A STUDY OF MEDITERRANEAN ANAEMIA

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I N D E X.

INTRODUCTION.

This study of Mediterranean Anaemia was made between July 1946 and March 1947, while I was serving as a Graded Physician in the R.A.M.C. in Cyprus. By way of introduction some features of conditions in Cyprus will be discussed, so that the problem there may be viewed in its natural perspective.

The existence of Mediterranean Anaemia in Cyprus has only been recognised in recent years. Arabantinos (1936) mentioned that he had seen cases from Cyprus, and two of the children described by Caffey (1937) were of Cypriot parentage. But in the island itself Fawdry, a District Medical Officer in the Colonial Government Medical Services, was the first to draw attention to the condition. He published a report on the subject in 1944. Up till then the condition had always been regarded as chronic malaria, despite the absence of parasites in the blood films and the failure of quinine therapy. The local medical practitioners are mostly Greek trained and, as pointed out later in this thesis, the Greek medical schools have been strong protagonists of malaria as the etiological factor. From conversation with local doctors it was clear that many still held these views. In the main, however, the Government Medical Department has enlightened medical opinion throughout the island, and it is now realised that far from being a rarity, this disease is very common. It is interesting to record that a technician working in one of the Government hospitals told me that he frequently saw target cells when he was examining blood films, but that per se he did not regard them as abnormal. The technician in question was a most able and competent worker, but he was not familiar with the trait of the disease. In view of what is subsequently said about the trait, this is an example of the adage that 'familiarity breeds contempt.' I was first introduced to the condition by Fawdry, and I must acknowledge the encouragement and guidance I received from him when I embarked on investigating the condition myself.

The area of the island is 3,572 square miles, and the population is about 400,000 - roughly the same population as in Edinburgh. About three-quarters of the population are Greek Cypriots, and belong to the Greek Orthodox Church. About one-fifth are Turks of the Moslem faith. These are a remnant of the era when the Turks ruled Cyprus. There are also a few Armenians, a small British community, and scattered families of other races, but all these comprise only a small percentage. The different sects live together quite harmoniously, though they keep much to themselves.

p.t.o.

INTRODUCTION (Continued).

Marriages between Greek and Turk are uncommon, but do take place. These points are important when one considers the hereditary aspect of Mediterranean Anaemia. Cyprus was an ideal place to study problems of heredity, since being a small island, relatives of patients were often quite easily available. In many cases whole families were located in the same village, and often in the same house. Life in Cyprus was very simple - in the country it had progressed little from Bible times - and the majority of the populace were subjected to the same environmental factors, and these had remained unchanged for very many generations.

The people themselves were friendly and co-operative. The Cypriots are a simple race, and though near the gateway of the East, are essentially European in their outlook. Their education is limited, and their intelligence below average judged by our standards. Often they did not know their own ages, and they would give different answers if questions were repeated. This made case history taking difficult, and accordingly details of events, particularly those happening years previously, were unreliable. In particular, I found it difficult to obtain details of family histories. Mothers often could not remember how many children they had had, and remembering miscarriages was usually quite beyond them. They were also less observant of symptoms than mothers in this country, and they would pass as normal, children which most mothers here would recognise as unwell. They were, however, most co-operative in allowing me to make my investigations. Perhaps their generosity in this respect should be recognised even further, since it is a popular belief in the island that all blood examinations have something to do with syphilis. Only in the third family which I recorded did I meet with opposition to my examining their blood. In this instance two sisters refused on the grounds that they were pregnant, and they believed that venepuncture would affect them adversely. I must acknowledge the enthusiasm and persuasive powers of my interpreter, whose helpwas invaluable. It was perhaps as well that I did not know some of the things he told my victims!

Certain diseases peculiar to the island are worthy of mention. As a result of an energetic Anopheles Eradication Scheme by the Government Medical Department, malaria is no longer a major problem, and the decline in its incidence is likely to continue. Hydatid disease is common and must be considered in the differential diagnosis of any tumour. In my nine months stay in Cyprus I saw hydatids affecting the liver, spleen, lung, kidney, thyroid and a coronary artery. Typhoid fever is endemic, particularly in the villages where sanitation is poor. Bacillary dysentery is common, and shows a normal seasonal increase. It has,

INTRODUCTION (Continued).

however, a lower incidence than elsewhere in the Middle East, probably because Cyprus is cleaner than the majority of places in that area. Amoebic dysentery is not seen.

Some interesting facts emerge regarding blood diseases other than Mediterranean Anaemia. Fawdry (1944) reviewed this problem, but other facts have come to light since his article was written. Idiopathic hypochromic anaemia, as we know it in this country, is never seen in Cyprus. In my stay there I never saw an example of koilonychia. Though the people are very poor, they live on the land, and apparently their diet is more adequate than that of the poorer classes in this country. The absence of this form of anaemia was fortunate in view of the similarities of the blood picture in this condition, and in Mediterranean Anaemia. In particular it would have complicated the detection of the trait of the disease. Fawdry records having seen cases of nutritional megalocytic anaemia, but I did not have the opportunity of seeing any such cases while I was on the island. A point of considerable interest is that he never saw a case of acholuric jaundice in all the seven years he was in Cyprus, despite the fact that specifically he was interested in blood disorders, and was on the look out for this condition. It may well be that since this disease, like Mediterranean Anaemia, is the result of a genetic mutation, the chance occurrence of one disease renders the other disease less likely. Haemolytic Anaemias of the allergic type are also seen. These result from sensitivity to certain beans. Cases are seen in the spring when there are flowers, and are presumably due to pollen sensitivity, and also in late summer when the beans have developed. I saw two cases in March 1947.

The investigations carried out in this study were essentially of the simplest nature. Technical assistance was limited and often had to be dispensed with owing to inaccuracy. No facilities were available for biochemical analysis. There are very many interesting problems connected with this disease which require the services of a biochemist. Such investigations may help to elucidate some of the obscure problems of iron metabolism, and also in the final issue, I feel that the etiology of Mediterranean Anaemia may have a biochemical basis.

Before describing cases of the disease a short introduction to the disease must first be given. It was first recognised by Cooley and Lee in 1925, though 1927 is usually accepted as the date when Cooley first described the condition as a clinical entity. He separated it from Von Jaksch's Anaemia with which it was originally confused. Cooley's name has often been associated with the condition, and there have been

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other synonyms. In view of the haematological response Cooley himself called the condition Erythroblastic Anaemia, but this term leads to confusion with other anaemias in which an erythroblastic reaction occurs. Whipple and Bradford (1936) called the condition Mediterranean Disease or Thalassaemia. This recognised that anaemia may be only one of the features of the disease and not the fundamental abnormality. Both the terms Mediterranean Disease and Mediterranean Anaemia are in common use. Thalassaemia has recently become popular, particularly in America. Lovel (1947) has pointed out that this is unfortunate, since 'Thalassa' is Greek for the sea and refers to the Mediterranean, and a literal translation means that the patient has the Mediterranean in his blood. This leaves the purists in no doubt as to why he should be anaemic! Damashek (1940) introduced the term Target Cell Anaemia, but this is unsatisfactory, since target cells are found in other conditions as well as in Mediterranean Anaemia.

An accurate definition of what constitutes the disease has not been given in the literature. There is in fact a gradual gradation from the trait not showing any anaemia, through the mild form of the disease, compatible with adult life, to the severe form of the disease, fatal in infancy. Clearly any distinction between one form and another is purely arbitrary. The terms Thalassaemia major and minor have been used to differentiate between the severe and mild forms of the disease. Classification is considered in greater detail under the heading of symptomatology.

In this thesis the following plan has been adopted. Fourteen cases of Mediterranean Anaemia together with haematological studies of their relatives are recorded. Examples of the trait of the disease are given and then the symptomatology and haematology of both the disease and the trait are discussed. A separate section is devoted to the radiological appearances. The results of investigations to determine the incidence of the trait of the disease in Cyprus are then recorded, and their significance is discussed. The results of a village survey are given to show how the trait may affect an isolated community. Various aspects of the etiology are next considered: the incidence of the disease in Cyprus, the defect in the erythrocyte, the development of the disease and finally the possible causes of the condition. The hereditary problem is considered in detail. Diagnosis, treatment and prognosis are dealt with in general terms, and a summary of the essential points concludes the thesis.

THE FIRST FAMILY.

CASE NO. 1. ESTATHIAS FRANDESCOU.

History.

The patient was a male infant, born on 10 Nov.45. When examined he was aged nine months. The family lived in Ayios Memnon, about one mile from Famagusta.

The infant was the parent's first child. He was born at full term following a normal delivery. No abnormality was noted in the infant at birth. Birth weight was not known, but the infant was of average size. No jaundice was noted after birth, and none since then. The baby was breast fed, and weaning had not been started when the examination was made. Feeds had been taken quite well.

At the age of three months, the mother noticed a gradual onset of pallor and weakness, which had become more marked. The child had appeared feverish at times, but the mother was unable to be precise regarding onset and duration of febrile periods. Fever was only of mild degree. At the age of six months, it was noticed that the abdomen was becoming more prominent. No other symptoms had been noticed. Digestion was normal. No petechial haemorrhages or bleeding from mucous membranes had occurred. There had been no respiratory, alimentary or other infections recognised.

Two teeth had been cut at the age of five months, but none since. The infant could hold its head up, but not sit up by itself. He could not talk, but would utter the word 'Mama!' The mother said the infant was contented, and did not cry unduly.

Family history.

The mother had always enjoyed good health, except for an attack of jaundice in adolescence. Two sisters and one brother were all healthy; none had died. The grandparents were alive and well, as were also the maternal great-grandparents.

The father had always been healthy. A brother and sister were both well. An elder brother had died of pulmonary tuberculosis at the age of 24. Another brother had died at the age of 15 months - cause unknown. The grandparents were alive and well.

Neither parent knew of any relative who had been at all pale.

The parents were peasant farmers. There did not appear to be any gross deficiency in their diet.

Examination.

General. The infant was small for its age. Weight 10 lbs. Mucous membranes were pale. The skin showed normal nutrition, but was pale and muddy and lacked a healthy bloom. No petechiae were seen.

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CASE NO. 1. CONTINUED.

No epicanthic fold was present. There was no frontal bossing, and the bridge of the nose was not depressed. The anterior fontanelle was not closed. There was no cyanosis, and no clubbing of the fingers. Muscle tone was poor. The facies did not show the so-called mongoloid expression. Slight icterus of the conjunctivae was noted.

Cardiovascular system. Pulse 130 per min., regular in time and force, and of good volume. A soft, localised systolic murmur was heard at the apex. Apex beat just outside the midclavicular line in the fifth intercostal space.

Respiratory and nervous systems. No abnormality.

Tongue. No atrophy or undue redness.

Mouth and throat. No cheilosis or angular stomatitis. Tonsils healthy. 2 incisor teeth were present.

Abdomen. Distended with a small umbilical hernia present. No free fluid in abdomen. The liver edge was palpable half way between the costal margin and the umbilicus. The edge and surface were smooth, firm and not tender. The spleen was palpable in the left flank. It extended downwards and forwards to the lateral edge of the left rectus sheath, about an inch below the umbilicus. It was firm, smooth and not tender. Its edge had a well defined notch.

Lymph glands. Not enlarged.

Urine. No protein or sugar. Urobilinogen present.

Blood examination.

Hb. 5.8 gm.% R.B.Cs. 2.59 million per cmm.
Reticulocytes 6%. W.B.Cs. 20,240 per cmm.
Nucleated R.B.Cs. 61 per 100 W.B.Cs. - 46 early normoblasts and 15 late normoblasts.
Differential W.B.Cs.

Neutrophils.....	38%.
Stab cells.....	1%.
Juveniles.....	5%.
Lymphocytes.....	53%.
Monocytes.....	1%.
Eosinophils.....	2%.
Basophils.....	0%.

Blood group. **A.**
Fragility. 0.42-0.15 gm.NaCl.%.
Film. Severe anisocytosis and poikilocytosis were present. Many target cells and ovalocytes were seen. Microcytes were also prominent. The cells were hypochromic, staining only at the periphery. Polychromasia and punctate basophilia were seen. Normoblasts were numerous. The picture was that of a severe degree of Mediterranean Anaemia.

Family studies.

The following members of the family were examined. The results are appended.

1. Mother. Savvou Frandesco. Age 23 years.
2. Father. Frandesco Andrea. Age 25 years.
3. Mother's sister, Photini Panayi. Age 32 years.
4. Mother's sister, Eleni Panayi, Age 22 years.

5.Mother's mother, Sotira Panayi, Age 50 years.
6.Mother's father's sister, Katerina Demetri, 58 yrs.
7.Mother's mother's mother,Eleni Antoni,Age c.85 yrs.
8.Mother's mother's father,Andonis Michael, 105 years.
9.Father's brother, Pandalis Andrea, Age 20 years.
10.Father's sister, Elizabeth Nicola, Age 22 years.
11.Father's mother, Milia Andrea, Age 50 years.
12.Father's father, Andreas Frandesco, Age 60 years.
13.Mother's brother, Michael Panayi, Age 26 years.

Patient.	1.	2.	3.
Hb.	5.8	14.7	13.4	13.0
RBCs.	2.59	4.95	5.02	3.98
Retics.	6%	3%	1%	0.5%
Nuc.RBCs.	61	0	0	0
A.and P.	+++	+	+	-
Target.	+	+++	+++	-
Oval.	+	+	+	-
Micro.	+	+	+	-
Frag.	0.42-0.15	0.42-0.21	0.36-0.21	0.45-0.33
Group.	A.	A.	O.	A.
Trait.	Disease.	Yes.	Yes.	No.

	4.	5.	6.	7.
Hb.	12.3	11.9	10.0	12.6
RBCs.	4.66	4.51	4.41	4.99
Retics.	1%	0.5%	1.5%	0.5%
Nuc.RBCs.	0	0	0	0
A.and P.	+	-	+	+
Target.	+	-	-	+
Oval.	+	-	+	++
Micro.	+	-	+	+
Frag.	0.39-0.21	0.42-0.27	0.39-0.18	0.42-0.24
Group	A.	A.	B.	A.
Trait.	Yes.	No.	Yes.	Yes.

	8.	9.	10	11.
Hb.	13.3	15.8	13.7	15.1
RBCs.	3.81	4.89	4.61	4.61
Retics.	1%	0.5%	0.5%	1%
Nuc.RBCs.	0	0	0	0
A. and P.	-	+	-	+
Target.	-	+	-	+
Oval.	-	-	-	++
Micro.	-	-	-	+
Frag.	0.42-0.30	0.42-0.27	0.45-0.30	0.42-0.21
Group.	O.	A.	A.	A.
Trait.	No.	Yes.	No.	Yes.

