherited in an autosomal dominant fashion and thus persons with the abnormal gene may suffer from the disturbance. The usual treatment

of a patient with symptoms and signs of the disorder, especially anaemia, splenomegaly and gallstones, is splenectomy. Persons presenting with attacks of cholecystitis and who have HS should not only undergo a cholecystectomy, but also a splenectomy to decrease haemolysis. Thus the diagnosis of the condition is important in order that the correct operation be performed.

One subject with HS in the survey was incorrectly treated for gallstones only, as the underlying cause (HS) was not diagnosed. As a result she only underwent a cholecystectomy. The accepted correct treatment would have been the removal of both the spleen and gall bladder at the same operation.

The diagnosis within a family is important so that they can be adequately counselled about the mode of inheritance, informed who is affected and given necessary details about the disorder. This knowledge is also important for the attending physician or surgeon in order that correct treatment be provided.

CHAPTER 25

ALPHA-THALASSAEMIA

In this chapter, pertinent points of discussion of alpha-thalassaemia which have not yet been dealt with are examined.

UNDERDIAGNOSIS OF ALPHA-THALASSAEMIA

The difficulty in diagnosing alpha-thalassaemia heterozygotes has previously been discussed. In summary, there are 4 genes for alpha-thalassaemia and if all are involved (i.e. homozygozity), it is incompatible with life and still birth with hydrops fetalis results. If 3 of the 4 genes are abnormal, the result is haemoglobin H disease in which the patient has a disease of medium severity. This is diagnosable on clinical grounds together with red cell parameters, red cell inclusion bodies and haemoglobin electrophoresis, where a characteristic H band is obtained. Persons with only one or two genes involved and who have the alpha-thalassaemia trait may or may not have abnormal clinical and haematological findings and thus be very difficult, if not impossible to diagnose. Diagnosis in these cases depends on analysis of the relative rates of synthesis of the alpha and beta chains.

The problems associated with the diagnosis of alpha-thalassaemia are well demonstrated by the survey. Because of the relative cost and time involved, only a certain number of persons were able to have alpha/beta chain ratios undertaken. These persons (as discussed in Chapter 15) were selected because they either had numerous red cell inclusion bodies or because they had red cell anomalies (e.g. decreased MCV and MCH), as well as a few inclusion bodies.

Three of the 6 so selected were finally diagnosed as having the alpha-

thalassaemia trait. None of these 3 were anaemic and all the red cell parameters of one respondent (Case 3) were normal. She did, however, have $\frac{60}{10\ 000}$ red cell inclusion bodies. Of the other 2 subjects, 1 had a moderate number of red cell inclusion bodies and abnormalities of his red cell indices, while the other also had red cell parameter anomalies with a small number of red cell inclusion bodies ($\frac{4}{10\ 000}$).

Of the remaining 3 who were not diagnosed as having the alpha-thalassaemia trait, it is seen that all had numerous red cell inclusion bodies and 1 had aberrant red cell indices. It is felt by the author that this latter individual may yet be found to carry alpha-thalassaemia, as her alpha/beta chain ratios were borderline. One of the remainder appears to have the beta-thalassaemia trait while the other patient is normal. These 3 cases are discussed in greater detail later in this chapter.

From the above, it can be seen that a diagnosis of the alpha-thalassaemia trait cannot be made where either both or one of these findings is abnormal and alpha/beta chain rate of synthesis must be measured.

Thirty-five subjects remain who had either one or the other of the above abnormalities (red cell parameter anomalies or haemoglobin H red cell inclusion bodies) but whom it was not possible for logistic reasons to investigate further. This group is made up of 20 with red cell inclusion bodies but no abnormalities of red cell indices, and 15 with anomalous red cell parameters (namely decreased MCV and MCH) but no red cell inclusion bodies. Of the latter group, 2 can be discarded as their decreased MCV and MCH can be explained on the basis of an iron deficiency anaemia. Of the former group, 1 subject had $\frac{36}{10\ 000}$

red cell inclusion bodies but no further red cell anomalies. He was not studied further as he was not contactable. His results are similar to Case No. 3 who was found to have alpha-thalassaemia and it is quite possible that he too had the alpha-thalassaemia trait.

It is recognized, and in fact illustrated by the survey, that normal persons may have some inclusion bodies in their peripheral blood smears. Nevertheless, it seems quite probable that of the remaining 19 with red cell inclusion bodies some, if investigated further, would be found to carry alpha-thalassaemia. Similarly, in the remaining 13 subjects with no red cell inclusion bodies but definitely decreased levels of MCH and MCV, it is very possible that a certain proportion do carry alpha-thalassaemia and that this is responsible for these alterations. This situation could only be clarified by undertaking alpha/beta chain synthetic rate ratios to ascertain whether these subjects carry either of the thalassaemias.

For the above reasons, it seems likely that the prevalence of heterozygous alpha-thalassaemia is significantly higher than the 1,2% quoted in the survey.

Alpha/Beta Chain Ratios

The results of alpha/beta chain ratios performed in the other 3 persons not diagnosed as having the alpha-thalassaemia trait are worth examining further.

M.J., a female aged 35 years, gave a history of an anaemia, the cause of which was not established. At the time of investigation, she had no clinical signs and her red cell indices and haemoglobin electrophoresis

were within normal limits. However, she did have $\frac{18}{10\ 000}$ red cell inclusion bodies and for this reason alpha/beta chain synthetic rates were measured. The result was 1,09 which is in keeping with normality.

M.D., a male aged 27 years, gave no medical history of an anaemia. His red cell parameters were all normal and haemoglobin electrophoresis and quantitation exhibited an A.A. pattern, HbF 0,1% and HbA_2 2,6%. However, on incubation with brilliant cresyl blue $\frac{20}{10\ 000}$ red cells had haemoglobin H inclusion bodies and it was, therefore, felt that he may carry heterozygous alpha-thalassaemia. Alpha/beta chain ratios were performed and gave a result of 1,38 which was repeated and found to be 1,47. This result is characteristic of beta-thalassaemia.

I.M., a female aged 26 years, gave a history of anaemia during pregnancy. Physical examination revealed a mild anaemia which we confirmed by laboratory examination. Her haemoglobin (Hb) level was 11,2 g per 100 ml, MCV 75 fl and MCH 24 pg. All these 3 parameters are decreased and in addition she had $\frac{8}{10\ 000}$ haemoglobin H red cell inclusion bodies which made the picture highly suggestive of the alpha-thalassaemia trait, as her haemoglobin electrophoresis and quantitation were both normal. However, her alpha/beta chain synthesis rate was 0,92, which falls just within the lower limit of normal and thus a diagnosis of the alpha-thalassaemia carrier state was not made. Nevertheless, it is the opinion of the author that this individual may yet be proved to have the alpha-thalassaemia trait and this is further evidence of the difficulty in diagnosing alpha-thalassaemia. This position could probably be clarified by family studies which, however, are not possible at present.

CHAPTER 26

BETA-THALASSAEMIA

THALASSAEMIA MAJOR

IMPLICATIONS

Thalassaemia major is a severe disabling disorder with important short and long term implications. Problems are those specific to the condition itself as well as those applicable to most chronic illnesses.

1. MEDICAL ASPECTS

In summary, these are problems arising from the disease itself, as well as its complications. These have previously been enumerated in the discussion of the disease in Chapters 7 and 8. These include the anaemia and its immediate effects, growth retardation, skeletal maldevelopment, endocrine failure, increased infections and hypersplenism.

2. HOSPITALISATION

Frequent hospitalisation is necessary from the time of diagnosis.

a) Initial Hospitalisation

This is usually to establish the diagnosis. If the clinician has a high index of suspicion this diagnosis is usually made shortly after 6 months of age. However, if the diagnosis is not apparent, hospitalisation may be prolonged while extensive investigations are undertaken. Implications now include cost and psychological factors which will be enlarged upon later.

b) From the time the condition is diagnosed frequent hospitalisation is required, mainly for treatment but also for follow-up

purposes. As the front line of treatment is blood transfusion, which is required frequently - initially approximately 1 monthly and thereafter 1 to 2 weekly, this has to be supervised in hospital. This, too, has important cost and psychological implications.

c) Due to the numerous complications that patients are prone to, hospitalisation is often necessary. These include infections, often due to unusual organisms, pericarditis, nephropathy and cardiac failure, all of which have occurred in the subjects included in the survey.

3. TREATMENT

Management consisting mainly of hypertransfusion plus chelation and supportive therapy which is frequent and lifelong. In addition to this it is also unpleasant and painful especially for the young who require regular and frequent intravenous infusion.

The other major disadvantage of treatment is that it has its own set of complications. As discussed earlier these are mainly iron overload and resultant haemochromatosis which is also a complication of the disease itself. The outcome includes cardiac failure, endocrine failure and hepatic cirrhosis.