	12.	13.	
Hb.	15.8	14.0	gm%
RBCs.	4.69	4.8	million per cmm.
Retics.	0.5%	0.5%	
Nuc.RBCs.	0	0	per 100 wbcs.
A. and P.	-	-	Anisocytosis & poikilocytosis.
Target.	-	-	Target cells.
Oval.	-	-	Ovalocytes.
Micro.	-	-	Microcytes.
Frag.	0.45-0.30	0.42-0.30	Fragility in NaCl. gm.%.
Group	A.	O.	
Trait.	No.	No.	

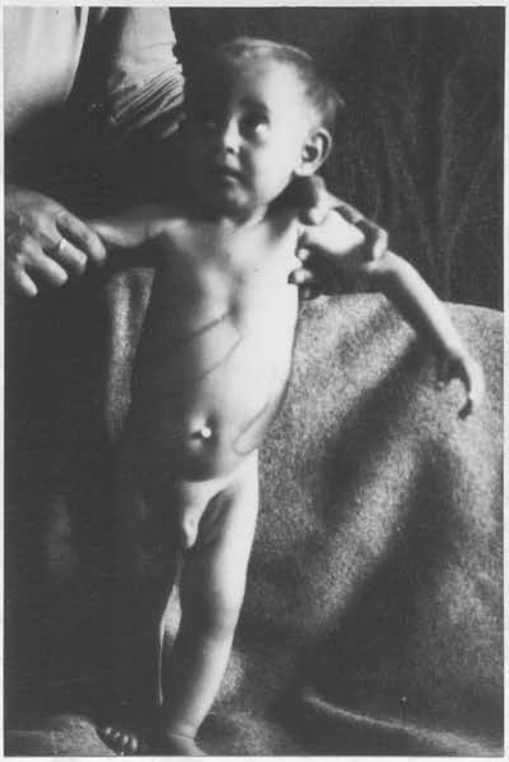

Plate I.

Case No.1. Estathias Frandesco.
The umbilical hernia, large liver and spleen are seen, but there is no abnormality of the facies.

CASE NO. 2. MARIE CHRISTODOULOU.

History.

The patient was a female child, born on 15 Aug.44. She was aged two years exactly when examined. The family lived in Nicosia.

The girl was the parent's second child. She was born at full term following a normal delivery. The mother had been quite well during pregnancy. The child was of average size at birth. Breast feeding had been continued until the age of fifteen months.

The mother noticed nothing wrong with the child until it was eight months old. She thought it was off colour and feverish. She said the fever was never marked and there was no shivering. Feverish attacks would last a few days, and recur at intervals of two to three weeks. They lasted in all about five months. At the same time the child was noticed to be paler than it had been. This pallor varied in intensity, but was never severe. As teething started at the same time, the mother attributed the illness to this. Enlargement of the abdomen had been noticed for the past three months. There was no jaundice after birth, and none since then. No petechial haemorrhages occurred and no bleeding from mucous membranes. There had been no difficulty with feeding, and digestion had been normal. Apart from the feverish attacks, which were never confirmed with a thermometer, there had been no recognisable infections. No bones had been fractured.

The first tooth had been cut at eight months, and the child had all her teeth except the second premolars at the time of the examination. She had walked at 18 months, and started to talk at the same time. She now had an average vocabulary for a child of her age. She was mentally alert, contented, and quite active.

Family history.

The patient was the second child. A boy had died five years previously, aged two and a half years, from the same condition. He had been much more severely affected by the condition than his sister.

The mother had had one miscarriage since the boy's death, at the third month. She herself was quite fit and had always enjoyed good health. Her father and mother, two brothers and three sisters were all alive and well. No brothers or sisters had died.

The father was in good health. He had three brothers and two sisters alive and well; none had died. His mother was alive and well. His father had died - age and cause of death unknown, though he was quite 'old.'

There was no history of any relatives being anaemic.

The father was a general labourer. No gross dietary deficiency was apparent.

Examination.

General. The patient weighed 28 lbs. Development was average. Skin showed normal nutrition. It was rather pale, and of muddy tint - slightly more than is usual in a Cypriot. Mucous membranes showed slight

The conjunctivae did not show any icterus. No ulcers on the shins. There was no frontal bossing. No epicanthic fold or depression of the bridge of the nose. The facies was not mongoloid. The anterior fontanelle was closed. There was no cyanosis and no clubbing of the fingers. Muscle tone was normal. There were no petechiae.

Cardiovascular system. Pulse 120 per min., regular in time and force, and of good volume. The apex was within the midclavicular line in the fifth intercostal space. No murmurs were heard.

Respiratory and nervous systems. No abnormality.

Tongue. Clean and moist. No atrophy or undue redness.

Mouth and throat. No cheilosis or angular stomatitis. Tonsils healthy. All teeth except second premolars were present. No dental decay.

Abdomen. No distension and no prominent veins over the abdomen. No umbilical herniae. No free fluid in the abdomen. The liver edge was palpable one inch below the costal margin. The edge was smooth, firm and regular. It was not tender. The tip of the spleen was just palpable below the left costal margin.

Lymph glands. Not enlarged.

Urine. No protein or sugar. Slight increase of urobilinogen.

Colour of eyes. Brown.

Blood examination.

Hb. 9.2 gm.%. R.B.Cs. 3.59 million per cmm.
Reticulocytes 15%. W.B.Cs. 21,700 per cmm.
Nucleated R.B.Cs. 9 per 100 W.B.Cs. - 2 early and 7 late normoblasts.
Differential W.B.Cs.

Neutrophils	26%.
Stab cells	2%.
Juveniles	1%.
Lymphocytes	65%.
Monocytes	2%.
Eosinophils	4%.
Basophils	0%.

Blood group. AB.
Fragility. 0.45-0.15 gm. NaCl.%.
Film. There was a moderately severe degree of anisocytosis and poikilocytosis present.
Ovalocytes and microcytes were seen and there were many target cells. Polychromasia was noted. Nucleated R.B.Cs. were present but were not numerous. Typical peripheral staining of a hypochromic anaemia was seen. The picture was that of a Mediterranean Anaemia of moderate severity.

Family studies.

The following members of the family were examined and the results are appended:

1. Mother. Angeliki Christodoulou. Age 26 years.
2. Father. Christodoulou Michael. Age 32 years.
3. Mother's sister, Anasthasias Thamis. Age 23 years.
4. Mother's sister, Christina Thamis. Age 18 years.
5. Mother's sister, Zinovia Thamis. Age 13 years.
6. Mother's brother, Georgeous Thamis. Age 23 years.
7. Mother's brother, Costas Thamis. Age 20 years.
8. Mother's mother, Maria Constantinou. Age 50 years.
9. Mother's father, Themistocles Constantinou. Age 55.
10. Father's brother, Abraham Michael. Age 24 years.

CASE NO. 2. CONTINUED.

11. Father's brother, Chrisostomos. Michael. Age 30 years.
12. Father's sister, Anastasia Michael. Age 26 years.
13. Father's sister, Meropi Michaelidou. Age 23 years.
14. Father's mother, Despina Christodoulou. Age 55 years.

	Patient.	1.	2.	3.
Hb.	9.2.	12.0.	12.3.	13.4.
RBCs.	3.59.	3.98.	4.64.	5.03.
Retics.	15%.	5%.	2%.	4.5%.
Nuc.RBCs.	9.	0.	0.	0.
A. and P.	++	+	+	+
Target.	+++	++	+	++
Oval.	+	++	+	+
Micro.	+	-	-	-
Frag.	0.45-0.15	0.39-0.18	0.39-0.18	0.39-0.18
Group.	AB.	B.	AB.	A.
Eyes.	Brown.	Brown.	Brown.	Brown.
Trait.	Disease.	Yes.	Yes.	Yes.
	4.	5.	6.	7.
Hb.	12.0.	14.8.	12.7.	12.3.
RBCs.	4.65.	5.7	4.57.	4.67.
Retics.	1%.	1%.	1%.	1%.
Nuc.RBCs.	0.	0.	0.	0.
A. and P.	+	+	+	+
Target.	+	-	-	++
Oval.	+	+	+	+
Micro.	-	+	-	-
Frag.	0.42-0.21	0.42-0.27	0.42-0.27	0.42-0.21
Group.	B.	A.	A.	A.
Eyes.	Brown.	Brown.	Hazel.	Brown.
Trait.	Yes.	Yes.	Yes.	Yes.
	8.	9.	10.	11.
Hb.	12.0.	12.4.	13.4.	14.8.
RBCs.	4.33.	4.34.	4.9.	4.9.
Retics.	1%.	1%.	2%.	1%.
Nuc.RBCs.	0.	0.	0.	0.
A. and P.	+	+	+	-
Target.	+	+	+	-
Oval.	+	+	+	-
Micro.	-	-	-	-
Frag.	0.45-0.18	0.39-0.21	0.36-0.24	0.36-0.27
Group.	B.	A.	B.	B.
Eyes.	Brown.	Hazel.	Brown.	Brown.
Trait.	Yes.	Yes.	Yes.	No.
	12.	13.	14.	
Hb.	13.4.	11.3.	14.1.	
RBCs.	4.1.	4.2.	4.67.	
Retics.	0.5%.	1%.	1%.	
Nuc.RBCs.	0.	0.	0.	
A. and P.	-	+	-	
Target.	-	+	-	
Oval.	-	+	-	
Micro.	-	-	-	
Frag.	0.39-0.30	0.39-0.24	0.42-0.27	
Group.	B.	B.	B.	
Eyes.	Brown.	Brown.	Blue.	
Trait.	No.	Yes.	No.	

CASE NO. 3. MARITSA IOANNOU.

History.

The patient was a young woman of twentyseven at the time of examination. She lived in the village of Ayios Ermolaos, about fifteen miles from Nicosia.

As far as she knew, she had been quite well up to the age of four or five, when she suffered from febrile attacks, which were called malaria. Her spleen enlarged and she became pale. She attended the two lowest classes at school, but was too weak to carry on, and could never play with the other children. She had been pale ever since.

She remembered that at the age of eleven her feet and ankles were swollen for about a month. Since then swelling occurred when she was tired, and it subsided with rest in bed.

At the time of examination the patient had no specific complaints. She admitted that she was easily tired, and could not do a hard day's work. She was breathless on exertion. There was no cough. Appetite and digestion were normal. There was no abnormality of micturition. Palpitations were noticed after exertion. There was permanent amenorrhoea, and she had only had four or five scanty vaginal bleedings in her life. These had occurred several years ago.

She was first admitted to hospital in 1941, and since then underwent several treatments for syphilis. The diagnosis was based on the configuration of her nose and a W.R. one plus on 6 Feb.41. She felt no subjective improvement from this therapy and her splenic enlargement persisted.

From available records it was found that her Haemoglobin in Nov.1941 was 4.4 gm %. At this time also a sternal marrow examination revealed no Leishman-Donovan bodies, and the formal gel reaction was negative. On 22 Nov.43, her W.R. was negative. A positive indirect and delayed direct Van den Bergh reactions were obtained. The blood picture was as follows:

Hb. 4.8 gm %. R.B.Cs 3.3 million per cu.mm.
W.B.Cs. 3000 per cu.mm.

Neutrophils.....	50%.
Lymphocytes.....	36%.
Monocytes.....	9%.
Eosinophils.....	4%.
Basophils.....	1%.

A transfusion of 800 cc blood was given on 5 Nov.43, but four months later on 3 Mar.44 her Hb. was again 4.8 gm %. and her R.B.Cs. 3.6 million per cu.mm. Hb. estimations had been made at intervals since that time, and showed this level had been constant over this period.

When she was a young child she fell off a donkey and broke her nose, thus giving the appearance of a depressed bridge of the nose.

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Family History.

The patient had three sisters and one brother alive and well. She was the youngest of the family; the brother was the eldest. A sister died at the age of twelve, cause unknown; another sister died at the age of seven from meningitis; a brother died at the age of three months, cause unknown. These three were all born after the brother and before the first living sister. Her brother had a child who died at the age of seventeen with a disease like her own - the child was always pale and had a large spleen which was removed by a surgeon.

Her father and mother were both alive and well. No grandparents were alive. The parents were peasant farmers. The patient helped on the farm, though had attempted domestic service for a short period. She had had to give it up as it was too much for her.

Examination.

General. The patient was of small average height and though thin was notwasted. There was marked pallor both of the skin and mucous membranes. The skin also showed an accentuation of the sallow colour of the Mediterranean races. It was normal in texture. No petechiae were present. Over the shins there were pigmented scars of old ulcers. There was no icterus of the conjunctivae. There was depression of the bridge of the nose, and a faint well healed scar over the root of the nose. There was no epicanthic fold, but there was slight bossing of the bones of the calvarium; otherwise no bony abnormality. A perforation of the nasal septum about one cm. in diameter was present. There was no cyanosis or clubbing of the fingers. Nails were normal. Axillary and pubic hair were scanty; the genitals were not examined. No oedema of the ankles was present at the time of the examination.

Cardiovascular system. Pulse 72 per min., regular in time and force, and of good volume. B.P. 125/80. The apex beat was half way between the midclavicular and anterior axillary lines, in the fifth intercostal space. A soft systolic murmur was heard at the apex; it was not propagated.

Respiratory and nervous systems. No abnormality.

Tongue. Clean and moist. No atrophy or undue redness.

Mouth and throat. No cheilosis or angular stomatitis. Tonsils healthy. Teeth in good condition.

Abdomen. There was marked distension, but no umbilical hernia present. No free fluid. Spleen was enlarged to half way between the umbilicus and left costal margin. It was firm, smooth, regular and not tender. It had a well-defined notch. The liver was enlarged three and a half inches below the right costal margin in the midclavicular line. The edge was smooth, firm, regular and not tender.

p.t.o.

Lymph glands. Not enlarged.
Urine. A trace of albumen was present, but no sugar. Urobilinogen was increased.
Colour of eyes. Brown.
Blood examination.
Hb. 4.9 gm.%. R.B.Cs. 2.8 million per cmm.
Reticulocytes 10%. W.B.Cs. 4,200 per cmm.
Nucleated R.B.Cs. 4 late normoblasts per 100 W.B.Cs.
P.C.V. 17%. M.C.V. 60.7 cu.microns.
M.C.H.C. 29.1%.
Fragility. 0.45-0.12. Blood group A.
Differential W.B.Cs.

Neutrophils	62%.
Stab cells	2%.
Juveniles	1%.
Lymphocytes	29%.
Monocytes	4%.
Eosinophils	2%.
Basophils	0%.

Film. Marked anisocytosis and poikilocytosis were present, with both microcytes and macrocytes. Target cells and ovalocytes were very numerous. In the midst of this bizarre picture there were several normal looking cells. The cells were hypochromic with peripheral staining, and many pale flattened cells were seen. Polychromasia and punctate basophilia were present. Late normoblasts were noted, but they were not numerous. The picture was that of Mediterranean Anaemia of moderate severity.
Sternal marrow. 500 identified cells.
Red series:

Pronormoblasts	7
Early normoblasts	105
Late normoblasts	314
Total	426

White series:

Myeloblasts	0
Promyelocytes	3
Neutrophil myelocytes	17
Eosinophil myelocytes	2
Neutrophil metamyelocytes	20
Neutrophils	25
Eosinophils	1
Lymphocytes	4
Monocytes	2
Total	74

White: red ratio - 1: 5.8.
The appearance and differential count were typical of a marked normoblastic response.

p.t.o.

CASE NO. 3 CONTINUED.

Family studies.

The following members of the family were examined and the results are appended:

1. Mother. Polyxeni Charalambos. Age 68 years.
2. Father. Zangaris Charalambos. Age 75 years.
3. Sister. Agathi Demosthenou. Age 36 years.

	Patient.	1.	2.	3.
Hb.	4.9	12.0	13.4	12.7
RBCs.	2.8	4.4	4.4	5.6
Retics.	10%.	2%.	1%.	3%.
Nuc.RBCs.	4.	0.	0.	0.
A. and P.	++	+	+	+
Target.	+++	+	+	+
Oval.	++	-	+	+
Micro.	++	-	+	-
Frag.	0.45-0.12	0.45-0.24	0.45-0.21	0.45-0.21
Group.	A.	A.	A.	A.
Eyes.	Brown.	Brown.	Hazel.	Brown.
Trait.	Disease.	Yes.	Yes.	Yes.

Sternal marrow of patient's mother. 500 identified cells.

Red series:

Pronormoblasts	8
Early normoblasts	103
Late normoblasts	71
Total	182

White series:

Myeloblasts	0
Promyelocytes	4
Myelocytes	31
Metamyelocytes	122
Neutrophils	108
Lymphocytes	48
Monocytes	3
Eosinophils	2
Total	318

White : Red ratio - 1.75 : 1.

The appearance and differential count were typical of a moderate normoblastic response.

CASE NO. 4. COSTAKIS KORFIOTIS.

History.

The patient was amale child, born on 12 Dec.'45. At the time of examination he was one year ten months old. The family lived in Pallouriotissa, a village about one mile from Nicosia.

The boy was his mother's first child. His father had a son by a previous marriage. The child was born at full term after a normal pregnancy and delivery. His birth weight was not known, but he was of average size. There was no jaundice after birth, and none had occurred since then. Breast feeding was carried on until he was nine months old.

The mother stated that the infant was quite healthy until the age of five months, when he was fevered and vomited his feeds repeatedly over the period of a week. At this time she first noticed that the child was pale. She was emphatic that the child was not pale before this. Since then the child was never well. His appetite diminished, and he vomited occasionally. There were repeated febrile attacks lasting a few days at a time, and these were noted in particular when teeth were cut. The temperature was not taken during these attacks. Though he grew in height he was always thin. He was weak and easily tired, and breathless on the slightest exertion. The mother noticed that his abdomen was prominent when he was thirteen months old, and a diagnosis of Mediterranean Anaemia was made at this time. Stools were dark brown in colour; no diarrhoea had occurred. Mentally the child had always been alert, but had appeared to the father to be anxious. He seldom cried and was far too placid. He did not sleep well. No petechial haemorrhages or bleeding from mucous membranes had occurred. No respiratory or alimentary infections had been recognised. At the age of five months the condition had been diagnosed as malaria, though no blood films had been examined, and no response to quinine therapy occurred.

The first tooth was cut at nine months, and, at the time of examination, all teeth were cut except the second premolars. He had started to talk at the age of eight months, but had made little progress since, and made little effort to talk. He had not sat up until he was over a year old, and could not yet stand without support. He could crawl, but no attempt had been made to walk.

Family history.

The mother had always enjoyed good health. She had an elder brother and an elder sister. Both were well. Her father was alive and well. Her mother was dead, cause unknown. She knew of no relatives who had died from this disease.

p.t.o.

The father had always been healthy. His two sisters and his father and mother were also in good health. His first wife had died, cause unknown. She had borne him a son, who had always been healthy. He knew of no relatives who had suffered from this disease.

The father's occupation was a clerk in the Colonial Government. He was an intelligent man with a good knowledge of English. Their standard of living was above that of the average Cypriot, and there was no dietary deficiency.

Examination.

General. The child was of average height for his age, but was under weight. Height: 30". Weight 20 lbs. The skin and mucous membranes were very pale. The skin was of normal texture, but had a sallow tint. This was probably the normal Mediterranean colouring shown up by the pallor. No petechiae or leg ulcers were seen. There was no icterus of the conjunctivae. A small epicanthic fold was present. Frontal bossing was well marked and the bridge of the nose was slightly depressed. The facies was characteristic of the condition, but could not be called mongoloid. The anterior fontanelle and cranial sutures were fused. There was no cyanosis or clubbing of the fingers. Muscle tone was below normal, and muscular development was well below average. The child looked weak and apathetic and lacking in energy.

Cardiovascular system. Pulse 144 per min., regular in time and force. Volume was poor. The apex was in the anterior axillary line, and percussion also revealed dilatation to the right - 1" to right of sternum in third right interspace. A soft systolic murmur was heard over the precordium, maximal at the apex. It was not propagated to the axilla. No thrills were present. No oedema of ankles or sacrum.

Respiratory system. Breathing was rapid after exercise, but was not laboured. A few coarse crepitations were heard at both bases. No other abnormality was found.

Tongue. Clean and moist. It was pale, but showed no atrophy or undue redness.

Mouth and throat. There was no cheilosis or angular stomatitis. Tonsils were healthy. All teeth were present except the second premolars. There was no dental decay.

Abdomen. There was distension and a small umbilical hernia was present. It could easily be reduced. There was no free fluid in the abdomen. The liver edge was palpable half way between the right costal margin and the umbilicus. It was smooth, regular, firm and not tender. The spleen was palpable in the left flank. It extended downwards and forwards to the level of the umbilicus almost to the midclavicular line. It was firm, smooth and not tender, and its edge had a well defined notch.

p.t.o.

CASE NO. 4. CONTINUED.

Nervous system. No abnormality.
Lymph glands. Not enlarged.
Urine. No protein or sugar. No increase of urobilinogen.
Colour of eyes. Hazel.
Circumference of skull. 19".

Blood examination.

Hb. 2.8 gm.%. R.B.Cs. 1.52 million per cmm.
Reticulocytes. 28%. W.B.Cs. 12,650 per cmm.
Nucleated R.B.Cs. 125 per 100 W.B.Cs. - 27 early and 98 late normoblasts.
Differential W.B.Cs.

Neutrophils	39%.
Stab Cells	9%.
Juveniles	9%.
Lymphocytes	40%.
Monocytes	2%.
Eosinophils	1%.
Basophils	0%.

Blood Group. B.
Fragility. 0.39-0.12 gm. NaCl. %.
Film. There was asevere degree of anisocytosis and poikilocytosis. Polychromasia and basophilic stippling were also present. Normoblasts were present in large numbers. Target cells were seen, but were not numerous; there were several ovalocytes and microcytes. Hypochromia was marked. The translucency of the cells suggested that they were thin. The picture was that of a severe degree of Mediterranean Anaemia.

Bone marrow. From tibial puncture. 500 identified cells.

Red series.

Pronormoblasts	18
Early normoblasts	181
Late normoblasts	215
Total	414

White series.

Myeloblasts	1
Promyelocytes	3
Neutrophil myelocytes	4
Eosinophil myelocytes	1
Neutrophil metamyelocytes	12
Eosinophil metamyelocytes	3
Neutrophils	33
Eosinophils	12
Lymphocytes	15
Monocytes	2
Total	86

White : Red ratio.- 1 : 4.8.

The appearance and differential count were typical of a marked normoblastic response.

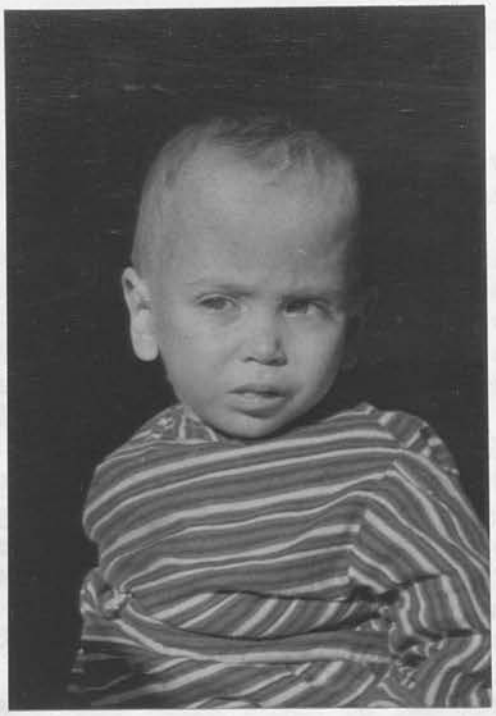

Plate II.
Case No.4. Costakis Korfiotis.
Bossing of the frontal bone and depression of the bridge of the nose are seen.

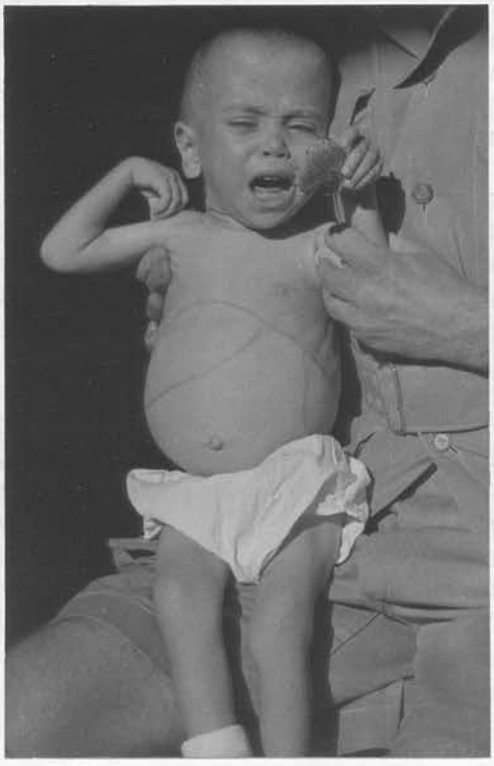

Plate III.
Case No.4. Costakis Korfiotis.
The umbilical hernia, large liver and spleen are seen, and the distended abdomen contrasts with the wasting of the limbs.

CASE NO. 5. STELLA KORFIOTIS.

History.

When Case No. 4 was first seen, it was noted that the mother was pregnant. She was quite healthy during her pregnancy, and was delivered at full term of a female infant weighing 6 lb. Delivery was normal. In view of the possibility that this child might develop Mediterranean Anaemia, opportunities were taken to examine the child whenever possible. The results of four examinations are recorded, and the changes in the blood picture are illustrated.

The child appeared quite normal at birth. She was not pale. Owing to a local superstition that Mediterranean Anaemia might be caused by breast feeding, the child was weaned straight away. No difficulty was experienced in feeding. The child was examined again when it was 85 days old. Up till this time the mother had not noticed anything wrong with the child. She was an intelligent woman, and as one child already suffered from the disease, she knew what to expect.

The next examination was carried out a month later, when the child was 116 days old. By this time the mother had noticed that the child was pale, and that its appetite was failing. It was listless and made no attempt to sit up on its own accord. There had been none of the fever or vomiting which she had noticed with her other child.

A month later, when the child was 158 days old, the mother stated that she was weaker and often appeared short of breath. No attempt had been made to sit up, and she had cut no teeth. Appetite was poor, and her mother thought she was losing weight. Prominence of the abdomen had not been noted.

A week later this patient and her brother developed upper respiratory tract infections. The brother recovered, but the little girl rapidly developed bronchopneumonia and died two days after the onset of her illness. Permission for post mortem examination was not obtained.

Examination.

3 days after birth the infant appeared quite normal. The posterior fontanelle was closed and the anterior fontanelle was of normal size. There was no pallor of skin or mucous membranes. No bossing of the skull was present, and the facies was normal. There was no icterus. The liver edge was palpable just below the costal margin, but not more than would be expected from the age. The spleen was not palpable. The features of the blood examination are tabulated below.

p.t.o.

When 85 days old there was slight pallor of skin and mucous membranes. No alteration in facies or skull was noted. There was no icterus. The liver edge was now palpable about one inch below the right costal margin. The tip of the spleen was palpable. Both organs were firm, regular, smooth and not tender.

At 116 days old there was an increase in the pallor. Early bossing of the frontal bone was suspected, but could not be called definite. The liver edge was now one and a half inches below the right costal margin, and the spleen was two inches below the left costal margin at its most prominent point. No notch could be felt. The character of the organs was as at the previous examination. No icterus was present. The heart rate was 170 per minute. The heart was not enlarged clinically, and there were no murmurs.

At 158 days old the pallor of skin and mucous membranes was very severe. The child looked weak and feeble, and its skin had lost its bloom. Slight icterus of the conjunctivae was noticed. No further changes in skull or facies were noted. The liver and spleen were as at the previous examination. There was no evidence of cardiac enlargement. The posterior half of the anterior fontanelle was now closed, and closure of the anterior half had commenced.

The patient was not seen again before she died. A description of her last illness was obtained from her parents, and it was clear that she succumbed to a descending respiratory infection.

Blood examinations.

	1.	2.	3.	4.
Hb. gm.%.	12.7	6.4	5.4	3.8
RBCs. mill. per cmm.	4.05	2.85	1.72	2.2
Retics.	8%.	12%.	16%.	14%.
Normoblasts - early	0.	27.	152.	125
per late	0.	6.	50.	137.
100wbcs. total	0.	33.	202.	262.
A. and P.	+	++	+++	+++
Target.	+	+	++	+
Oval.	+	+	+	++
Micro.	-	+	++	++
Frag.	Constant - 0.42-0.12 gm. NaCl.%.			
Group				0
Total W.B.Cs.	7,000.	17,400.	24,500.	24,400.
Neutrophils	62%.	20%.	26%.	33%.
Stab Cells	3%.	6%.	3%.	8%.
Juveniles.	5%.	8%.	3%.	7%.
Lymphocytes	18%.	62%.	66%.	39%.
Monocytes	3%.	2%	1%.	3%.
Eosinophils	9%.	1%.	1%.	10%.
Basophils	0%.	1%.	0%.	0%.