4. SCHOOLING AND EDUCATION

Absenteeism occurs often as numerous hospitalisations are required for treatment and complications of the illness. This may contribute toward academic failure even though mental retardation is not an associated problem of the condition or in the 2 cases in this study.

Sport is an integral part of life in most South African schools. Patients with thalassaemia major, even if they feel physically capable of participating are advised not to due to possible problems such as traumatic rupture of the spleen. This can lead to a feeling of inferiority and abnormality. This was a problem with the younger of the 2 investigation subjects who was particularly keen to play rugby football and judo.

5. PSYCHOLOGY

a) Parents

It is important that the parents of an affected individual be fully informed about the disorder, its inheritance, treatment and prognosis. They must firstly not be made to feel guilty that they carry a recessive gene and must therefore be supported in adjusting to an offspring who is going to require frequent and lifelong treatment and will probably have a limited lifespan. It is important that they be informed of the latest developments as regards antenatal diagnosis and prevention in future pregnancies and any new therapy which might be of benefit to their child.

It is known that parents carrying a disorder may feel tainted and have powerful guilt feelings. This may create significant tensions within the individual and result in neurotic reactions as well as psychosomatic illnesses. This in turn leads to the parents being overprotective towards the affected child and overindulging him or her thereby creating further problems.

If there are siblings they too will be affected by the abnormal

family situation. With more attention being lavished on the patient, sibling rivalry and resentment often arises.

Education, understanding and support are the most important ways in which doctors can be of assistance to the parents of an affected individual. They should also be guided in their relationship with normal offspring.

b) Patient

Psychological problems can complicate the whole life of one suffering from thalassaemia major. The first problems are encountered during initial hospitalisations when the parent and child are separated. The patient finds himself in unfamiliar surroundings and being cared for by strangers, which results in 'separation anxiety', an entity well recognized when children are hospitalised.

During hospitalisation, nursing care often entails control of bodily functions, such as washing and feeding which the child may have already mastered and he is treated in an inappropriate way for his age. This loss of ego control usually results in regressive behaviour such as bedwetting which may persist after discharge from hospital. However, as defence against this regression the child may become a difficult, intractible patient, not reverting to or accepting helpless infancy. It may be necessary that the child be reweaned.

In chronic illnesses such as thalassaemia major the child may feel discriminated against, passively deprived and thus lose confidence in himself. This is often a problem during convalescent periods

when neurotic disorders may manifest. Typical reactions are mood swings, temper tantrums, bedwetting, phobias and sleeping problems. These do not necessarily occur as a result of hospitalisation. In addition, feelings of abnormality and thus inferiority and rejection may be present for life.

The need to take medicines on a long term basis has been recognized as a cause of repressed ideas of the patient being attacked by the mother. When motor restraint is placed on a child the reasons may not be apparent to him and result in behavioural problems such as irritability, bad language and temper tantrums.

Schooling difficulties, although not presenting as a problem in the 2 cases in the survey, may occur. This is especially so in a child who is average or below average intellectually and who has to miss lessons because of the illness. The patient's peers may consider him 'not normal' and reject him because of the frequent absenteeism, his inability to participate in all the normal school activities such as sport and possibly because of the characteristic appearance. This may result in depression even to the extent of severe withdrawal (Minde, et al, 1972). V.P., who wanted to play 'body contact' sport but was not allowed to, has been mentioned previously.

The child might quite understandably have ambivalent feelings towards his parents. On the one hand there is the natural love bond between child and parent, while on the other hand he may resent his parents for 'giving' him his ailment and then in addition curbing his activities. Feelings of abnormality, inferiority and rejection may all play a part in the development of

the character of the patient and result in lifelong neuroses.

V.P., the younger patient with thalassaemia major in the survey, has manifested certain of these behaviour patterns. While at a young age he blamed his parents for his condition and asked why it had happened to him. Later on he presented with a problem of temper tantrums and aggressive behaviour at home. Enuresis is still an intermittent problem with him.

Problems of a sexual nature may develop. According to Freudian thought, boys in the oedipal stage may be unable to cope. They may react passively (as do females) to the bodily pain they are forced to suffer and not with expected masculine contempt (Freud, 1960). This may result in lifelong sexual identity confusion. Further difficulties can be envisaged especially in boys at puberty and further into life. These would be due chiefly to the delayed physical development of the individual. Disturbed endocrine function is a well recognised complication of thalassaemia major. This includes malfunction at the level of the pituitary and gonads, with resultant inadequate production of the testerones in males and oestrogens in females. The overall outcome is delayed and possible absence of normal puberty. Decreased secondary sexual characteristics are common. In fact, they were illustrated by the older of the 2 patients with thalassaemia in the survey. In this milieu sexual identity problems may be a feature of the person. This may result, in addition, in neurotic reactions.

Logothetis, et al (1971), after evaluating 138 patients with thalassaemia major, reported that there was no apparent difference

in the I.Q. mean and distribution of their patients and the normal population. However, they did note a positive correlation between I.Q. and total blood transfused since birth and a negative correlation between I.Q. and parameters that characterize the severe stigmata of the disease.

In this series, 96 of the subjects had behavioural problems manifest as impulsiveness, capriciousness and uncontrolled temper or withdrawal, dependence and seclusiveness. In addition, 67 had abnormalities of affect,usually depression and floating anxiety. It was found that patients whose parents were very anxious and overly protective were more likely to have psychological problems.

6. ECONOMICS

Thalassaemia major, by virtue of the high cost of treatment and frequent hospitalisation, has important economic considerations. These may have to be initially borne by the parent of the affected child but after diagnosis the financial burden is carried by the state.

As an example of the high costs involved, P.C., the older of the 2 Greek patients with thalassaemia major in Cape Town, will be quoted.

TABLE 26 - 1

TREATMENT COST OF SUBJECT WITH THALASSAEMIA MAJOR

SERVICE	COST/UNIT	TOTAL ANNUAL COST
Blood (frozen washed cells)	R32.00	R2,400,00
Desferrioxamine	R70,91	R4,350,00
Hospital admission	R53,68	R 500,00
		R7,250,00

These costs, which are approximate, have been calculated for the state. A private individual would have to pay about 50% more. These figures will now be explained. The blood is given in the form of frozen washed cells and the patient being discussed receives about 5 units every 3 to 4 weeks. He takes about 20 g of desferrioxamine, the iron chelating agent, per week. This costs R20,91 for 5 g. Hospital admission for blood transfusion is necessitated approximately once per month for 1 day, and furthermore about 10 days annually for complications of the disorder. The cost per bed per day at Groote Schuur Hospital is R53,68. However, this includes treatment and investigations which the subject does not always need. Therefore, when calculating the above, an approximate estimate of his true cost to the state was made.

The final annual total cost in this individual, though possibly slightly higher than most, is representative of the relative costs to the state of maintaining management of a patient with thalassaemia

major. In Cape Town, there are 2 Greek and 3 patients of other population groups suffering from this severe form of the disorder. Thus the probable cost to the state in Cape Town to treat these patients is approximately R30 000 per annum. From knowledge of the Greek population in Cape Town and that of the rest of South Africa, especially considering origins and size, there are probably a minimum of another 5-10 individuals with thalassaemia major. The cost of maintaining them is thus in the region of R30 000-R70 000 per annum.

As a result the total cost of maintaining patients with thalassaemia major in South Africa, where there are relatively few of these cases, is in the region of R100 000 per annum.

Greece has a population of about 9 000 000. The prevalence of beta-thalassaemia heterozygotes varies from 4% in certain areas of the mainland to 6%-15% on Cyprus and 20% on certain islands, with an overall prevalence of probably about 10%. Therefore, a large number of new cases must be born annually. Greece is a poor country and is not able to treat as intensively as would be ideal. Nevertheless, extrapolating from the costs in South Africa, it is clear that Greece must spend a large amount of her health budget on treating patients with thalassaemia major.

To rectify this situation, it is most necessary to prevent the birth of patients with thalassaemia major. This is currently being done in Greece by compulsory premarital screening of each couple intending to marry. The cost of screening is insignificant compared to the heavy financial burden to the state of new cases.

SCREENING PROCEDURES FOR BETA-THALASSAEMIA

In this survey persons tested were screened fully for any inherited haematological disorder. When making a diagnosis of beta-thalassaemia cognisance was taken of all the red cell parameters, the osmotic fragilities, peripheral blood smear and haemoglobin electrophoresis. In the literature reports have appeared discussing the efficacy of various parameters for use in large scale surveys. Tests which have been suggested for population screening include MCV, MCH, osmotic fragilities and haemoglobin electrophoresis. The ascertainment of the MCV and MCH can either be done by a fairly lengthy method such as followed in this investigation or by automatic measuring on a large counter. Using this latter method it would be possible to investigate a large number of blood specimens per day. However, for economic reasons this could only be done at a large centre which would have a large counter.

In evaluating the results produced by the author's laboratory it is apparent that the MCV and MCH are most reliably correlated with an increase in HbF and/or HbA_2 and thus beta-thalassaemia. The MCV was decreased in all but 3 and the MCH in all but 2 persons with heterozygous beta-thalassaemia. Thus in screening the Greek population of Cape Town, 2 respondents with beta-thalassaemia would have been missed if only the MCH and MCV were measured. On the other hand, another 18 individuals had a decreased MCH and MCV without an increase in HbF or HbA_2. Two of these persons had iron deficiency anaemia from another cause, while 3 patients had inclusion bodies on their peripheral smears and were further investigated for alpha-thalassaemia.

A pool of 13 persons who had a decreased MCV and MCH with no apparent

cause, still remains. One of these had an HbA_2 of 2,8% and possibly could have a mild form of beta-thalassaemia. The remainder can only be accounted for as possibly having a mild form of heterozygous alpha-thalassaemia or possibly delta-beta-thalassaemia. They might also be at one end of the spectrum for the normal population or there may have been minor experimental errors.

The osmotic fragility has previously been used in large scale screening procedures in Greece and elsewhere with apparent success (Rucknagel, 1966). This method has been perfected and simplified to enable large scale screenings to be undertaken on one drop of blood obtained by finger puncture (Silvestrone and Bianco, 1975). In this survey, of the 22 persons found to have the thalassaemic trait, only 6 had a decreased osmotic fragility. The remainder, although within normal limits appeared at the lower end of the scale for normality. On this basis it appears that osmotic fragility studies alone, as undertaken in the author's study, are inadequate for use in large population surveys. The probable reason for this is that the normal distribution curve which was used was shifted too far to the left. If the curve was to be shifted further to the right the majority of the respondents carrying the thalassaemia trait would be found to have a decreased osmotic fragility. However, were this to be done there would be a further body of persons with a decreased osmotic fragility and no other abnormalities such as has also occurred with the MCV and MCH.

Taking a broad viewpoint, it is apparent that the most efficient tests in large population screenings are the MCH together with the MCV which would pick up the majority of thalassaemia carriers. Persons with decreased MCH and/or MCV would subsequently have to be fully screened

by electrophoresis and in certain situations by alpha-beta-chain ratios to determine if the abnormalities of MCV and/or MCH were actually due to the thalassaemia trait.

METHOD USED FOR ESTIMATION OF HAEMOGLOBIN A_2

After the survey had been completed and the results formulated it was suggested that the method of Black, et al (1960), which was used for estimating haemoglobin A_2, was antiquated. This method was used for economy and to ensure that the experimental technique was indeed accurate, a random 10% of all specimens as well as all abnormal specimens were retested by elution from cellulose acetate. There were no discrepancies and the author is confident that the method used and the results obtained were accurate.

PREVALENCE OF THE BETA-THALASSAEMIA GENE IN THE GREEK PEOPLE

The prevalence of heterozygous beta-thalassaemia in the Greek population of Cape Town is relatively high (9,2%). From existing knowledge of the gene frequency of beta-thalassaemia in various regions in Greece, this result is much as expected, as the overall prevalence in Greece is in the region of 10%.

At present it is generally accepted that heterozygous beta-thalassaemia protects against malaria. Recent research has produced findings to suggest that the high HbF found in thalassaemia heterozygotes in the first year of life confers resistence against falciparum malaria (Pasvol, Weatherall and Wilson, 1977). During this period the infant is particularly susceptible to Plasmodium falciparum which results in a high mortality and thus a selective advantage at this stage would in-

fluence the prevalence of the carrier state of thalassaemia. This probable selective advantage has resulted in a high incidence of heterozygous beta-thalassaemia in areas of malarial and former malarial endemicity, such as Greece. In Cape Town where malaria is not endemic, individuals with heterozygous beta-thalassaemia are no longer at a selective advantage and it is therefore to be expected that the gene frequency of beta-thalassaemia will decrease in Cape Town. However, as the detrimental effects of heterozygous beta-thalassaemia are so mild, the disadvantage of having the disorder are insignificant and the decline in the gene frequency of the disorder will take very many generations.

There are regions, such as the north-eastern Transvaal in South Africa, where malaria is still endemic. Individuals with heterozygous beta-thalassaemia living in these regions might be expected to have a selective advantage against malaria and the incidence of beta-thalassaemia would therefore be expected to remain static or increase. However, with modern medicinal therapy and public health regulations there should no longer be a significant mortality or morbidity from malaria. For these reasons the gene frequency of beta-thalassaemia would be expected to remain relatively static.

HETEROGENEITY OF THE THALASSAEMIAS

The great heterogeneity of the thalassaemias is well illustrated by the findings of the survey and significant variations in clinical, haematological and electrophoretic manifestations were found. Certain individuals with heterozygous beta-thalassaemia had symptoms referrable to an anaemia, while others were asymptomatic. Likewise, certain beta-

thalassaemia heterozygotes had marked haematological abnormalities while others had only mild stigmata of the disorder.

In addition, the severity of the clinical manifestations did not necessarily correlate with the degree of the haematological or electrophoretic abnormalities. Similarly, the magnitude of the haematological findings was not proportional to those of the electrophoretic findings.

Certain subjects with symptomatic anaemia had only minor elevations of their haemoglobin F or A_2 whereas others with more significantly elevated levels of haemoglobin A_2 or F were totally asymptomatic. In a similar manner, persons with significantly decreased haematological parameters and highly abnormal red cell morphology did not necessarily have a symptomatic anaemia, while certain persons with minor red cell changes and haematological parameters were found with a symptomatic anaemia. Lastly, the degree of elevation of haemoglobins A_2 and F are not matched by a similar reduction in MCV and MCH or an alteration in red cell morphology.

Further evidence for the heterogeneity of the thalassaemias is that individuals with beta-thalassaemia have elevated levels of both haemoglobin A_2 and F, whereas others have only an elevation of haemoglobin A_2. A certain group have elevated levels of haemoglobin F with normal haemoglobin A_2. The diagnosis in this latter category, who form a subdivision under the broad heading of thalassaemias, is delta-beta-thalassaemia. These levels of haemoglobins A_2 and F tend to be constant within families. Further examples of the heterogeneity of the condition will be presented in the following discussion.

INTRA-FAMILIAL SEGREGATION OF HAEMOGLOBIN A_2 AND F

It has been previously noted that there is significant intra-familial segregation of haemoglobin F and A_2 values (Weatherall, 1964). Presented below are the haemoglobin A_2 and F levels of 1 of the respondents included in the survey, as well as his or her sibling or child.

	FAMILY	HbF %	Hb A_2 %
1.	O.T.	4,8	2,5
	C.T.	2,5	2,4
2.	A.K.	1,8	3,9
	G.K.	2,2	5,3
	V.K.	2,1	3,6
3.	G.P.	1,0	5,8
	S.P.	1,3	3,9
4.	S.S.	0,8	4,8
	G.S.	3,6	5,0
5.	G.P.	0,4	4,8
	M.C.	0,9	3,6

Family 1 demonstrates a raised haemoglobin F with a normal haemoglobin A_2 value in both subjects and is probably an example of delta-beta-thalassaemia.

Families 3 and 5 have a heterozygous beta-thalassaemia with a normal haemoglobin F and a raised haemoglobin A_2 level.

Family 2 has a form of beta-thalassaemia with raised both haemoglobin F and A_2 values.

In family 4 the father's haemoglobin F is normal, while his son's is raised. The haemoglobin A_2 is increased in both father and son. The

explanation for the son's raised haemoglobin F is probably that the child was 18 months old at the time of testing and had not decreased his gamma-chain production to normal adult levels. It can be interpreted that his haemoglobin F would be within normal limits by adulthood. It is thus demonstrated that members of families with the thalassaemia trait tend to have similar levels of haemoglobin F and haemoglobin A_2 which may differ from other families. This illustrates intra-familial segregation of haemoglobin F and haemoglobin A_2. It is also further proof of the heterogeneity of the thalassaemias.

HEREDITARY PERSISTENCE OF FETAL HAEMOGLOBIN (HPFH)

As defined earlier (Chapter 8), HPFH occurs where a raised haemoglobin F is found in the absence of other abnormal haematological parameters, and it is diagnosed incidently during screening procedures and family studies.

It is possible that subject 21 (see Chapter 16) falls into this category. His complaints of the symptoms of anaemia were not borne out by the findings of a haemoglobin of 16,1 g/100 ml. His MCV was normal, but the MCH was minimally decreased, the value being 26 pg. The only abnormality of his peripheral blood smear was anisopoikilocytosis, a minimal defect. Finally, his haemoglobin F was 2,7% on consecutive occasions.

The haemoglobin F in HPFH is usually in the region of 20-30%, whereas that of heterozygous beta-thalassaemia varies from normal to 6% (Weatherall and Clegg, 1972). This is further evidence for respondent 21 having the thalassaemia trait and not HPFH, if one were to differentiate between the two. His diagnosis of thalassaemia heterozygosity hinged on his abnormal haemoglobin F, plus the 2 other minor haemato-

logical abnormalities and this gives further credence to Clegg and Weatherall's (1976) theory that the HPFH syndromes are in fact a mild form of thalassaemia. This further illustrates the wide heterogeneity of the thalassaemia.