Bone marrow. From tibial puncture. 500 identified cells.

Red series.

Pronormoblasts.....	28
Early normoblasts.....	162
Late normoblasts.....	152
Total	342

p.t.o.

CASE NO. 5. CONTINUED.

White series:

Myeloblasts	2
Promyelocytes	8
Neutrophil myelocytes	43
Eosinophil myelocytes	3
Neutrophil metamyelocytes	13
Neutrophils	51
Eosinophils	5
Lymphocytes	28
Monocytes	3
Basophils	2
Total	158

White : red ratio - 1 : 2.2.

The appearance and differential count were typical of a normoblast response.

Family studies:

The following members of the family were examined with the results as tabulated.

1. Mother. Elena Korfiotis. Age 35 years.
2. Father. Thrassos Korfiotis. Age 36 years.
3. Mother's brother. Demetrios Constantinou. Age 34 yrs.
4. Mother's sister. Irini Constantinou. Age 30 years.
5. Mother's father. Costas Demetri. Age 65 years.

	Case 4.	1.	2.	3.
Hb.	2.8	14.8	14.1	13.4
RBCs.	1.52	4.2	5.98	4.95
Retics.	28%.	0.5%.	1%.	1%.
Nuc.RBCs.	125	0	0	0
A. and P.	+++	+	+	+
Target.	++	+	++	++
Oval.	++	+	+	+
Micro.	++	+	+	+
Frag.	0.39-0.12	0.42-0.12	0.33-0.18	0.39-0.24
Group.	B.	O.	B.	O.
Eyes.	Hazel	Brown	Brown	Hazel
Trait.	Disease	Yes	Yes	Yes

	4.	5.	Case 5.
Hb.	13.4	12.7	
RBCs.	4.25	4.2	
Retics.	1%.	0.5%	Already
Nuc.RBCs.	0	0	tabulated.
A. and P.	+	+	
Target.	+	++	
Oval.	+	+	
Micro.	+	+	
Frag.	0.39-0.24	0.39-0.24	
Group	O.	O.	
Eyes.	Hazel	Brown.	
Trait.	Yes.	Yes.	

THE FIFTH FAMILY.

CASE NO. 6. DIMOS IOANNOU.

History.

The patient was a young man aged 31 years. He lived in the village of Kolokoshi, about five miles from Nicosia.

He stated that he had always been fit, and had had no illnesses. He had always led an active life and often cycled from Alithinou, his father's village, to Nicosia, a distance of 33 miles, and the journey had caused him no distress. On the day he was examined he had cycled into Nicosia, and showed no distress.

Twelve years previously he had recurrent ulceration of his legs over his shins, and this took about twelve months to heal up. His general health was not in any way affected.

There had been no attacks of jaundice; no petechiae in the skin. Appetite was good. There was no breathlessness on exertion.

Family history.

The patient had three brothers and three sisters. Two of the brothers were known to have the same disease. The other brother and sisters considered themselves to be healthy. These relatives will be considered in more detail later. One sister had died the day of her birth. One brother had died at the age of twelve from 'anaemia'.

The father, his two brothers and one sister were all well. One sister and one brother had died in late middle age. The cause was unknown. Grandparents also were dead. There had been no other cases of anaemia in the family.

The mother was alive and well. Seven brothers and one sister were alive and well. One brother died at the age of sixty from 'old age'. Two sisters had died aged forty and thirtyfive, cause unknown. She knew of none of her relatives being anaemic. Her father and mother were both dead, cause unknown.

The father was village Mukhtar, local village headman. He was a farmer in an isolated village in the hills. This village will be referred to subsequently, as a survey of the whole village was made. There was no evidence of any dietary deficiency. The son just referred to worked in the NAAFI at one of the military camps, and certainly had a good diet!

Examination.

General. The patient was of average weight, height and build. Pallor of skin and mucous membranes was present. In addition the skin had a muddy appearance. The conjunctivae showed a slight icteric tinge. Texture of the skin was normal, and there were no petechiae.

p.t.o.

Both shins showed pigmented scars of old ulcers. There was slight frontal bossing, but no depression of the bridge of the nose, and no epicanthic fold. The mongoloid facies was not apparent. There was no clubbing of the fingers and no cyanosis. Nails were normal. No oedema of the ankles was present.

Cardiovascular system. The pulse was 80 per min., regular in time and force, and of good volume. B.P. 120/80. The apex beat was in the midclavicular line in the fifth intercostal space. The cardiac impulse was normal. No thrills were present. A soft systolic murmur was heard at the apex, but was not propagated elsewhere.

Respiratory and nervous systems. No abnormality.

Tongue. Clean and moist. No atrophy or undue redness.

Mouth and throat. No cheilosis or angular stomatitis. Tonsils healthy. Teeth satisfactory.

Abdomen. Not prominent. No free fluid present. The spleen was palpable in the left flank. It was three inches below the left costal margin. It was firm, smooth, regular, not tender, and its border had a well-defined notch. The liver was palpable about half an inch below the right costal margin. It was firm, smooth, regular and not tender.

Lymph glands. Not enlarged.

Urine. No protein or sugar. Slight increase of urobilinogen.

Circumference of skull. $21\frac{3}{4}$".

Colour of eyes. Brown.

Blood examination.

Hb. 9.5 gm.%. R.B.Cs. 4.2 million per cmm.
Reticulocytes. 9%. W.B.Cs. 5,100 per cmm.
P.C.V. 37%. M.C.V. 88.1 cu. microns.
M.C.H.C. 25.7%. Nucleated R.B.Cs. 2 per 100 W.B.Cs. - 1 early and 1 late normoblast.
Differential W.B.Cs.

Neutrophils	60%.
Stab Cells	4%.
Juveniles	5%.
Lymphocytes	28%.
Monocytes	3%.
Eosinophils	0%.
Basophils	0%.

Blood group. O. Rhesus positive.
Fragility. 0.51-0.18.
Film. There was severe anisocytosis and poikilocytosis. Ovalocytes and microcytes were seen, and target cells were numerous. Basophilic stippling and polychromasia were noted. Marked hypochromia was present. Normoblasts were scanty. The picture was that of Mediterranean Anaemia of moderate severity.

Sternal marrow. 500 identified cells.
Red series.

Pronormoblasts	28
Early normoblasts	226
Late normoblasts	114
Total	368

p.t.o.

White series.

Myeloblasts	0
Promyelocytes	4
Neutrophil myelocytes	18
Neutrophil metamyelocytes	24
Neutrophils	52
Eosinophils	1
Lymphocytes	30
Monocytes	3
Total	132

White : red ratio - 1 : 2.8.

The appearance and differential count were typical of a moderate normoblastic response.

CASE NO. 7. CHARALAMBOS IOANNOU.

History.

The patient was a young man aged 25 or 26; he did not know which. He lived in the village of Kolokoshi, about five miles from Nicosia. He worked in the NAAFI of a military camp there.

He was unable to give an accurate history, and could not vouch for the details of his ea rly life. So far as he knew he had been fit up to the age of five years. At that time he had a bout of fever lasting about six weeks. The cause was unknown. He was said to have had an attack of jaundice in infancy, but he knew nothing of this; there had been no attacks since.

Since the age of 7 or 8 he had never been well. He complained that he was weak and easily tired. This prevented him from playing games with other children. He was told that he had an enlarged spleen at that age. There had been no pain or discomfort in connection with this spleen. He had been pale as long as he could remember. Pallor had been persistent, and had not varied much. There had always been breathlessness on exertion.

Appetite and digestion were good. Bowels were regular; motions were normal colour. There was no upset of micturition. He slept well; no headaches. There were no motor or sensory symptoms.

He had had recurrent ulcers on the legs, which had been slow to heal. There had been no petechiae or bleeding from mucous membranes. No swelling of the ankles had occurred. There had been no respiratory, alimentary or other infections. Since his attack of fever at the age of five, he had only been febrile on one occasion at the age of twentyfour; this attack lasted only for a few days. For this he had been treated with twelve injections of quinine.

p.t.o.

Family history.

This has already been given. The patient was the brother of cases Nos. 6 and 8.

Examination.

General. The patient was of average height and weight. Development was normal. There was pallor of both skin and mucous membranes. The skin was of a sallower tinge than is normal for the Cypriot race. The conjunctivae showed a mild icteric tinge. Texture of the skin was normal. There were no petechiae. Both shins showed pigmented scars of old ulcers. There was no epicanthic fold, no frontal bossing or depression of the bridge of the nose. There was no clubbing of the fingers or cyanosis. Nails were normal. No oedema of the ankles was seen.

Cardiovascular system. Pulse 82 per min., regular in time and force, and of good volume. B.P. 115/65. The apex beat was in the fifth space, just outside the midclavicular line. No thrills were present. Cardiac impulse was normal. A soft systolic murmur was heard at all areas, but was maximal at the apex.

Respiratory and nervous systems. No abnormality.

Tongue. Clean and moist. No atrophy or undue red-
Mouth and throat. No cheilosis or angular ness.
stomatitis. Tonsils healthy. Teeth satisfactory.

Abdomen. It was prominent. No free fluid was present. The spleen was palpable in the left hypochondrium. It extended down to 2" above the iliac crest, and forwards to 1" from the umbilicus. It was firm, smooth, regular and not tender. A well-defined notch was present. The liver was palpable 2" below the right costal margin in the midclavicular line. The edge was firm, smooth and not tender.

Lymph glands. Not enlarged.

Urine. No protein or sugar. No increase of urobilinogen.

Circumference of skull. 21½".

Colour of eyes. Brown.

Blood examination.

Hb. 8.5 gm.%. R.B.Cs. 4.15 million per cmm.
Reticulocytes. 11%. W.B.Cs. 6,150 per cmm.
Nucleated R.B.Cs. 4 per 100 W.B.Cs. - 1 early and 3 late normoblasts.
P.C.V. 30%. M.C.V. 72.8 cu. microns.
M.C.H.C. 28.2%.
Differential W.B.Cs.

Neutrophils	48%.
Stab Cells	5%.
Juveniles	5%.
Lymphocytes	41%.
Monocytes	0%.
Eosinophils	1%.

Fragility. 0.45-0.15 gm. NaCl.%.
Blood group O. Rhesus positive.

p.t.o.

Film. A moderately severe degree of anisocytosis and poikilocytosis was present. Target cells, ovalocytes and microcytes were seen. Polychromasia and basophilic stippling were also noted. Hypochromia was noted owing to the peripheral staining of the cells. There were, however, a number of well-filled cells of normal shape and size. Normoblasts were present, but not numerous. The picture was that of Mediterranean Anaemia of mild degree.

Sternal marrow. 500 identified cells.

Red series.

Pronormoblasts	22
Early normoblasts	237
Late normoblasts	102
Total	361

White series.

Myeloblasts	1
Promyelocytes	3
Neutrophil myelocytes	22
Neutrophil metamyelocytes	40
Neutrophils	30
Eosinophils	5
Basophils	1
Lymphocytes	34
Monocytes	3
Total	139

White : red ratio - 1 : 2.6.

The appearance and differential count were typical of a moderate normoblastic response.

CASE NO. 8. PAVLOS IOANNOU.

History.

The patient was a youth aged thirteen years. He lived in Nicosia.

His parents stated that he had been pale since the age of one year, when their doctor told them that his spleen was at fault. Though weak he had always played games at school, though he was not as good as the other children. He was quite a good scholar, and well able to compete with others of his age.

Several bouts of fever had occurred. They were not severe, and lasted about a week at a time. He had not been jaundiced at such times. It had been noticed that he had a slight icteric tinge, and that this varied from time to time, but this variation was not related to his febrile attacks. Shortness of breath occurred on exertion, though he could walk two miles slow ly without distress.

Appetite and digestion were good. Bowels were regular; motions were normal in colour. There was no upset in micturition. He slept well and had no headaches. There were no motor or sensory symptoms.

p.t.o.

CASE NO. 8 CONTINUED.

Family history.
This has already been given. The patient was the younger brother of cases Nos. 6 and 7.

Examination.
General. The patient looked younger than his years. He had the build of a boy of ten. His skin was pale and had a sallow tint. Mucous membranes were pale and the conjunctivae were mildly icteric. Texture of the skin was normal. There were no petechiae. Large non-pigmented scars were present on both shins. There was no epicanthic fold, no frontal bossing, and no depression of the bridge of the nose. There was no clubbing of the fingers, and no cyanosis. Nails were normal. No oedema of the ankles was present.

Cardiovascular system. Pulse 100 per minute, regular in time and force, and of good volume. B.P. 120/80 mm.Hg. The apex beat was in the fifth intercostal space, just outside the midclavicular line. Normal cardiac impulse; no thrill present. A soft systolic murmur was heard at all areas; it was not propagated to the axilla.

Respiratory and nervous systems. No abnormality.

Tongue. Clean and moist. No atrophy or undue red-
Mouth and throat. No cheilosis or angular (ness. stomatitis. Tonsils healthy. Teeth satisfactory.

Abdomen. It was protruberant. There was no free fluid present and no umbilical hernia. The spleen was enlarged, downwards to the level of the iliac crest, and forwards to one inch to the left of the umbilicus. It was firm, smooth, regular and not tender. A well-defined notch was present. The liver was palpable two inches below the right costal margin. The edge was firm and smooth, but not tender.

Lymph glands. Not enlarged.

Urine. No protein or sugar. Urobilinogen was increased.

Fundi. Normal.

Circumference of skull. 21".

Colour of eyes. Brown.

Blood examination.

Hb. 4.5 gm.%. R.B.Cs. 2.8 million per cmm.
Reticulocytes 8%. W.B.Cs. 7,100 per cmm.
Nucleated R.B.Cs. ~~m~~ 2 per 100 W.B.Cs. - 1 early and 1 late normoblast.
P.C.V. 17%. M.C.V. 60.7 cu. microns.
M.C.H.C. 25.8%.
Fragility. 0.48-0.24 gm. NaCl.%.
Blood group.A. Rhesus positive.
Differential W.B.Cs.