DELTA-BETA-THALASSAEMIA

Three of the 23 respondents documented as having heterozygous beta-thalassaemia had a raised haemoglobin F but a normal haemoglobin A_2. Subjects 1 and 4 (see Chapter 16) had clinical and haematological findings which were typical of the thalassaemia trait. They were not anaemic but had low MCVs and MCHs. Examination of the peripheral blood smear revealed microcytosis, anisopoikilocytosis and target cells in subject 1. The haemoglobin A_2 in both was 2,4% (normal). The haemoglobin F in respondent 1 was 7,9%, while that in subject 4 was 2,5%.

The son of case 4 also had a normal haemoglobin A_2 and the haemoglobin F of 4,8%. These individuals in fact do not have beta-thalassaemia but delta-beta-thalassaemia where there is a defect in delta- as well as beta-chain production. The haemoglobin levels of 13 g and 12,3 g per 100 ml and the MCH of 21 pg in the 2 subjects are very similar to the mean haemoglobin level of 12,4 g per 100 ml and the mean MCH of 22,4 pg found by Stamatoyannopoulos, Fessas and Papayannopoulos (1969) in their series of 68 cases of delta-beta-thalassaemia. However, their mean haemoglobin F value of 10,9% is higher than that of the subjects studied in this series.

Subject 21 had minor red cell abnormalities consisting of a marginal decrease in MCH (26 pg) and anisopoikilocytosis and probably falls into

this category. His haemoglobin A_2 level was 2,4% and his haemoglobin F 2,7%, which is definitely elevated. The mildness of his stigmata suggest the possible diagnosis of hereditary persistence of fetal haemoglobin (HPFH). However, as he does have some red cell abnormalities and as it is now thought that the HPFH is probably a mild expression of the thalassaemic gene (Clegg and Weatherall, 1976), it is reasonable to classify him as having the thalassaemia trait. As he has a normal haemoglobin A_2 but a raised haemoglobin F, he probably has delta-beta-thalassaemia, with a very mild expression of the gene.

A fourth subject, patient 23, was diagnosed on alpha/beta-chain synthetic rates as having delta-beta-thalassaemia. He is discussed in detail later in this chapter under the heading "Heterozygous beta-thalassaemia with normal haemoglobin pattern".

The source of the abnormality in delta-beta-thalassaemia is that there is not only a quantitative defect in beta-chain, but also of delta-chain synthesis. The depression of delta-chain production is not significant or important and as seen from the investigated subjects, the clinical abnormalities are no more severe than in mild heterozygous thalassaemia.

HETEROZYGOUS BETA-THALASSAEMIA WITH A NORMAL HAEMOGLOBIN PATTERN

Subject 23 (see Chapter 16) of the cases with heterozygous beta-thalassaemia was a late addition to this group as the results of the alpha/beta chain synthetic rate studies were only made available to the author 6 months after the rest of the laboratory work had been completed. He presented in an interesting fashion. He had normal red cell parameters but as he had $\frac{20}{10\ 000}$ haemoglobin H inclusion bodies,

it was felt that he may carry the alpha-thalassaemia trait. Alpha/beta chain synthetic rates were undertaken and the result was 1,47, which signifies a defect in beta-chain production. As this was unexpected, this latter investigation was repeated with an outcome of 1,38, which is within keeping with a diagnosis of beta-thalassaemia.

On examining his red cell indices, he was not anaemic and his MCV and MCH were within normal limits. These 2 parameters are the most reliable simple indicators of the beta-thalassaemia trait. In this study, when considering both, they are accurate in 92% of cases. His other red cell indices were also normal. Haemoglobin electrophoresis and analysis reveal a HbA_2 of 2,6% and a HbF level of 0,1%. These results are also normal.

Thalassaemia with a normal haemoglobin pattern has previously been recognized (Silvestroni, et al, 1978). This may be the phenotypic manifestation of the alpha-thalassaemia carrier state, beta-thalassaemia with severe iron deficiency or the association of beta- with delta-thalassaemia in the same individual.

Case 23 was clearly not iron deficient and alpha/beta chain synthetic rates excluded the diagnosis of alpha-thalassaemia and in fact substantiated the diagnosis of the beta-thalassaemia trait.

Subjects with normal haemoglobin levels and delta-beta-thalassaemia have usually been diagnosed on investigating the parents of a patient with thalassaemia major (Stammaloyanopoulos, Fessas and Papayannopoulos, 1969; Weatherall and Clegg, 1972; Fessas and Loukopoulous, 1974, and Weatherall, et al, 1974). The diagnosis of delta-beta-thalassaemia has then been made and confirmed by alpha-beta chain synthetic rates and on

this basis the author has diagnosed the delta-beta-thalassaemia trait in respondent 23.

The subject who has normal red cell indices was only diagnosed as having the thalassaemia trait on alpha/beta chain synthetic rates and it is therefore possible that other subjects in the survey who were considered normal may actually also have the thalassaemic trait. This particularly applies to those persons who had abnormal red cell parameters such as a decreased MCV and/or MCH. This matter could only be clarified by measuring alpha/beta chain synthetic rates to exclude a defect in beta chain production (or, in the case of the alpha-thalassaemia trait, a defect in alpha chain production as discussed earlier). This was not possible in this survey for logistic reasons and can, in fact, only be done in highly specialized laboratories.

A particular source of concern, as illustrated by this data, is that persons can remain undiagnosed following routine screening for the thalassaemia trait. The unpleasant implications are that a child with thalassaemia major may be born after the parents have been assured antenatally that this will not occur.

RATIO OF THE BETA TO THE DELTA-BETA-THALASSAEMIA TRAIT

The ratio of the beta-thalassaemia trait to the delta-beta-thalassaemia trait is 10 : 1 in Greece when calculated from the data of Malamos, et al (1962) and Kattamis, et al (1978). The ratio in this series is of the order of 5 : 1, which is higher than expected. Reasons for this include the relatively small size of the sample as well as the fact that 2 of the subjects diagnosed as having the delta-beta-thalassaemia trait would possibly not have been diagnosed in the former series because of the minor nature of their haematological abnormalities.

CHAPTER 27

GLUCOSE-6-PHOSPHATE DEFICIENCY

DIFFICULTY IN DIAGNOSING THE FEMALE HETEROZYGOTE

As G-6-PD deficiency is inherited in an X-linked manner, female carriers may manifest the disorder to a greater or lesser extent, depending on the proportions of the two X chromosomes which are inactivated. If a large proportion of the X chromosomes directing the synthesis of normal G-6-PD is inactivated, and thus more of the X chromosomes directing abnormal qualitative or quantitative G-6-PD are operative, the person will be G-6-PD deficient to a greater extent than in the opposite set of circumstances. This situation will also be mirrored in the tests for G-6-PD deficiency and thus the Motulsky (brilliant cresyl blue dye) tests will be grossly abnormal in some carrier females, whereas completely normal in others. In the latter situation, it is possible to miss the diagnosis of the carrier state in certain females and thus figures of the gene frequency of the disorder might be erroneous to a certain extent where females are taken into account. In this survey, females were included and thus the results may be slightly inaccurate. In persons where the Motulsky test was equivocal, the position was clarified in a number of cases by using the more specific methaemoglobin reduction test. This particular problem is illustrated in two of our respondents, Nos. 3 and 10 (see Chapter 17).

Subject 3 was demonstrated to be grossly G-6-PD deficient, his Motulsky test being more than 5 hours. On testing his daughter, who is by definition an obligatory carrier, her Motulsky test was 39 minutes, which is considered normal. Respondent 10 had a Motulsky test of 65

minutes, which is not grossly elevated and is more characteristic of the Negro than the Greek variety of G-6-PD deficiency. His daughter had a Motulsky of 49 minutes, which is abnormal, but when her methaemoglobin reduction was estimated it was found to be normal.

This illustrates that neither of the tests used in this survey are infallible in the case of women, and it is quite conceivable that certain of the female respondents were reported as normal whereas, in fact, they carried G-6-PD deficiency. The overall prevalence was 6% with that of women being 5% and that of men 6,7%. It is quite possible that the discrepancy between the sexes is due to some females being missed in the screening and that the true prevalence of G-6-PD deficiency in the Greek population of Cape Town is 6,7% (with a gene frequency of 0,067) and not 6%.