Neutrophils.....	60%.
Stab Cells.....	1%
Juveniles.....	1%
Lymphocytes.....	28%.
Monocytes.....	6%.
Eosinophils.....	3%.
Basophils.....	1%.

p.t.o.

CASE NO. 8. CONTINUED.

Film. Anisocytosis and poikilocytosis of moderate severity were present. Target cells were numerous, and ovalocytes and microcytes were also seen. Polychromasia and basophilic stippling were noted. Normoblasts were infrequent. The cells were very hypochromic. The picture was that of a moderately severe case of Mediterranean Anaemia.

Sternal marrow. 500 identified cells.

Red series.

Pronormoblasts	17
Early normoblasts	180
Late normoblasts	173
Total	370

White series.

Myeloblasts	0
Promyelocytes	1
Neutrophil myelocytes	9
Neutrophil metamyelocytes	39
Neutrophils	55
Eosinophils	2
Basophils	1.
Monocytes	2
Lymphocytes	21
Total	130

White : red ratio - 1 : 2.9.

The appearance and differential count were typical of a moderate normoblastic response.

Family studies.

The following members of the family were examined and the results are appended:

1. Brother. Charilaous Ioannou.	Age 17 years.
2. Sister. Sofia Ioannou.	Age 24 years.
3. Sister. Loukia Ioannou.	Age 20 years.
4. Sister. Kyriakou Ioannou.	Age 12 years.
5. Wife of D.I.Eleni Christodoulou.	Age 25 years.
6. Daughter of D.I.Iannoula Dimos.	Age 3 years.
7. Daughter of D.I.Maroula Dimos.	Age 10 months.
8. Mother. Andreanna Ioannou.	Age 50 years.
9. Father. Ioannis Nicola.	Age 54 years.

Sternal marrow of 1. Charilaous Ioannou.

500 identified cells.

Red series.

Pronormoblasts	1
Early normoblasts	97
Late normoblasts	43
Total	141

White series.

Myeloblasts	2
Promyelocytes	7
Neutrophil myelocytes	30
Eosinophil myelocytes	4
Neutrophil metamyelocytes	93
Eosinophil metamyelocytes	10
Neutrophils	125

p.t.o.

Eosinophils	5
Lymphocytes	76
Monocytes	7
Total	359

White : red ratio - 2.6 : 1.

The appearance and differential count were quite normal.

	Case 6.	Case 7.	Case 8.	1.
Hb.	9.5	8.5	4.5	12.1
RBCs.	4.2	4.15	2.8	5.1
Retics.	9%.	11%.	8%.	3%.
Nuc.RBCs.	2.	4	2	0
A. and P.	+++	+++	+++	+
Target.	++	+	++	+
Oval.	+	+	++	+
Micro.	+	+	+	+
Frag.	0.51-0.18	0.45-0.15	0.48-0.24	0.48-0.27
Group.	0.	0.	A.	0.
Rhesus.	+	+	+	+
Eyes.	Brown	Brown	Brown	Brown
Trait.	Disease	Disease	Disease	Yes

	2.	3.	4.	5.
Hb.	14.0	12.7	12.7	13.3
RBCs.	4.45	4.37	4.2	4.25
Retics.	1%	1%	0.5%	0.5%
Nuc.RBCs.	0.	0.	0.	0.
A.and P.	-	+	-	-
Target.	-	+	-	-
Oval.	-	+	-	-
Micro.	-	+	-	-
Frag.	0.45-0.30	0.45-0.24	0.45-0.30	0.45-0.33
Group.	0.	A.	0.	A.
Rhesus	+	+	+	
Eyes.	Brown	Brown	Brown	Brown
Trait.	No	Yes	No	No

	6.	7.	8.	9.
Hb.	12.2	11.1	13.5	14.0
RBCs.	3.79	4.6	4.33	4.67
Retics.	3%	1%	1%	3%
Nuc.RBCs.	0.	0.	0.	0.
A.and P.	+	+	+	+
Target.	+	+	+	+
Oval.	+	+	+	+
Micro.	+	+	+	+
Frag.	0.45-0.27	0.45-0.24	0.45-0.24	0.45-0.27
Group.	0.	A.	0.	A.
Rhesus.			+	+
Eyes.	Brown	Brown	Brown	Brown
Trait.	Yes	Yes	Yes	Yes.

CASE NO. 9. ANDREAS BARNABA.

History.

The patient was a boy, eight years old. He was born on 15 Aug. 1938. He was the youngest child in the family. He was born at full term following a normal pregnancy and delivery. He was of average size at birth, though his birth weight was not known. No jaundice was noted after his birth, and there had been none since. He had been breast fed.

At the age of about five months the mother noticed that he was pale, and that his abdomen was prominent. Feverish attacks also occurred. As he had commenced to cut his teeth at this time his symptoms were attributed to this cause. At this time his doctor noticed that his spleen was enlarged. The febrile attacks lasted up till the age of three years, and did not recur. They lasted a few days at a time. The mother could not be sure how frequent they were.

Frontal bossing was first noticed by his mother at the age of three years. It gradually became more prominent. His mother stated that he was easily tired and was breathless on exertion. Mentally he was quite bright and was able to keep up with the boys in his class at school.

His appetite was good, and digestion normal. Bowels were regular. No upset of micturition. No headaches. No petechial haemorrhages or bleeding from mucous membranes had occurred. There had been no ulceration of the ankles or shins and no swelling of the ankles.

In the winter months he had been more subject to colds than his healthy brothers and sisters. He had had no serious illnesses.

Family history.

The mother had always been healthy. She had two brothers alive and well. Her parents were dead, cause unknown. In addition to the patient, the mother had five healthy children - two daughters and then three sons. Two daughters had died aged two months and three months, cause unknown. Both these were born after the first son. Two abortions had occurred - one after the first daughter, and the other before the patient was born, after the third son.

The father was alive and well. He had two brothers and two sisters, all alive and well. His parents were dead, cause unknown. There was no history of anaemia in the family.

The parents lived in Pallouriotissa, a village about one mile from Nicosia. They were peasant farmers. Their diet appeared quite satisfactory.

p.t.o.

CASE NO. 9. CONTINUED.

Examination.

General. The patient was of average height for his age. Apart from his prominent abdomen, he was of slender build, though he was not wasted. Skin was pale and sallow. Mucous membranes were pale. There was no icterus of the conjunctivae. There was depression of the bridge of the nose, and marked bossing of the frontal bone. The parietal bones also showed marked bossing. This gave the appearance of depression of the suture lines. This feature was most striking. A slight epicanthic fold was present. The facies was typical of the condition, and in this case a 'spot diagnosis' was made on seeing the child in the street. There was no cyanosis and no clubbing of the fingers. Nails were normal. No ulceration of the shins was seen, and no scars were present. There was no oedema of the ankles.

Cardiovascular system. Pulse was regular in time and force; rate 96 per minute. B.P. 110/70 mm. Hg. Apex was in the fifth left intercostal space ½" outside the midclavicular line. The cardiac impulse was forceful. There was visible pulsation of the carotid vessels in the neck. A soft systolic murmur was heard at the apex. It was not propagated.

Respiratory system. The chest was increased in its antero-posterior diameter, with beading of the sternum. This was not marked. No other abnormality.

Tongue. Clean and moist. Papillae well developed.

Mouth and throat. No cheilosis or angular stomatitis. Many carious teeth of first dentition were present. Tonsils healthy.

Abdomen. This was very protruberant; no umbilical hernia was present. No free fluid was present. The liver was palpable 2½" below the right costal margin. The spleen was palpable in the left flank; it extended down to the level of the umbilicus, and forwards to the midclavicular line. Both organs were firm, smooth, regular and not tender. No notch could be felt on the spleen.

Nervous system. No abnormality.

Lymph glands. A few small glands were felt in the neck. These were not considered abnormal.

Urine. No protein or sugar. Urobilinogen was increased.

Circumference of skull. 21".

Colour of eyes. Brown.

Blood examination.

Hb. 4.5 gm.%. R.B.Cs. 1.83 million per cmm.
Reticulocytes 15%. W.B.Cs. 6,400 per cmm.
Nucleated R.B.Cs. 20 per 100 W.B.Cs. - 13 early and 7 late normoblasts.
P.C.V. 17%. M.C.V. 92.9 cu. microns.
M.C.H.C. 26.2%.
Fragility. 0.45-0.21 gm. NaCl.%.
Blood group. O.

p.t.o.

CASE NO. 9. CONTINUED.

Differential W.B.Cs.

Neutrophils	46%.
Stab Cells	3%.
Juveniles	3%.
Lymphocytes	40%.
Monocytes	4%.
Eosinophils	3%.
Basophils	1%.

Film. Severe anisocytosis and poikilocytosis were present with many macrocytes and microcytes. Ovalocytes and target cells were also numerous. Peripheral staining was evidence of hypochromia. Polychromasia and basophil stippling were also noted. Many normoblasts were seen. Despite this bizarre picture there were a number of normal looking cells. The picture was that of a moderately severe degree of Mediterranean Anaemia.

Sternal marrow. 500 identified cells.

Red series.

Pronormoblasts	19
Early normoblasts	153
Late normoblasts	227
Total	399

White series.

Myeloblasts	2
Promyelocytes	7
Neutrophil myelocytes	11
Neutrophil metamyelocytes	22
Neutrophils	25
Eosinophils	3
Basophils	0
Lymphocytes	28
Monocytes	3
Total	101

White : red ratio - 1 : 4.

The appearance and differential count were typical of a moderately pronounced normoblastic response.

Family studies.

The following members of the family were examined, and the results are appended:

1. Brother. Vassus Barnaba. Age 20 years.
2. Brother. Kyriacou Barnaba. Age 12 years.
3. Brother. Thrassovoulos Barnaba. Age 9 years.
4. Sister. Maria Barnaba. Age 24 years.
5. Sister. Elenia Barnaba. Age 22 years.
6. Father. Barnabus Kyriakou. Age 45 years.
7. Mother. Orania Barnaba. Age 47 years.
8. Father's sister. Eftikia Papakyriakou. Age 46 yrs.
9. Mother's brother. Aristodinius Spannou. Age 51 yrs.

p.t.o.

CASE NO. 9. CONTINUED.

Patient.	1.	2.	3.
Hb.	4.5	11.4	10.8	10.2
RBCs.	1.83	4.78	4.2	4.56
Retics.	15%.	2%.	3%.	2%.
Nuc.RBCs.	20	0	0	0
A. and P.	+++	+	+	+
Target.	++	+	-	+
Oval.	+	+	+	+
Micro.	+	+	+	+
Frag.	0.45-0.21	0.45-0.21	0.45-0.27	0.45-0.24
Group.	0.	0.	0.	0.
Eyes.	Brown	Hazel	Brown	Brown
Trait.	Disease.	Yes.	Yes.	Yes.

	4.	5.	6.	7.
Hb.	10.8	12.1	11.4	10.8
RBCs.	4.52	4.9	4.3	4.36
Retics.	0.5%.	2%.	2%.	2%.
Nuc.RBCs.	0.	0.	0.	0.
A. and P.	+	-	+	+
Target.	-	-	-	+
Oval.	-	-	+	-
Micro.	-	-	+	+
Frag.	0.48-0.33	0.48-0.33	0.45-0.24	0.42-0.21
Group.	A.	A.	0.	A.
Eyes.	Brown	Brown	Hazel	Brown
Trait.	No.	No.	Yes.	Yes.

	8.	9.
Hb.	12.1	12.7
RBCs.	4.55	5.4
Retics.	0.5%.	0.5%.
Nuc.RBCs.	0.	0.
A. and P.	-	-
Target.	-	-
Oval.	-	-
Micro.	-	-
Frag.	0.48-0.27	0.48-0.27
Group.	0.	A.
Eyes.	Brown	Hazel
Trait.	No.	No.

Plates IV, V, and VI.

Case No.9. Andreas Barnaba.

The frontal and parietal bossing with the outlining of the suture lines, and the depression of the bridge of the nose are seen. There is also protruberance of the abdomen with enlargement of the liver and spleen.

Case No. 10. LOUIS LOIZOU.

History.

The patient was a young man aged 32 years. He lived in Limassol. He was the manager of the Adelphotis Motor Company.

From childhood his complexion had always been pale. At school there was no limitation of activity, and he was able to play football with the other children. He was rather backward at lessons, but attended the secondary school for a time. He stated, however, that he was easily tired on exertion. Appetite had always been good.

At the age of 15 he had an attack of fever, which was treated as malaria. At the age of 20 it was first noticed that his spleen was enlarged, and this increased gradually. The effect of anti-malaria therapy on this splenic enlargement was uncertain. At the age of 25, on account of weakness and pallor, he was advised to go to Athens for splenectomy. It was decided, however, to delay operation until the results of medical treatment had been assessed.

In 1941, at the age of 27, he was admitted to the Cyprus Mines Corporation Hospital at Pendayia for treatment of a wound on the leg, close to the site of an old osteomyelitis. The wound failed to heal for two and a half months. It was decided to perform a splenectomy, and ten days after this operation his leg healed rapidly.

Following the splenectomy the patient had felt stronger. Dr. Fawdry, who gave me the past blood examination reports, stated that his condition had shown slight improvement, but there had been no dramatic improvement. The icterus, after a preliminary increase, diminished, but never disappeared completely.

His past history included an empyema, which was treated by rib resection in 1925. He had an operation for osteomyelitis of his right leg in 1928.

Family history.

The patient was married. His wife and two children were alive and well. Nine brothers and sisters were also in good health. His father and mother were both alive and well. So far as the patient knew, no relative had ever suffered from anaemia.

Examination.

General. The patient was of average height and weight. Weight was 10 stone 12 lbs. Slight pallor of skin and mucous membranes was present. The skin was sallow, and his conjunctivae had a mild icteric tint. No petechiae were present. Scars were seen on both shins, otherwise the skin was normal in texture. The facies was not abnormal and there was no bossing of the frontal bone. There was no clubbing of the fingers and no cyanosis. Nails were normal. There was no oedema of the ankles.

p.t.o.

Case No. 10 Continued.

Cardiovascular system. Pulse 80 per minute, regular in time and force, and of good volume. B.P. 125/85 mm. Hg. The apex was in the fifth left interspace in the midclavicular line. Cardiac impulse was normal. No thrills were present. There were no murmurs.

Respiratory and nervous systems. No abnormality.

Tongue. Clean and moist. No atrophy of papillae.

Mouth and throat. No cheilosis or angular stomatitis. Tonsils healthy. Teeth satisfactory.

Abdomen. There was no distension. A well healed left paramedian scar was present. No free fluid was present. The liver edge was palpable one and a half inches below the right costal margin. It was smooth, regular, firm and not tender.