A SEVERE FORM OF G-6-PD DEFICIENCY IN LESBOS

During the Second World War, subject 10 (see Chapter 17) and a brother ate fava beans in Greece and, as a result, had what was probably an acute haemolytic attack. C.V. received a blood transfusion, his father having donated the blood, whereas for reasons unknown to the author, his brother did not receive a transfusion and subsequently died. In South Africa, 25 years later, respondent 10's mother, after taking salicylates for an influenca-like illness, suffered from an attack of haemolysis which necessitated hospital admission. It therefore seems that this family, who originate from Lesbos, suffer from a particularly severe form of G-6-PD deficiency. Doxiadis, et al (1964) have previously noted that G-6-PD deficiency neonates on Lesbos have a particularly severe form of jaundice and that the non-G-6-PD deficient

newborn infants suffer from a more marked form of neonatal jaundice than those in other areas of Greece. They thus postulated a second icterogenic factor which worsens the jaundice when occurring with G-6-PD deficiency.

This factor might be environmental or genetic. From this one family, no final conclusions could be drawn. However, it would support the concept that the additional icterogenic factor is genetic and not environmental.

HETEROGENEITY OF G-6-PD DEFICIENCY

The heterogeneity of G-6-PD deficiency has been well recognized and documented. There are clinical differences with certain population groups having a more noted deficiency than others, e.g. the Mediterranean type results in a more severe condition than the Negro variety. There are also differences in electrophoretic mobility and other laboratory parameters which have been discussed earlier. These are all examples of the heterogeneity of the disorder. Heterogeneity not only exists between the various population groups but also within the communities having the anomalous gene.

There is evidence for this phenomenon in this study. Clinically, there were differences in how certain of the individuals with the condition reacted to it. Some of the subjects had genuine symptoms of a haemolytic attack on taking a medicine, such as acetyl salicylic acid, known to precipitate haemolysis, while others could take the same preparation without any untoward effects. The reaction was so severe in two of the subjects that hospital admission was necessary and extensive special investigations were undertaken to enable the diagnosis to be made.

Similarly, 4 of the cases in the study had the physical sign of splenomegaly, while others did not. However, this would depend on the rate of haemolysis, which in turn would depend on the frequency with which the drugs inducing haemolysis were ingested. Therefore, this sign could be stimulated by the persons themselves by, for example, regularly taking acetyl salicylic acid on getting headaches.

The laboratory results also illustrate this heterogeneity (see Table 17 - 5). The brilliant cresyl blue test is a measure of the activity of the enzyme G-6-PD. The results in the males varied from 59 minutes to more than 5 hours. In the majority of the men studied, the results were grossly abnormal. However, in 2 of the men the results were 59 and 65 minutes, which is only slightly abnormal. In subject 9, electrophoresis was undertaken to typify the enzyme. The result was that the G-6-PD was of the B variety, i.e. the Mediterranean type which is what one would expect in an individual of Greek origin. There is, therefore, evidence that there is also heterogeneity within this large group.

CHAPTER 28

THALASSAEMIA IN ASSOCIATION WITH GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY

THALASSAEMIA AND G-6-PD DEFICIENCY IN NON-GREEK CAPE TOWN RESIDENTS

Thalassaemia is encountered reasonably commonly amongst the Cape Coloured population of Cape Town. This population group has a mixed gene pool, with Caucasian, African Negro, Bushman, Nama (Hottentot) and Asian contributions.

Caucasians of Mediterranean origin and individuals from south east Asia have a high prevalence of beta-thalassaemia while the beta-thalassaemia gene is found to a lesser extent in the West African negro. The beta-thalassaemia gene in the Cape Coloured population therefore possibly has origin from these 3 sources. Non-Greek patients with beta-thalassaemia who were encountered in Cape Town were predominantly of Malay stock and it is therefore reasonable to assume that their beta-thalassaemia gene came mainly from south-east Asia. There are in addition a few Cape Coloured families with south Mediterranean antecedents who possibly contributed the beta-thalassaemic gene to this population's gene pool.

There are Caucasian individuals of non-Greek stock in Cape Town with heterozygous beta-thalassaemia. These people are mainly Italian in origin and to a lesser extent, Portuguese. Caucasian individuals are also present in Cape Town who have English names and heterozygous beta-thalassaemia. However, on more detailed examination it is now evident there is an antecedent of southern Mediterranean origin in these families. It is reasonable to assume that the beta-thalassaemia in

these individuals came from this source.

There have been reports of individuals of English/Dutch stock in South Africa with sickle-cell haemoglobin C disease and haemoglobin S beta-thalassaemia (Dunston, et al, 1972). Gene flow from populations containing the beta-thalassaemic as well as HbS and HbC genes to the English and Dutch during colonial times has been suggested as a source of these genes

G-6-PD deficiency is also found in Cape Town in the same population groups discussed above and for the similar reasons. The incidence of G-6-PD deficiency in the African Negro of South Africa ranges from 0-10% (Tobias, 1974). This is significantly higher than that for beta-thalassaemia in the same people. The result is that there is a higher incidence of G-6-PD deficiency than beta-thalassaemia in the Coloured population in Cape Town as there is an added source for the G-6-PD deficiency gene, which is typical of the Negro variety.

THALASSAEMIA, G-6-PD DEFICIENCY, GEOGRAPHY AND MALARIA

Previous mention has been made concerning the relationship between malaria and G-6-PD deficiency and thalassaemia. The selective advantage of G-6-PD deficiency against malaria has been well established and although it has not been proven in the case of thalassaemia, the background of the disorder strongly suggests that thalassaemia also protects against malaria.

Examining the places of origin in Greece of the respondents with heterozygous beta-thalassaemia, it is evident that all except one originates

from a low-lying town where it is reasonable to assume that malaria was endemic in the past. However, taking note of the place of origin of all the respondents it becomes obvious that most of the subjects included in the survey come from low-lying areas. For this reason it would be falacious to state that from the figures obtained in this survey there is a direct relationship between the low-lying areas, malarial endemicity in the past and thalassaemia.

It is possible, however, to compare the incidences in the various areas in Greece with those found amongst respondents living in Cape Town. The Greek subjects in Cape Town with the highest incidence of the beta-thalassaemia gene are those from Cyprus, who have a prevalence of 30%. The comparative figure for Greek Cypriots in Cyprus is 15% (Ashiotis, et al, 1973). The discrepancy is probably the result of the small number of subjects, who numbered 15, from Cyprus who were included in this survey. The prevalence of 10,5% in persons from Lesbos obtained in the survey compares well with the 10% incidence found on Lesbos (Fessas, 1976). The good correlation is probably due to the relatively large number of subjects sampled from Lesbos. The prevalence of 9,5% in persons from the Peloponnese is somewhat higher than the 6% found there (Rucknagel, 1966), whereas no subjects from the Dorian Islands were found with the beta-thalassaemia gene. However, this finding is probably also spurious due to the relatively small number of persons tested from these latter areas.

Whereas the relationship between low-lying areas, malarial endemicity and incidence of thalassaemia has not been clearly demonstrated (Stamatoyannopoulos and Fessas, 1964), the relationship between the above factors and the G-6-PD deficiency has been well established

(Plato, Rucknagel and Gershowitz, 1964; Siniscalco, et al, 1964). It has been demonstrated by the above authors that the incidence of G-6-PD deficiency is significantly higher in coastal areas where malaria was endemic than in higher mountainous regions where malaria was not found. Thus, in an area such as the Arta area of central Greece, there is a striking difference in the incidence of G-6-PD deficiency between non-malarial endemic regions (2,8%) and places where malaria was endemic (16,4%) (Stamatoyannopoulos and Fessas, 1964).

In examining the figures of the survey, the regions of origin of respondents with the highest incidence of G-6-PD deficiency are the various Greek islands, in particular Lemnos (20%) and Cephalonia, an Ionian island (11,8%). Due to the geographical structure of the islands, malaria was endemic there to a greater extent than on the mainland and one could therefore expect there to be a high incidence of G-6-PD deficiency. The 4,3% prevalence found in subjects originating from Lesbos compares favourably with the values 4,6% and 3,9% found on Lesbos (Doxiadis, et al, 1964). Due to the relatively small number of respondents tested from some regions the seemingly high incidence may be somewhat misleading as it is possible that the true incidence in these areas is in reality lower.

INTERACTION OF THALASSAEMIA AND G-6-PD DEFICIENCY

As thalassaemia and G-6-PD deficiency are both very common in Greece and in people of Greek origin, it is likely that the two conditions will sometimes occur in the same individual.

It is known that there is no direct genetic relationship between G-6-PD deficiency, which is X-linked, and the thalassaemia gene which is auto-

somal. Thus they occur independently and by multiplying the prevalence of each, the number of persons expected to have both disorders can be calculated. In the survey it would be expected that there would be one such individual (as discussed earlier) while there are in fact two. As the numbers are relatively small, such a discrepancy is not totally unexpected. The two respondents in the survey with both beta-thalassaemia and G-6-PD deficiency illustrate the point that the one disorder has no deleterious effect on the other (see Chapter 17).

A.P. (see Chapter 17), who was aged 65 at the time of diagnosis, had never had any symptoms referable to G-6-PD deficiency or thalassaemia and would probably never have been diagnosed had it not been for the survey. He has subsequently demised from carcinoma of the bladder.