Lymph glands. Not enlarged.

Urine. No protein or sugar. There was an increase of urobilinogen.

Colour of eyes. Brown.

Blood examinations.

28 Feb.'41. R.B.Cs. 5.5 million per c.mm. Hb. 11 gm.% Normoblasts present in the blood film.

7 Mar.'41. Splenectomy performed.

10 Apr.41. R.B.Cs. 4.3 million per c.mm. Hb. 11.7 gm.%. Normoblasts 12,400 per c.mm.

30 Aug.42. R.B.Cs. 5.5 million per c.mm. Hb. 12.4 gm.%. Normoblasts 36,500 per c.mm.

15 Oct.43. R.B.Cs. 5.9 million per c.mm. Hb. 12.4 gm.%. Normoblasts 28,500 per c.mm. W.B.Cs. 6,400 per c.mm.

Neutrophils	34%.
Lymphocytes	44%.
Monocytes	17%.
Eosinophils	4%.
Basophils	1%.

Fragility test.

2 Feb.41. Haemolysis began at 0.50%, complete 0.1%. Van den Bergh reaction: Positive indirect.

2 Nov.46.

Hb. 12.0 gm.%. R.B.Cs. 4.55 million per cmm. Reticulocytes 29%. W.B.Cs. 6,600 per cmm. Nucleated R.B.Cs. 488 per 100 W.B.Cs. - 168 early and 320 late normoblasts.

P.C.V. 44%. M.C.V. 96.7 cu. microns.

M.C.H.C. 27.3%.

Fragility. 0.48-0.09 gm. NaCl.%.

Differential W.B.Cs.

Neutrophils	42%.
Stab Cells	1%.
Juveniles	3%.
Lymphocytes	46%.
Monocytes	4%.
Eosinophils	3%.
Basophils	1%.

p.t.o.

Case No. 10 Continued.

Blood group. O.
Film. There was marked anisocytosis and poikilocytosis with both microcytes and macrocytes. Target cells and ovalocytes were numerous, but the most prominent feature of the film was the large number of normoblasts. The cells were hypochromic and many pale flattened cells were seen. Polychromasia and punctate basophilia were present. The picture was that of Mediterranean Anaemia of mild degree.

Sternal marrow. 500 identified cells.

Red series.

Pronormoblasts	5
Early normoblasts	59
Late normoblasts	300
Total	364

White series.

Myeloblasts	2
Promyelocytes	4
Neutrophil myelocytes	11
Eosinophil myelocytes	1
Neutrophil metamyelocytes	40
Eosinophil metamyelocytes	5
Neutrophils	42
Eosinophils	5
Basophils	1
Lymphocytes	18
Monocytes	7
Total	136

White : red ratio - 1 : 2.7.

The appearance and differential count were typical of a moderate normoblastic response.

Family studies.

The following members of the family were examined and the results are appended.

1. Wife. Frossoula Loizou. Age 34 years.
2. Brother. Georgiou Loizou. Age 20 years.
3. Sister. Lida Loizou. Age 22 years.
4. Mother. Dora Loizou. Age 55 years.
5. Father. Paraskevos Loizou Age 66 years.

	Patient.	1.	2.
Hb.	12.0	12.7	13.2
RBCs.	4.55	4.35	4.8
Retics.	29%.	1%.	1%.
Nuc.RBCs.	488.	0.	0.
A. and P.	++	-	+
Target.	++	-	-
Oval.	++	-	+
Micro.	++	-	+
Frag.	0.48-0.09	0.45-0.33	0.42-0.27
Group.	O.	A.	O.
Eyes.	Brown.	Brown.	Brown.
Trait.	Disease.	No.	Yes.

p.t.o.

Case No. 10 Continued.

	3.	4.	5.
Hb.	13.4	13.4	15.5
RBCs.	4.65	4.77	5.65
Retics.	1%.	1%.	2%.
Nuc.RBCs.	0.	0.	0.
A. and P.	+	+	+
Target.	-	+	+
Oval.	++	+	+
Micro.	+	-	+
Frag.	0.42-0.24	0.42-0.24	0.42-0.21
Group.	A.	A.	O.
Eyes.	Brown	Hazel	Hazel
Trait.	Yes	Yes	Yes

THE EIGHTH FAMILY.

Case No. 11. STELLAKIS DIKAIOS.

History.

The patient was a male child born on 13 Oct. 1944. He was aged two years when examined. The family lived in Ayios Domedros, a village about 3 miles from Nicosia.

The boy was the parent's first child. He was born at full term following a normal pregnancy and delivery. He was of average size at birth. Breast feeding had been continued until the age of ten months.

The mother stated that the child had been healthy until the age of six months. During the next three months attacks of fever occurred. The mother could not be very precise about them. They were never severe. They lasted about a day and recurred every few days. At the age of ten months, the abdomen was noticed to be prominent and the child pale. Appetite was poor and the mother said the child did not gain weight, though he was not in fact weighed. No more febrile attacks were noted after the age of thirteen months, and the appetite improved. Since then the child made better progress.

There had been no jaundice after birth and none since then. No petechial haemorrhages had occured, and there had been no bleeding from mucous membranes. Digestion had been good, and during the second year of life the appetite had been good.

The first tooth had been cut at the age of seven months, and the first dentition had now been completed. He had started to walk at twentyone months and could walk quite well at the time of examination. He had

p.t.o.

started to talk at the age of nineteen months. Though he never spoke much, he was quite alert and mental development was not retarded.

Apart from the febrile attacks, which were of obscure origin, there had been no recognisable respiratory or alimentary infections.

Family history.

The mother had always enjoyed good health. She had had no miscarriages. Two sisters and two brothers were alive and well, as were also both his parents.

The father was alive and well. He was receiving antiluetic therapy. He had one brother, who was in good health, and one sister, who was ill. The cause of her illness was not known, but she had never been anaemic. Her father died at the age of seventyfive, cause unknown. Her mother was alive and well.

There was no history of any relative being anaemic.

The father was a carpenter. No gross dietary deficiency.

Examination.

General. The child was of average height for his age, but was a little under weight. Height: 31". Weight: 25 lbs. The skin and mucous membranes were pale. The skin was of normal texture, but was more sallow than is normal in the Mediterranean races. No icterus of the conjunctivae was present. A slight epicanthic fold was present. Very slight frontal bossing was present, and the bridge of the nose was depressed. The facies was characteristic of the condition. No cyanosis or clubbing of the fingers was present. Muscle tone was normal and muscular development fair. The patient was a lively alert child.

Cardiovascular system. Pulse 100 per minute, regular in time and force and of good volume. The apex was just outside the midclavicular line in the fourth left intercostal space. A soft systolic murmur was heard at the apex. It was not propagated to the axilla. No thrills were present. There was no oedema of the ankles or sacrum.

Respiratory and nervous systems. No abnormality.

Tongue. Clean and moist. No atrophy or undue redness.

Mouth and throat. No cheilosis or angular stomatitis present. Tonsils were healthy. Teeth were satisfactory.

Abdomen. This was prominent and a small umbilical hernia was present. No free fluid was detected. The liver was palpable half way between the right costal margin and the umbilicus. The spleen was palpable one inch below the left costal margin. In both cases the edge was smooth, regular, firm and not tender.

Lymph glands. Not enlarged.

Urine. No protein or sugar. Increase in urobilinogen was noted.

Colour of eyes. Brown.

p.t.o.

Case 11. Continued.

Blood examination.
Hb. 6.6 gm.%. R.B.Cs. 2.55 million per cmm.
Reticulocytes. 10%. W.B.Cs. 14,900 per cmm.
Nucleated R.B.Cs. 18 per 100 W.B.Cs. - 10 early and 8 late normoblasts.
Differential W.B.Cs.

Neutrophils	46%.
Stab Cells	1%.
Juveniles	10%.
Lymphocytes	46%.
Monocytes	3%.
Eosinophils	1%.
Basophils	1%.

Blood group. A.
Fragility. 0.39-0.18 gm. NaCl.%.
Film. There was a severe degree of anisocytosis and poikilocytosis. Target cells, ovalocytes and microcytes were seen. Many of the target cells were macrocytic. A moderate degree of hypochromia was present. Normoblasts were seen, many of them being very early forms. In the white series juveniles were prominent. The picture was that of Mediterranean Anaemia of moderate severity.

Case No. 12. IANNOULA DIKAIOS.

History.
Case No. 11 was examined when the mother was pregnant. She had a normal pregnancy, and was delivered at term of a female infant weighing six and a half pounds. The child was born on 2nd.October, 1946, and it was examined first on 6th. November, 1946. She was seen on two further occasions and the results of these examinations are recorded.

The infant's birth was a normal one. She was 35 days old when she was first examined. At this time the mother had noticed nothing wrong with the child. She had been breast feeding it and the feeds had been taken quite well. It was a contented child and slept well. Weight had been gained.

The next examination was carried out when the infant was 71 days old. The mother was still satisfied with her infant's progress, though she had noticed that there was pallor. No difficulty had been experienced with feeding.

The girl was not seen again until she was 151 days old. The mother was still satisfied with the child's progress, and would not have sought medical attention but for the fact that I had specifically asked her to bring the child back to see me. The pallor was quite

p.t.o.

Case No. 12 Continued.

obvious to the mother, but there had been no diminution of appetite, no fever, and the child was quite contented. No teeth had been cut, and no attempt had been made to sit up. It had smiled and its behaviour was regarded as normal for its age.

Examination.

When first seen, the child's general condition was quite good. There was slight pallor, but otherwise nothing abnormal was made out on examination. The liver edge was palpable just below the right costal margin. The spleen was not palpable. There was no abdominal distension. Facies was normal. The posterior fontanelle was closed, and the anterior fontanelle was open and of normal dimensions. The results of the blood examination are tabulated below.

At the age of 71 days definite changes were noted. Pallor had increased. There was no jaundice. The spleen could now be felt one inch below the left costal margin. It was firm, smooth, regular and not tender. No increase in size of liver had occurred.

At the age of 151 days pallor had increased, but the spleen was still the same size. The posterior margin of the anterior fontanelle had commenced to close, but was not in advance of the normal rate. Otherwise the child's condition was as at the previous examination. The colour of the eyes was brown.

Blood examinations.

	1.	2.	3.
Hb. gm.%.	8.9	7.1	6.5
RBCs. mill.per cmm.	3.4	2.8	3.2
Reticulocytes.	7%.		
Normoblasts - early	0.	2.	4.
per late	0.	3.	3.
100 WBCs. total	0.	5.	7.
A. and P.	+	++	+++
Target Cells.	++	++	+
Ovalocytes.	+	+	++
Microcytes.	+	++	++
Fragility.	Constant.	0.36-0.18.	
Group.			O.
Total WBCs.	7,200	9,000	12,900.
Neutrophils.	27%.	17%.	37%.
Stab cells.	0%.	1%.	1%.
Juveniles.	2%.	5%.	4%.
Lymphocytes.	68%.	73%.	54%.
Monocytes.	2%.	2%.	2%.
Eosinophils.	1%.	1%.	2%.
Basophils.	0%.	1%.	0%.

p.t.o.

Case No. 12 Continued.

Bone marrow. From tibial puncture. 500 identified cells.

Red series.

Pronormoblasts	6
Early normoblasts	207
Late normoblasts	175
Total	388

White series.

Myeloblasts	0
Promyelocytes	2
Neutrophil myelocytes	7
Neutrophil metamyelocytes	21
Eosinophil metamyelocytes	3
Neutrophils	41
Eosinophils	3
Basophils	1
Lymphocytes	33
Monocytes	1
Total	112

White : red ratio - 1 : 3.5.

The appearance and differential count were typical of a marked normoblastic response.

Family studies.

The following members of the family were examined and the results are tabulated below.

1. Mother Eli Dikaios. Age 22 years.
2. Father Apostolos Dikaios. Age 26 years.
3. Mother's sister. Polyxeni Georghiou. Age 26 years.
4. Mother's sister. Theodora Krio. Age 10 years.
5. Mother's mother. Irene Krio. Age 55 years.
6. Father's mother. Panayiota Dikaios. Age 57 years.

	Case 11.	Case 12.(3)	1.	2.
Hb.	6.6	6.5	11.3	12.7
RBCs.	2.55	3.2	4.35	4.65
Retics.	10%.		2%.	1%.
Nuc.RBCs.	18.	7.	0.	0.
A. and P.	+++	+++	+	+
Target.	+	+	++	+
Oval.	+	++	+	+
Micro.	+	++	+	+
Frag.	0.39-0.18	0.36-0.18	0.36-0.21	0.39-0.21
Group.	A.	O.	O.	A.
Eyes.	Brown	Brown	Hazel	Brown.
Trait.	Disease	Disease	Yes	Yes

	3.	4.	5.	6.
Hb.	10.6	10.6	10.6	12.0
RBCs.	3.6	4.45	3.9	4.1
Retics.	1%.	2.5%.	1%.	0.5%
Nuc.RBCs.	0.	0.	0.	0.
A. and P.	+	+	+	+
Target.	+	++	+	+
Oval.	+	+	+	+
Micro.	+	+	+	+
Frag.	0.39-0.24	0.36-0.21	0.39-0.24	0.36-0.21
Group.	A.	A.	O.	A.
Eyes.	Hazel	Brown	Brown	Brown
Trait.	Yes.	Yes.	Yes.	Yes.

THE NINTH FAMILY.

Case No. 13. PARASKEVOU CHARALAMBOS.

History.

The patient was a female child aged three years old. The family lived in Massari, a village about 25 miles from Nicosia.

The girl was born at full term following a normal pregnancy and delivery. She appeared quite normal at birth. Breast feeding was continued until the age of seven months.

The infant appeared quite normal until she was seven months old. At this time she had an attack of dysentery and it was noticed that she was pale. This pallor persisted. The mother thought there had been days when the child was febrile, but she could not precise details. Though the child's abdomen was prominent, she could not remember when this developed. Appetite was always good, but the child did not gain weight satisfactorily.

There had been no jaundice after birth, and none since then. No petechial haemorrhages had occurred, and there had been no bleeding from mucous membranes. Digestion had been normal. Since the attack of dysentery the child had always been easily tired, and had been breathless whenever she exerted herself.

The first tooth was cut at the age of six months, and the first dentition was completed by the age of two years. Owing to weakness, the child had not learnt to stand without assistance. She only made feeble attempts to crawl. Her vocabulary consisted of a few simple words, but she did not attempt to talk much. She was a placid child and cried but little.