A second patient, O.V. (see Chapter 17), had previously been diagnosed as having both disorders as he had been investigated fully following an attack of favism. He suffers from a particularly severe form of G-6-PD deficiency and has had a cholecystectomy for gall stones, which were conceivably the result of increased haemolysis on the basis of his G-6-PD deficiency. Apart from previous attacks of acute haemolysis and his cholecystectomy, he has otherwise been in good health.

CHAPTER 29

HEREDITARY SPHEROCYTOSIS

DIFFICULTY IN THE DIAGNOSIS

McKusick, in his classical work on Mendelian inheritance, quotes the prevalence of hereditary spherocytosis as 2,2 per 10 000. In the population included in the survey, the prevalence has a minimum frequency of 1,2 per 100 and occurs possibly as frequently as 3,2 per 100. The diagnosis of HS is usually initially made on clinical and haematological grounds. Presenting features include a history of cholecystitis, an anaemia and an unexplained splenomegaly. Contributory laboratory evidence is a possible decreased haemoglobin value, typical spherocytic red blood cells on the peripheral blood smear, and an increased reticulocyte count. Finally, the diagnosis is made when an increased osmotic fragility of the red blood cells is found and autohaemolytic studies reveal characteristic abnormalities. However, in this study the initial abnormality found in most cases was an increased osmotic fragility. Thereafter, other distinctive features of the disorder were sought. In a significant proportion of these individuals typical clinical and haematological features were absent, which further complicated their diagnosis of HS. Experimental techniques were checked in an attempt to explain the paucity of contributing evidence. However, no experimental error was found and the results were considered to be in order. Therefore, it appears probable that these subjects have HS but with a very mild expression of the gene. Family studies were done where possible to elucidate the situation but this was unfortunately not possible in a number of these patients. McKusick maintains that an increased osmotic fragility of the red blood

cells is the most characteristic abnormality in this disorder and this too lends weight to the diagnosis of HS in these cases. These findings are increased evidence for the heterogeneity of the disorder.

HIGH PREVALENCE OF HEREDITARY SPEROCYTOSIS IN THE GREEK POPULATION OF CAPE TOWN

The prevalence of hereditary spherocytosis is quoted as 2,2 per 10 000. No data is available for southern Europe and more specifically Greece. The gene frequency found in the Greek population of Cape Town in this survey is far in excess of this figure (see Chapter 18). It is reasonable to suggest that the founder effect has been responsible for these figures in Cape Town. It is known that the HS gene is present in Greece. However, no population survey has been undertaken to determine the frequency of the gene (Fessas, 1978) and therefore we do not know whether the high frequency found in the Greek community of Cape Town is representative of that in Greece.

If these findings are a reflection of the situation in Greece, there are important implications. The characteristic abnormalities of the red blood cell in G-6-PD deficiency, sickle cell trait and probably thalassaemia minor have been considered to confer resistance to malaria. The important question which now arises is whether persons with HS also have a decreased susceptibility to malaria and hence a selective advantage.

The primary defect in HS appears to lie in the red cell membrane which has been found to have an increased permeability to sodium ions (Jacob and Jandl, 1964). In addition, an abnormal red cell membrane protein

has been reported (Amsden and White, 1971). If the primary defect is indeed in the red cell membrane, it is possibly reasonable to hypothesize that this defect may render the red cell membrane resistant to the malarial parasite. However, at present there is no evidence to refute or confirm this suggestion.

These early findings are therefore of great interest and would bear further exhaustive investigation to clarify the position completely.

CHAPTER 30

CONCLUSION AND RECOMMENDATIONS

The prevalence of certain of the inherited haematological disorders in the Greek population of Cape Town were found to be as follows:-

1.	Alpha-thalassaemia trait	1,2%
2.	Beta-thalassaemia trait	9,2%
3.	G-6-PD deficiency	6,7%
4.	Hereditary spherocytosis	1,2%
5.	Sickle cell trait	0,4%

From our knowledge of the gene frequency of these disturbances in Greece and by extrapolating to the situation in Greeks in Cape Town, the prevalence of the thalassaemia and G-6-PD deficiency is much as expected. However, the prevalence of hereditary spherocytosis is unexpectedly high.

The gene frequencies for these haematological conditions are significant and justify further thought and action. G-6-PD deficiency and hereditary spherocytosis, as encountered in the survey, were relatively mild conditions and do not warrant any prophylactic action. However, the thalassaemias in the homozygous state, result in a severe, crippling disease with important socio-economic implications. This form of the disorder should be prevented from occurring where possible.

This survey did indeed start this process. The first important step is education. Firstly, the medical profession should be aware of the dangers of the disorders for the population at risk. In addition the

Greek population should be aware that they are prone to certain conditions which are preventable.

A circular was sent to members of the Greek community informing them of the disorders and their implications and giving basic information as to current medical treatment. The author also spoke to groups at social gatherings as well as to individuals requesting information and advice. Persons volunteering to participate in the survey were, without exception, told exactly why the study was being done.

Further education is still necessary. It is important that persons do not feel it a stigma to be a carrier of the disorder and to come forward of their own accord to have their blood screened for these disturbances. This is particularly important premaritally in order that persons know whether they are heterozygotes for thalassaemia. If both the man and the woman are carriers they can be informed of the possible outcome of any pregnancy. In appropriate circumstances they can be referred to an overseas clinic where antenatal diagnosis is performed and have the pregnancy tested.

While undertaking the survey the author attempted to set up a service whereby persons could be voluntarily tested. The laboratory and hospital side of this was organized but volunteers were not forthcoming. The author then contacted the ecclesiastical authorities and requested that they inform couples premaritally of the service to be offered. Unfortunately, although appreciation of the work was expressed, the authorities felt that they could not be part of it. However, because of the risks of the severe disease it is nevertheless imperative that such a service be offered.

This testing can be justified from an economic point of view if one considers that it costs the State over R7000 per annum to maintain one patient with thalassaemia major. It is thus obviously worthwhile for the State to provide a service to prevent the recurrence of this disorder.

It is compulsory in Greece for couples to be tested premaritally for any haemoglobinopathy and the thalassaemias. This is because of the severity of the disorders and their harsh economic consequences. This problem has also been realized in other countries to which large numbers of Greeks have emigrated, such as Australia (Editorial,1974) and it has been suggested that comprehensive voluntary screening programmes be instituted in these countries.

However, as opposed to Greece where premarital testing is compulsory, it is preferable that the public at risk be an informed, enlightened one, seeking testing of their own free will. To attain this, education is most important as there is the tendency for anxieties, fears and guilt feelings to be raised as well as possible interference with moral, personal and religious beliefs. In order not to invade the rights of the individual it is important that the results of the tests be confidential. Thereafter it is necessary that individual advice and counselling be given where requested.

Probably the next most important group who should be screened are mothers in early pregnancy.

With the present organization of the South African health services only a few indivuals would be screened at Provincial hospitals. The majority of persons are seen and treated privately. In these circumstances

screening would have to be offered by the general practitioner or obstetrician. Arrangements would then have to be made for the testing to be done by the Provincial Laboratories. The importance that the public be informed and enlightened to minimize anxieties and guilt feelings and that confidentiality be maintained is emphasized here.

The choice of possibilities for an at-risk pregnancy found in this manner in Cape Town are, if the patients can afford transport overseas, referral to a large, specialized unit which can undertake antenatal diagnosis with the understanding that an affected pregnancy be terminated; that the pregnancy be terminated locally, or that the pregnancy go to term if the parents so wish.

In the case of G-6-PD deficiency the anomaly is not quite as severe. However, it is nevertheless important that the medical fraternity be aware of the population at risk in order that rapid diagnosis may be made when a patient presents with the side effects of the disorder. Although the condition is usually mild it may necessitate hospital admission. It is thus recognised that where possible, the communities that have a high prevalence of this disorder be tested for it. Such arrangements have been made for the Greek community in Cape Town. It is thereafter advisable that persons with the disorder wear a medic-alert disc. Antenatal diagnosis of the disorder is not indicated in utero, but maternal prenatal and neonatal testing is advisable in certain instances and ideally should be undertaken in all possible carriers of the disorder.

Finally, the gene frequency of hereditary spherocytosis far exceeded that expected. This is felt to be reasonably high as a result of the founder effect and may not be representative of the true picture of

the Greek people. It is recommended that further study be undertaken to clarify this matter as the implications are far reaching. These include the possibility of a selective advantage against malaria.

Antenatal diagnosis is not indicated in HS although knowledge of the presence of the disorder is important as it might occur in the Greek community with a significant prevalence. Diagnosis is important in order that the correct treatment may be given. Essentially, this is splenectomy. This surgical procedure ought to have been done in one of the survey subjects in addition to a cholecystectomy, but as the correct diagnosis of the underlying disorder was not made, it was not performed.

It is also important that there be awareness of the possibility of haemoglobinopathies in persons of Greek origin. One person with the sickle cell trait was found in Cape Town.