Apart from the one attack of dysentery, there had been no recognisable infections.

Family history.

The mother had always enjoyed good health. She had three brothers and two sisters alive and well. Her mother was alive and well, but her father had died, cause unknown.

The father was alive and well. His brother and two sisters were also in good health. Both his parents were alive and well.

The patient had two brothers and one sister, all in good health. The parent's first child, a boy, had died from Mediterranean Anaemia at the age of two. A healthy brother and sister then followed in the family, and then a sister died of the same condition, aged two years two months. The patient was the next child and then followed the second healthy brother.

p.t.o.

Case No. 13. Continued.

No other relatives had been anaemic, so far as the parents knew.

The parents were peasant farmers. The father was the village Mukhtar, and the family circumstances were above average. There was no evidence of dietary deficiency.

Examination.

General. The patient was of average height for her age, but was under weight. Height: 31". Weight: 26 lbs. The skin and mucous membranes were very pale. The skin was of normal texture, but had a sallow tint. Slight icterus of the conjunctivae was noted. No petechiae or leg ulcers were seen. Muscle tone and development were much below average, and the child's thin arms and legs formed a contrast to her prominent abdomen. Frontal bossing was present, and there was depression of the bridge of the nose. A slight epicanthic fold was also noted. The facies was characteristic of the condition. The anterior fontanelle and suture lines were closed. There was no depression at the suture lines. There was no cyanosis or clubbing of the fingers. The child was weak, apathetic and lacking in energy. It would sit in its cot quietly, listlessly for hours on end.

Cardiovascular system. Pulse 120 per minute, regular in time and force. Volume was poor. The apex beat was in the fifth intercostal space, just outside the midclavicular line. No murmurs could be heard. There was no oedema of the ankles or sacrum.

Respiratory and nervous systems. No abnormality.

Tongue. Clean and moist, but pale. No atrophy.

Mouth and throat. There was no cheilosis or angular stomatitis. Tonsils were healthy. The first dentition was complete. There was no dental decay.

Abdomen. There was gross distension and a small umbilical hernia was present. There was no free fluid. The spleen was grossly enlarged. It extended downwards as far as the level of the iliac crest, and forwards to the lateral margin of the left rectus sheath. A well defined notch was present. The edge of the liver was felt below the right costal margin, one and a half inches down in the midaxillary line. Both organs were smooth, firm, regular and not tender.

Lymph glands. Not enlarged.

Urine. No protein or sugar. An increase of urobilinogen was present.

Circumference of skull. 17".

Colour of eyes. Brown.

p.t.o.

Case No. 13. Continued.

Blood examination.
Hb. 3.2 gm.%. R.B.Cs. 1.92 million per cmm.
Reticulocytes 24%. W.B.Cs. 17,500 per cmm.
Nucleated R.B.Cs. 167 per 100 W.B.Cs. - 143 early and 24 late normoblasts.
P.C.V. 15%. M.C.V. 78.1 cu. microns.
M.C.H.C. 21.2%.
Fragility. 0.42-0.21 gm. NaCl.%.
Blood group. A. Rhesus positive.
Differential W.B.Cs.

Neutrophils	46%.
Stab cells	2%.
Lymphocytes	44%.
Monocytes	7%.
Eosinophils	1%.

Film. There was marked anisocytosis and poikilocytosis. Ovalocytes and microcytes were present and target cells were also seen. Peripheral staining was evidence of marked hypochromia, and the transparency of the cells suggested that they were very thin. Basophilic stippling and polychromasia were present. Normoblasts, especially the early type, were numerous. The picture was that of a severe case of Mediterranean Anaemia.

Sternal marrow. 500 identified cells.
Red series.

Pronormoblasts	22
Early normoblasts	199
Late normoblasts	194
Total	415

White series.

Myeloblasts	0
Promyelocytes	4
Neutrophil myelocytes	15
Neutrophil metamyelocytes	20
Neutrophils	21
Eosinophils	2
Basophils	2
Monocytes	1
Lymphocytes	20
Total	85

White : red ratio - 1 : 4.9

The appearance and differential count were typical of a marked normoblastic response.

Family studies.

The following members of the family were examined and the results are tabulated below.

1. Mother.	Eleni Charalambos.	Age 30 years.
2. Father.	Charalambos Panayi.	Age 30 years.
3. Patient's sister.	Millon Charalambos.	Age 10 years.
4. Patient's brother.	Michaelagis Charalambos	Age 6 years.
5. Patient's brother.	Andreas Charalambos.	Age 6 months.
6. Father's mother.	Irene Charalambos.	Age 62 years.
7. Father's father.	Georghiou Charalambos.	Age 65 years.

p.t.o.

Plate VII.

Case No. 13. Paraskevou Charalambos.
The facies with the depressed bridge of the nose, and the protruberant abdomen are clearly shown.

Case 13.	1.	2.	3.
Hb. 3.2	12.7	13.4	12.7
RBCs. 1.92	4.7	4.5	4.35
Retics. 24%.	2%.	1%.	1%.
Nuc.RBCs.167.	0.	0.	0.
A. and P.+++	+	+	+
Target. +	+	++	+
Oval. +	+	+	+
Micro. ++	+	+	+
Frag. 0.42-0.21	0.45-0.24	0.42-0.24	0.48-0.27.
Group. A.	A.	A.	A.
Rhesus. +	+		
Eyes. Brown	Brown	Brown	Brown
Trait. Disease	Yes	Yes	Yes

4.	5.	6.	7.
Hb. 12.0	11.3	14.8	13.4
RBCs. 3.77	3.8	5.5	4.11
Retics. 1%.	1%.	1%.	3%.
Nuc.RBCs. 0.	0.	0.	0.
A. and P. +	+	+	+
Target. +	-	+	+
Oval. +	+	+	+
Micro. -	+	+	+
Frag. 0.39-0.24	0.45-0.24	0.42-0.27	0.42-0.27
Group. A.	A.	O.	A.
Eyes. Brown	Brown	Brown	Hazel
Trait. Yes	Yes	Yes	Yes

THE TENTH FAMILY.

Case No. 14. NICOS STEPHANIDES.

History.

The patient was a young man aged 25 years. He lived with his parents in Nicosia. His father owned a silversmith's shop.

He was unable to give an account of himself. Dr. Fawdry informed me that the man had suffered from hebephrenic schizophrenia since 1943. His mother told me that he had been pale all his life, but that his pallor had been more noticeable during the preceding four years. During this time also, it was thought that he had been slightly jaundiced. He had had occasional febrile attacks, but his mother could give no details of these. He was mildly dyspnoeic on exertion, and was easily tired. Appetite was poor, but

p.t.o.

Case No. 14. Continued.

there was no digestive upset. He had never had any ulceration of his legs, and there had been no swelling of his ankles. No petechiae or bleeding from mucous membranes had ever occurred.

At the age of ten he was said to have had rheumatic fever, and at the age of sixteen typhus.

Family history.

The patient had one brother and one sister. Both were in good health. His father and mother were also alive and well. All grandparents were dead. The parents had lost one boy at the age of sixteen months from dysentery. They said that he had never been pale.

Examination.

General. The patient was of average height, but was of asthenic build. Posture was poor. There was slight pallor of both skin and mucous membranes. The skin had a sallow appearance, but this was most probably an exaggeration of the normal racial colour. The conjunctivae showed no icterus. There were no petechiae and no ulceration of the shins. No facial abnormality and no bossing of the frontal bones was present. There was no clubbing of the fingers and no cyanosis. Nails were normal. No oedema of the ankles was seen.

Cardiovascular system. Pulse 90 per minute, regular in time and force and of good volume. B.P. 135/80 mm. Hg. The apex was in the fifth left interspace in the midclavicular line. Cardiac impulse was normal. No thrills were present. No murmurs were heard.

Respiratory and nervous systems. No abnormality.

Tongue. Clean and moist. No atrophy of papillae.

Mouth and throat. No cheilosis or angular stomatitis. Tonsils healthy. Teeth: some carious.

Abdomen. It was not prominent. No free fluid was present. The liver was not palpable. The spleen was palpable one inch below the left costal margin. It was firm, smooth, regular and not tender. Its notch could not be felt.

Lymph glands. Not enlarged.

Urine. No protein or sugar. No increase in urobilinogen.

Circumference of skull. $21\frac{3}{4}$".

Mental condition. The patient was a typical hebephrenic schizophrenic. He was detached from reality, and co-operation was extremely difficult.

Blood examination.

Hb. 13.1 gm.%. R.B.Cs. 5.7 millions per cmm.

The patient refused further examination. I am indebted to Dr. A.L.Fawdry for the following haematological details.

p.t.o.

Case No. 14. Continued.

The film showed anisocytosis and poikilocytosis + , target cells + , and circulating normoblasts + .
Fragility. 0.40-0.15 gm.NaCl.%.

Sternal marrow. 500 identified cells.

Red series.	
Pronormoblasts	2
Early normoblasts	104
Late normoblasts	227
Total	333
White series.	
Myeloblasts	1
Promyelocytes	6
Neutrophil myelocytes	17
Eosinophil myelocytes	4
Neutrophil metamyelocytes	38
Eosinophil metamyelocytes	3
Neutrophils	87
Eosinophils	5
Lymphocytes	6
Monocytes	0
Total	167

White : red ratio - 1 : 2.0.

Family studies.

1.Mother Elengo Stephanides. Age 46 years.
2.Father.Georghiou Stephanides. Age 60 years.
3.Patient's brother. Savvas Stephanides.Age 19 years.

Patient.		1.	2.	3.
Hb.	13.1	14.8	14.0	14.6
RBCs.	5.7	4.86	4.86	5.3
Retics.		0.5%.	0.5%.	0.5%.
Nuc.RBCs	+	0.	0.	0.
A. and P.	+	+	+	+
Target.	+	+	+	+
Oval.		+	+	+
Micro.		+	+	+
Frag.	0.40-0.15	0.45-0.27	0.45-0.27	0.42-0.24
Group.		A.	O.	O.
Eyes.	Hazel	Brown	Hazel	Brown
Trait.	Disease.	Yes	Yes	Yes

EXAMPLES OF THE TRAIT.

Apart from the examples of the trait demonstrated in the families of the cases, three Cypriots showing a severe degree of the trait were examined and submitted to sternal puncture. The results of these investigations are as follows.

No. 1. Charalambos Ioannides.

This man was aged 42 years. He was a L/Corporal serving on the HQ staff of Cyprus Forces. He considered himself a fit and active man. He came under observation as he was examined as one of the hundred controls described later. He had come to the military hospital on account of recurrent ulceration of his legs. Apart from indefinite febrile attacks in his youth - diagnosed as malaria, but without blood examination - he had always been fit.

His father and mother were both dead, cause unknown. He had two brothers and two sisters, all alive and well. He was married. His wife was quite well, and he had two boys, aged eight and six, both in good health. No children had died, and there had been no miscarriages.

On examination, there were no facial changes, and no frontal bossing. The spleen was palpable one and a half inches below the left costal margin. The liver edge could also be felt just below the right costal margin. Both organs were firm, smooth, regular and not tender. Urine showed no increase in urobilinogen. No radiological changes were apparent in the skull, hands or pelvis.

Blood examination.

Hb. 13.7 gm.%. R.B.Cs. 5.25 million per cmm.
Reticulocytes 1%. W.B.Cs. 6,600 per cmm.
No nucleated R.B.Cs. seen.
Fragility. 0.36-0.15 gm. NaCl.%.
Film. A mild degree of anisocytosis and poikilocytosis were seen. Target cells and ovalocytes and many microcytes were present.

Sternal marrow.

Results are tabulated later. There was a normoblastic response of mild degree.

No. 2. Costas Christodoulou.

This man, aged 26 years, was a private in 1022 Coy, Cyprus Regiment. He was a healthy man who came under observation solely on routine examination looking for the trait of Mediterranean Anaemia. He said he had never had any illnesses.

So far as he knew, none of his family had ever been anaemic.

Examination revealed no abnormality. There were no facial or bony changes. Liver and spleen were not enlarged. The urine did not contain excess of urobilinogen.

p.t.o.

Blood examination.
Hb. 14.0 gm.%. R.B.Cs. 5.1 million per cmm.
Reticulocytes 1.5%. W.B.Cs. 7,200 per cmm.
No nucleated R.B.Cs.
Fragility. 0.42-0.21 gm. NaCl.%.
Film. A mild degree of anisocytosis and poikilocytosis were present. Target cells, ovalocytes and microcytes were all seen. There were no malarial parasites.

Sternal Marrow.
A mild normoblastic response was noted. Detailed figures are tabulated later.

No. 3. Theodorou Klitos.

This man, aged 24 years, was a private in 2 Coy., P.T.C., Cyprus Regiment. He came under observation as a result of routine examination, searching for examples of the trait. He stated that he had always been healthy, and that so far as he knew, none of his relatives had ever suffered from any form of anaemia.

General examination was negative. No facial or bony changes. No enlarged liver or spleen. No increase in urine urobilinogen.

Blood examination.
Hb. 12.7 gm.%. R.B.Cs. 4.6 million per cmm.
Reticulocytes 1%. W.B.Cs. 5,600 per cmm.
No nucleated R.B.Cs.
Fragility. 0.39-0.24 gm. NaCl.%.
Film. A mild degree of anisocytosis and poikilocytosis was present. Target cells, ovalocytes, and microcytes were seen.

Sternal marrow.
A mild normoblastic response was noted. Detailed figures are tabulated later.

Sternal Marrows. 500 identified cells.

	1.	2.	3.
Red series.			
Pronormoblasts.....	8	3	2
Early normoblasts.....	143	133	85
Late normoblasts.....	83	110	95
Total.....	234	244	182
White series.			
Myeloblasts.....	3	0	0
Promyelocytes.....	7	2	1
Neutrophil myelocytes.	20	5	12
Eosinophil myelocytes.	5	1	1
Neutrophil metamyelocytes.	71	64	84
Eosinophil metamyelocytes.	4	1	1
Neutrophils.....	122	152	184
Eosinophils.....	8	4	4
Basophils.....	1	1	0
Monocytes.....	2	2	4
Lymphocytes.....	23	24	27
Total.....	266	256	318
Ratio. W.B.Cs. per 1 R.B.C.	1.14	1.05	1.75

SYMPTOMATOLOGY.

Severity of the condition.