APPENDIX A

Unless otherwise indicated the laboratory techniques are based on those as described by Dacie and Lewis (1975).

1. PERIPHERAL BLOOD SMEARS

a) Red Cell Morphology

A drop of well-mixed, fresh E.D.T.A. blood is smeared onto 2 clean slides and then rapidly air dried.

Staining procedure:

0,5 ml Wright's stain is added to the slide and after 1 minute 1 ml of Wright's buffer at pH 6,8 is added. This is well mixed on the smear and left on the slide for 12 minutes before being washed off with distilled water and air dried. The slide is now ready to be examined under an oil immersion lens under 40 objective magnification for red cell morphology.

2. SUPRAVITALLY STAINED PREPARATIONS

a) Reticulocytes

0,2 ml of brilliant cresyl blue and an equal quantity of blood are well mixed and then incubated at $37^{o}C$ in a waterbath for 30 minutes. Two smears are then made from this solution. Five hundred cells are counted from each slide and note is taken of the number of reticulocytes under 100 objective magnification. Thus a total of 1000 cells are counted. The total number of reticulocytes is then divided by 10 to allow the reticulocyte count to be expressed as a percentage. To obtain the corrected reticulocyte count the following formula is used:-

$$\frac{\text{Observed reticulocyte count x patient's PCV}}{\text{normal PCV}}$$

The PCV is sex-dependent and the normal reticulocyte count ranges from 0,5 to 2%.

b) Haemoglobin H inclusion bodies

0,2 ml brilliant cresyl blue is added to 3 drops of blood and mixed well. This is then incubated for 30, 60, 90 and 120 minutes at 37°C in a waterbath. Two smears are made of each at 30, 60, 90 and 120 minutes.

The smears are then examined under 100 objective magnification and 5000 cells counted on each slide, noting the number of red cell inclusion bodies. Thus, 10 000 cells are counted and the final result is expressed as number of inclusion bodies per 10 000 cells.

The above method was employed for the first half of the survey. During the second half of the survey a similar procedure was followed, with the exception that the solution was incubated for 4 hours and only 1 set of smears was made.

3. BLOOD GROUPING

a) ABO

b) Rhesus

c) Duffy blood grouping was performed. (Routine methods were used and will not be discussed further.)

4. RED CELL PARAMETERS

a) Haemoglobin

The cyanmethaemoglobin method as described by Dacie and Lewis

(1975) was used.

PRINCIPLE

By diluting the blood in a solution containing potassium cyanide and potassium ferricyanide, haemoglobin, methaemoglobin and carboxyhaemoglobin are all converted to the stable compound cyanmethaemoglobin. The optical density of this solution is then measured in a photoelectric colorimeter and compared to that of a known standard.

REAGENTS

Drabkin's cyanide ferricyanide : KCN 0,05 g, K3 FE $(CN)_6$ 0,20 g and distilled water to 1 litre.

Standard of concentration 15,2 per 100 ml.

PROCEDURE

0,02 ml of blood is added to 4 ml of modified Drabkin's cyanide-ferricyanide solution in a tube which is then inverted several times. The solution is then compared with the standard in a Klett-Sommerson photoelectric colorimeter.

Calculation:

$$\text{Haemoglobin (g per 100 ml)} = \frac{\text{Test}}{\text{Standard}} \times \frac{\text{concentration of STD x dilution factor}}{1000}$$

b) PACKED CELL VOLUME (PCV)

The microhaematocrit method was followed.

A microhaematocrit tube has well mixed E.D.T.A. blood added to two-thirds of its capacity. One end of the tube is sealed with plasticine or by flame and the tube is spun from 3 to 5 minutes at 12 000 gravs on a Hawksley centrifuge. The result is read off directly as a percentage on a Hawksley microhaematocrit reader. Normal value for a male is 45% while for a female 40%.

c) RED CELL COUNT

0,05 ml of well mixed blood is added to 25 ml isoton, a balanced isotonic modified medium which maintains the integrity of the cell for at least 30 minutes. 0,1 ml of this solution is then added to 10 ml isoton giving a 1 : 50 000 strength. This solution is now used to measure the red cell count. The model F Coulter counter, a particle counter, then automatically counts the number of red cells in the solution.

Where the red cell count is high there is an increased possibility of 2 cells being counted as one. To compensate for this an incidence chart is supplied for correction of the red cell count. Caution must be exercised to ensure that the blood is well mixed and that the system is dust-free. If these precautions are not taken the counts will be erroneous.

d) MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION (MCHC)

The MCHC is calculated by dividing the haemoglobin by the PCV and multiplying by 100 ($\frac{Hb}{PCV} \times \frac{100}{1}$). The result is expressed as a percentage with normal values ranging from 32 to 36 per cent.

e) MEAN CORPUSCULAR VOLUME (MCV)

The MCV is calculated by dividing the PCV by the red cell

count and multiplying by 10. The result is recorded in femtolitres. The normal range is 78 to 98 femtolitres (fl).

$$\frac{PCV}{RCC} \times \frac{10}{1}$$

f) MEAN CORPUSCULAR HAEMOGLOBIN (MCH)

The MCH is obtained by dividing the haemoglobin by the red cell count and multiplying by 10.

$$\left(\frac{Hb}{RCC} \times \frac{10}{1}\right)$$

The result is expressed in picograms (pg) and the normal range is 27 to 32 pg.

5. OSMOTIC FRAGILITY

Basis

The method followed is slightly modified from that described by Dacie and Lewis (1975) and based on that of Parpart, et al (1947). Blood is added to hypotonic saline buffered to pH 7,4 in the proportion of 1 to 100. The test is carried out at room temperature and lysis is measured photoelectrically.

Reagents

A stock solution of buffered sodium chloride, osmotically equivalent to 100 g/l Nacl is made up as follows:-

NaCl - 90 g, Na_2HPO_4 - 13,6 g and NaH_2PO_4, $2H_2O$ - 2,43 g are dissolved in water and the final volume is adjusted to 1 litre. It is stored as a 10 g/l stock solution at 4^oC. This is titrated to 1 g/l accurately for use when needed. Serial dilutions of 8,5 are convenient concentrations. In

addition a 13 g/l dilution is used after incubation.

METHOD

The test is performed within 2 hours of collection of the blood. 0,02 ml volumes of the well mixed blood to be tested are added to 5 ml volumes of the range of hypotonic solutions and immediately mixed by inverting several times. The tubes are allowed to stand at room temperature from 30 minutes to 2 hours, then remixed and centrifuged for 5 minutes at 1200-1500 g. The amount of lysis in each tube is then compared with that in the 100% lysis tube (1,0 g/l NaCl) using a photoelectric colorimeter with a yellow-green filter or at a wavelength setting of 540 nm. The supernatant from the 9,0 g/l NaCl tube is used as the blank or that from a 13 g/l NaCl tube if there is lysis in the 8,5 g/l NaCl.

6. ELECTROPHORESIS

Although any method suitable for electrophoresis of serum proteins can be used for haemoglobins, paper electrophoresis by the vertical (hanging strip) method has been found to be most suitable for routine use, such as large scale screening procedures.

a) DETECTION AND PRELIMINARY CLASSIFICATION OF ABNORMAL HAEMOGLOBINS

The method followed is that described by Lehmann and Ager (1960).

BUFFER

Standard barbiturate buffer pH8,6: Ionic strength

Sodium diethylbarbiturate	20,6 g and
Diethylbarbituric acid	1,84 g

are added to distilled water to make up 2 litres. This buffer has half the concentration of diethylbarbituric acid used routinely for serum protein electrophoresis as it gives wider separation in the region where the haemoglobins are found.

PROCEDURE

Whatman No. 3 mm filter paper cut to strips measuring 36,5 cm by 8,7 cm are folded gently in midline and this is marked lightly with a pencil. The strip is hung dry in the tank and 10 to 15 ml samples of haemolysate are streaked with a saline pipette along the pencilled line, about 1 cm wide. Fresh buffer is now applied by pipette to the paper to about 0,3 cm below the haemolysate and electrophoresis is performed for 17 to 20 hours at 12 milliamperes. The papers are then removed and the heavily soaked unwanted ends cut off to produce more clearly defined zones. The papers are dried in an incubator at about 120°C. The haemoglobin bands are clearly visible and any abnormal bands will require further investigation. A small amount of non-haemoglobin protein may become visible between the line of application and the region where haemoglobin A_2 is found: this is thought to be the enzyme methaemoglobin reductase.

b) QUANTITATION OF HAEMOGLOBIN A_2

The method used is that described by Black, et al (1966).

Buffer:

1,2 g Barbituric acid
6,8 g Sodium barbital
40,4 Tris
4,0 g Disodium EDTA
3,0 g Boric acid
with distilled water to make up a 2-litre solution.

Two Whatman 3 mm filter paper strips measuring 14½ by 1½ inches have a pencil line drawn 1¼ inches from the midline. 0,1 ml of haemolysate is pipetted to the line to form a band approximately 1 cm in breadth. The ends of the strip are dipped into buffer to within 1/8 inch of each edge of the haemolysate and excess buffer is removed by blotting. The strip is hung in the tank with the applied band toward the anode. The run is started at 12 milliamperes and allowed to proceed for 17 to 20 hours. Filter papers should not touch each other and the box must be full. The A_2 band and its tail are cut from the two strips and are placed in 5 ml of Drabkin's reagent for elution. The A band is cut from the 2 strips and placed in 5 ml Drabkin's solution for elution and left for 2 hours. After 2 hours, the elutes are centrifuged to pack the paper and the optical density (OD) measured on a Klett-Sommerson photoelectric colorimeter with filter 540 nm.

Percentage of haemoglobin A_2 is determined by the formula:

$$\frac{OD\ A_2}{OD\ A_2 + OD\ A} \times \frac{100}{1} = \text{Percentage haemoglobin } A_2$$

The range in non-thalassaemic individuals is 1,0 to 2,9 per cent, with a mean of 2,03 $\pm$ 0,37.

7. THE ESTIMATION OF ALKALI-RESISTANT HAEMOGLOBIN-FETAL HAEMOGLOBIN

A convenient method of haemoglobin F (HbF) estimation is the "one minute alkali denaturation test" of Singer, Chernoff and Singer (1951). However, this technique is not altogether reliable at low concentrations of HbF.

Adult haemoglobin is denatured by alkalis whereas haemoglobin F is

resistant to this process. The denatured haemoglobin is removed by precipitation with ammonium sulphate and the remaining haemoglobin is measured photometrically.

REAGENTS

1) Ammonium sulphate: 152 g is dissolved in distilled water to give a final volume of 400 ml with 1 ml 10 N HCL added.
2) N12 NAOH: 20 ml IN NAOH is added to 220 ml distilled water to give a final volume of 240 ml.
3) Ammonium hydroxide: 4 ml of concentrated ammonia is added to 1 litre of distilled water.

The reagents should be kept and the test should be performed at about 20°C. 0,2 ml of the haemolysate is added to 3,2 ml N12 sodium hydroxide, mixed thoroughly and left for precisely 1 minute. 6,8 ml of the ammonium sulphate reagent is then added and after inverting several times and waiting about a minute the mixture is filtered through No. 42 Whatman filter paper and measured on the Klett-Summerson photoelectric colorimeter at 540 nm. If it is colourless, no measurable amount of HbF is present. A solution which is termed "total" is now made up by adding 0,05 ml of the original haemolysate to 5 ml of ammonium hydroxide solution and this solution is measured photometrically. A control solution is made up of 6,8 ml ammonium sulphate added to 3,2 ml sodium hydroxide. This solution is now filtered before being measured photometrically. The Klett-Summerson colorimeter is zeroed on a 5 ml ammonium hydroxide solution.

Calculation:

$$\text{Percentage haemoglobin F} = \frac{\text{Test - Control}}{2 \times \text{Total}} \times \frac{100}{1}$$

Results of below 1,7 per cent alkali-resistant haemoglobin are not significant, whereas over 1,7 per cent are suggestive of some degree of red cell pathology.

8. THE BRILLIANT CRESYL BLUE DYE TEST OR MOTULSKY TEST

This test is for G-6-PD deficiency and is essentially that developed by Motulsky and Campbell-Kraut (1961).

PRINCIPLE

Haemolysates are incubated in the presence of excess glucose - 6-phosphate (G6P) as substrate and NADP as coenzyme, together with brilliant cresyl blue dye. Dehydrogenation of G6P as a result of the presence of G-6-PD leads to reduction of NADP to NADPH. The added dye is reduced to a colourless compound in proportion to the amount of NAPPH formed. The dye also stimulates the activity of the shunt (oxidative) pathway so that decolourization occurs rapidly. Deficiency of the enzyme thus results in a prolonged dye-decolourization time.

REAGENTS

1) BUFFER DYE SOLUTION

44,75 g Tris (2-amino-2-hydroxymethyl propane-1,3 diol) and 160 mg of brilliant cresyl blue are dissolved to form a solution of 400 ml. The pH is adjusted to 8,5 with concentrated hydrochloric acid. The solution is mixed, made up to a final volume of 500 ml divided into 4,5 ml volumes and frozen.

2) NADP

50 g are dissolved in 100 ml distilled water. The solution is divided into 1 ml volumes and stored frozen.

3) SODIUM GLUCOSE-6-PHOSPHATE

895 mg of the salt is dissolved in 100 ml of distilled water.

This solution is also divided into 1 ml volumes and frozen.

COMBINED REAGENT SOLUTION

This is prepared fresh daily by mixing 4,5 ml buffer-dye solution, 1 ml of NADP solution and 1,0 ml of glucose-6-phosphate solution.

PROCEDURE

0,02 ml of fresh whole blood is added to 1,0 ml of distilled water. The suspension is well mixed in water to lyse the cells before mixing with 0,65 ml of the combined reagent solution. The whole is then covered with liquid paraffin and incubated at 37°C without agitation. After 30 minutes the solution is checked each minute for dye-decolorization. The decolourization produces subtle changes in the appearance of the contents of the tube. The reddish colour of haemoglobin shows through the blue dye giving an appearance rather like a striped marble.

RESULTS

Normal for women : 30-40 minutes

Normal for men : less than 45 minutes

SUPPLEMENTARY TESTS

9. METHAEMOGLOBIN REDUCTION TEST

This test for G-6-PD deficiency is used in the case of female carriers for G-6-PD deficiency where the results of the Motulsky test are equivocal and there is doubt as to whether or not the female is a carrier.

PRINCIPLE

The test developed by Brewer, Tarlov and Alving (1962) involves the oxidation of haemoglobin to methaemoglobin by sodium nitrite and its subsequent enzymatic reconversion to haemoglobin in the presence of methylene blue. This redox dye affects the pentose phosphate shunt and activates reduced NADP-methaemoglobin reductase in normal, but not G-6-PD deficient erythrocytes.

REAGENTS

0,18 M sodium nitrite and 0,28 M dextrose solution

0,0004 M methylene blue chloride solution

TUBES

1. Normal reference tube : has no reagent added.
2. Positive reference tube : has exactly 0,1 ml of the combined sodium nitrite-dextrose reagent added.
3. Unknown sample tube : has exactly 0,1 ml of the sodium nitrite-dextrose reagent and 0,1 ml of the methylene blue reagent.

PROCEDURE

2 ml of blood (either normal, drug-sensitive or unknown) is added to both the positive reference and the normal reference tubes. The contents are well mixed by inverting each tube about 15 times. Add 2 ml of blood from the individual under investigation to the unknown sample tube and mix. The tubes are incubated unstoppered in a waterbath at $37^{o}C$ for 3 hours. A 0,1 ml aliquot from each of the 3 tubes is then pipetted into separate clear glass test tubes, to each of which 10 ml of phosphate buffer has previously been added. The unknown tube is then compared spectrophotometrically to the positive and normal test

tubes. In individuals showing full expression of the G-6-PD deficiency trait, more than 70% methaemoglobin remains, while in normal individuals less than 5% persists. In female heterozygotes the amount of methaemoglobin persisting varies between 5 and 70%.

10. SOLUBILITY TEST FOR HAEMOGLOBIN S (ITANO, 1953)

This is a test for haemoglobin S (HbS) and is based on the unique low solubility of HbS in its reduced form.

BUFFER: (Concentrated phosphate) pH6,8

KH_2PO4 and K_2HPO4 are dried to constant weight at 100°C. Then 40,12 g KH_2PO4 and 70,47g K_2HPO4 are dissolved overnight in 200 ml water in a 250 ml volumetric flask. Next morning the volume is made up to 250 ml and the solution filtered through Whatman 42 filter paper.

TEST

100 mg sodium hydrosulphite ($Na_2S_2O_4$) is weighed with a 10 ml cylinder and 8 ml of the buffer added. After shaking to dissolve, 0,8 ml distilled water is layered over this solution. A volume of the approximately 10 g/100 ml oxyhaemoglobin solution chosen to contain exactly 50 g haemoglobin is carefully pipetted into the water layer. The volume is made up to 10 ml with distilled water without mixing and the flask incubated for 15 minutes in a 25°C water bath. If no HbS is present the solution will be clear. However, if HbS is present, a precipitate proportionate to the amount of HbS present will form.

The following tests were not done routinely and were highly specialized. The author, therefore, did not familiarize himself with the techniques

used and thus the names and references of the tests are all the information given.

11. AUTOHAEMOLYSIS STUDIES (Where hereditary spherocytosis was suspected)

(Dacie and Lewis, 1975)

The method followed was a combination of 2 described by Dacie and Lewis in order that it be more sensitive.

12. ALPHA/BETA CHAIN RATIOS

To enable the diagnosis of the alpha-thalassaemia trait to be substantiated (Weatherall, et al, 1969).

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