The classical features of the disease have been described in the literature on numerous occasions. It is proposed, therefore, to discuss the more interesting points brought out by this study. The fourteen cases described illustrate the different typesof case which may be present. Until 1940 only the severe form of the disease had been recognised. Wintrobe et al.(1940) then described cases of a relatively mild anaemia occurring in adolescents and adults in several Italian families. These cases resembled Mediterranean Anaemia as it was then recognised, but they were much less severe. Their observations have been repeatedly confirmed, and the occurrence of this mild form in Cyprus was reported by Fawdry (1946), who recorded six cases. My impression is that every grade from the mildest to the severest occurs, and the series here described bears this out.

Presenting symptoms.

Pallor and febrile attacks are usually the first recorded symptoms, though fever is by no means constant. I did not have the opportunity to confirm the pyrexia myself, but the symptom is well authenticated. In this series of cases fever was found to be a variable feature. Lapse of time made the histories of patients and their relatives unreliable, but in general it seemed that fever often ushared in the condition, lasted for a number of months, and if the patient survived it did not recur. Unfortunately it was not observed in the two cases followed from birth, else it might have been possible to correlate the fever with the development of the anaemia. These febrile attacks can be explained in either of two ways: a) Fever may be due to an intercurrent infection which increases the anaemia, and draws attention to the condition. Protagonists of the infective theory, particularly the Greek schools, who have supported the malarial etiology, have claimed that this fever is the result of the infection causing the condition. b) Fever may be due to rapid haemolysis precipitating the condition. This is similar to the crises occurring in acholuric jaundice or pernicious anaemia. On this hypothesis febrile attacks occur while the Hb. is falling, and cease when the Hb. is maintained at a fairly constant level. While this latter is the more likely explanation, it is perhaps significant that jaundice was not noticed in any of the febrile attacks recorded in the series of cases. The degree of icterus would be mild, however, and could be easily missed by an uneducated Cypriot whose racial pigmentation would tend to obscure the jaundice.

Pallor was a regular presenting symptom and was recognised by the parents of the patients. It is worth recording that in both the cases followed from birth

p.t.o.

SYMPTOMATOLOGY (Continued).

the mothers had not recognised that pallor was present even though it was very obvious to a trained eye, and blood examination showed a marked anaemia. This slow recognition on the part of the mothers was all the more surprising, since they both already had children suffering from the disease. The age of onset of the disease is usually determined by the recognition of pallor by the mother, and this is clearly open to considerable fallacy.

Protruberance of the abdomen is noticed later than the pallor. Owing to the firmness of the spleen, enlargement of this organ is often recognised by the patient's mother.

Symptoms due to the anaemia.

Most of the symptoms are due to anaemia and are dependent on its degree. Weakness is present to a greater or lesser extent. Costakis Korfiotis, for example, could not stand unaided at the age of two years. General retardation of physical development is a natural sequel. No retardation of dentition and no delay in the appearance of ossific centres was observed. Mental development was not affected, and some of the children seemed unusually bright. Breathlessness on exertion was proportional to the degree of anaemia - it was linked with the cardiac dilatation and high output failure which occurs. Loss of appetite was noticed in the more severe cases.

The characteristic facies.

The development of the characteristic facies is essentially a slow process, linked with the osseous changes. Parents were unable to state when they considered their children's faces to be abnormal, and many did not even recognise the change. The gradual development of the facies occurs at the same time as the radiological changes in the skull are recognised. The facies is by no means constant. It appears to depend partly on the severity of the disease and partly on its duration. It is absent from the milder cases. The term mongoloid facies was introduced into the early descriptions of the disease, and has been retained uncritically ever since. The facies is indeed characteristic, but I feel the term mongoloid should be discarded. The only featuresin common with a mongol are the exaggeration of the epicanthic folds, and the broad bridge of the nose. The eyes, which are the dominant feature in expression, do not resemble those of a mongol at all. They are intelligent and do not have the slope found in mongolism. The shape of the skull is also different. Plates II, IV and VII show typical examples of the facies, and to describe these as mongoloid would be inaccurate.

Ulceration of the legs.

The accounts in the literature make no mention of ulceration of the legs as a feature of the disease.

p.to.

SYMPTOMATOLOGY (Continued).

With the exception of case No. 14, Nicos Stephanides, all the adult cases in the series gave a history of recurrent ulceration of the shins, which had been slow in healing. This was not seen in any of the cases during infancy or childhood. Fawdry (1947) also made this observation. The cause of this symptom is not known, but it is of interest since chronic leg ulcers occur both in acholuric jaundice and sickle cell anaemia, conditions which in many ways are allied to Mediterranean Anaemia. Quite fortuitously Charalambos Ioannides, recorded as an example of the trait, was admitted to the Military Hospital, Nicosia, on account of chronic leg ulcers. This is only one example of leg ulcers and the trait, and there was no opportunity to follow up the problem. It is possible that cases of the trait may show an increased incidence of this complaint. The significance of chronic leg ulceration was only appreciated shortly before I left Cyprus, and so I could not investigate the problem further.

Premature closure of the fontanelle.

Fawdry (1947) also observed premature closure of the anterior fontanelle and he found it was frequently completely ossified when the infant was one year old. The recorded cases were not of suitable age to add evidence to this observation. Case No. 5, Stella Korfiotis, who died at the age of five months, did show closure of her fontanelle greatly in advance of what would be expected for her age.

Other clinical observations.

Splenomegaly and hepatomegaly occurred in all the cases except No. 14, Nicos Stephanides, a mild case in which liver enlargement was absent.

The enlargement of the heart and the systolic murmurs depend on the anaemia. Hunter (1946) discusses this problem of the heart in anaemia and Nemet and Gross (1936) describe the cardiac hypertrophy and dilatation seen post mortem in a case of Mediterranean Anaemia.

No neurological signs were found in any of the cases. Gatto (1939) is the only observer to have recorded neurological signs.

The skeletal and radiological changes are described in a subsequent section, as are also the haematological changes.

The urine showed increase in urobilinogen in the severer cases, but not in the milder cases or in cases of the trait. Ehrlich's aldehyde reagent was used in this test.

Analysis of gastric contents was not done, but other workers have reported them normal.

Mild cases.

There may be no symptoms at all in the mild cases. Case No. 6, Dimos Ioannou, illustrates this. He had always considered himself fit, and his ability to cycle 33 miles without undue distress shows that he had no

p.t.o.

SYMPTOMATOLOGY (Continued).

limitation of exercise tolerance. Despite this he had the characteristic features of the disease. Both he and Case No. 10, Louis Loizou, had married women without the trait, who had given birth to healthy children. The children of Louis Loizou were not examined; those of Dimos Ioannou both showed the trait.

The trait of the disease.

As has been stated, it is very difficult to decide what constitutes the trait, and what the disease itself. Wintrobe (1940) described mild forms of the disease compatible with adult life referring to them as thalassaemia minor to contrast them with thalassaemia major, the severe form found in infancy. He did not state, however, any clear cut differentiation, and some of his mild cases were what are now referred to as the trait. Smith (1943) discussed the diagnosis of the trait, but he did not clarify the differentiation. For the purpose of discussion I shall refer to the trait when there is no anaemia or only mild anaemia and no circulating normoblasts, and to the disease when the anaemia is more severe and circulating normoblasts are seen. This distinction is a purely arbitrary one, since one is dealing with a gradation and not with two distinct entities. Nor is it entirely satisfactory, since under conditions of stress, such as an infection, cases of the trait may become more anaemic and circulating normoblasts may appear. Wintrobe (1940) showed that an injection of adrenaline caused the appearance of normoblasts in the peripheral blood in severe cases of the trait. Pietroni (1946) told me of a case with the haematological features of the trait of the disease, who while suffering from rheumatic fever, became anaemic and circulating normoblasts appeared in the peripheral blood. I was not able to get details of this case. The problem of the trait or mild form of the disease to infections offers considerable scope for further investigation. I did not have the opportunity to follow up this point.

Apart from the haematological examination, the clinical features of the trait were not studied in detail. In general the cases of the trait appeared to be healthy individuals. The leg ulceration shown by Charalambos Ioannides has already been referred to. He also had an enlarged spleen. Routine examination for the spleen was not made on all cases of the trait - it was not practical to disrobe all the Cypriots examined for the trait, but the case just quoted was the only one in which it was found. Fawdry (1947) had occasionally seen an enlarged spleen in cases of the trait, and Smith (1943) did not find it in the families of the cases he examined, but he recorded that it had been observed in two of the relatives at some previous period.

The blood changes will be discussed together with the changes found in the disease.

p.t.o.

SYMPTOMATOLOGY (Continued).

Familial pattern.

The severity of the disease appears to follow a definite familial pattern in the families recorded. Thus in the fourth family, the sister of Case 4, became severely anaemic like her brother. Their Hb. was 2.8 and 3.8 gm.% respectively. In the eighth family a similar state of affairs was noted. Cases 11 and 12 had Hb. levels of 6.6 and 6.5 gm.% respectively. In the fifth family two cases had Hb. levels of 9.5 and 8.5 gm.% respectively, but the third case did not follow this pattern and had a Hb.of 4.5 gm.%. In the ninth family case 13 had a Hb. of 3.2 gm.%. and a brother had died previously of this condition at the age of two years; presumably this had also been a severe case. It will be noted that both the fifth and the seventh families are large. Both show only mild cases of the disease, with the exception of case 8, Pavlos Ioannou, and both families have members showing no evidence of the trait - two daughters in each family. It appears, therefore, that there is a tendency to follow a definite familial pattern. Where there are children with this disease in a family, any further affected children will tend to manifest the disease with roughly the same severity. While the series recorded here is obviously too small to be significant, the tendency is clear enough to warrant further investigation. In a paper by Smith (1943) on familial blood studies in cases of Mediterranean Anaemia, three of his families have two cases of the disease in each of them. These cases add further evidence to the hypothesis.

No conclusions could be reached regarding the severity of the disease in the offspring compared with the severity of the trait in the parents. It appeared that there was no definite relationship. Severe cases might result when the parents had a severe degree of the trait, as in the fourth family, and mild cases from parents with a mild degree of the trait, as in the tenth family. A consideration of the other families in this series was so inconclusive, however, that no conclusions could be drawn.

HAEMATOLOGY.

The features of the blood changes have been recorded very fully in the literature, and there is little fresh to add. The cases described in this study show the various changes very clearly, and they emphasise how these changes may vary in intensity. The changes in the disease and in the trait will be discussed together, since their origin is the same and they vary only in degree.

The degree and type of anaemia.

In some cases of the trait there may be no anaemia at all, in others anaemia is only slight. Anaemia is present in all cases of the disease, and it varies from a mild anaemia, associated with no symptoms as in case 6, Dimos Ioannou, to a severe anaemia incompatible with life, as in case 5, Stella Korfiotis. In the cases recorded the anaemia was of the hypochromic, microcytic type in most instances. The M.C.H.C. varied between 21.2 and 29.1% in the seven cases in which it was estimated. The M.C.V. was usually subnormal (4 cases) ranging from 60.7 to 78.1 cu. microns; it was normal in one case, and raised in one case to 96.7 cu. microns. This will be referred to later. Haematocrit studies were not carried out on cases of the trait, as this problem had been studied in detail by Smith (1943). He showed that in the majority of cases of the trait both the M.C.V. and the M.C.H.C. were lowered, but not to the same extent as in the disease.

Changes in the blood film.

Normoblasts. The presence of circulating normoblasts is a characteristic feature of the disease. As has been stated already their presence is a useful criterion for differentiating between the disease and the trait. The number of normoblasts present is roughly related to the degree of the anaemia. Case 10, Louis Loizou is an exception, but in his case a splenectomy had been performed, and this is said to increase the number of circulating normoblasts. Case 5, Stella Korfiotis, the case followed from birth, showed a gradual increase in normoblasts as the anaemia became more severe. This case had 64,000 normoblasts per cmm. shortly before death, and this was the highest figure reached in this series. Figures as high as 125,000 per cmm. have been recorded by Baty et al (1932). The proportion of early to late normoblasts bore no relationship either to the severity of the condition or to any variation in the clinical condition which could be observed. Examination of the blood films of the same patient taken at different times, showed a striking variation in the proportion of early and late normoblasts.

Anisocytosis and Poikilocytosis. Cases of the disease show a severe degree of anisocytosis and poikilocytosis. This is out of proportion to the degree of anaemia, since it is severe, even in cases with only a

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moderate degree of anaemia. Plate XIV - blood film of case 6, Dimos Ioannou, - illustrates this point. His Hb. was 9.5 gm.%. Cases of the trait show a mild degree of anisocytosis and poikilocytosis, but there is a gradation between the mildest changes and the more severe ones.

Abnormal forms. Various types have been described. Microcytes are a prominent feature of films of the disease, and they are also found in most cases of the trait, though they are then less conspicuous. They are illustrated in plates X, XI, XII, and XIV. Ovalocytes are often seen, but they are probably a part of the poikilocytosis rather than a specific abnormality of their own. Plates VIII and XI show a number of them. Macrocytes are seen in some films, but they are inconstant and are far less numerous than microcytes. Three types have been described by Smith (1940): target cells, hypochromic macrocytes, and large pale leaflike erythrocytes containing irregularly distributed haemoglobin. This leaflike appearance will be discussed later on. There are illustrations of each type. Plate IX shows a macrocytic target cell near the top left corner. A hypochromic irregular macrocyte is seen near the extreme left at the top of plate XI. A pale, leaflike macrocyte is seen behind the normoblast half way down the right border of plate XV. Smith (1943) stated that macrocytes are greatly multiplied following splenectomy, and this point is illustrated in case 10, Louis Loizou. Many macrocytes were seen in his blood film, and his was the one case with an M.C.V. greater than normal. Rous and Robertson (1923) considered poikilocytosis and microcytes to be morphological evidence of blood destruction. Wintrobe (1946) supported this contention, since erythrocytes can be torn apart by microdissection without Hb. loss, and poikilocytes are absent from the site of blood formation, i.e. the bone marrow. It may be, then, that the macrocytes seen after splenectomy are due to the removal of one of the main sites of blood destruction. Target cells will be considered separately.

Target cells. These cells form one of the most interesting problems of Mediterranean Anaemia. They were described by Bywaters (1938) and by Smith (1940) and by Damashek (1940). Though present in the illustration of a blood film in his paper, Caminopetros (1938) did not comment on them in the text. Damashek called the condition Target Cell Anaemia, but the term was not adopted, because target cells have been observed in other conditions. Barrett (1938) recorded that he had observed target cells in 27 cases of jaundice, 45 cases of hypochromic anaemia, 8 cases of individuals after splenectomy, and in 9 cases of steatorrhoea. Haden and Evans (1937) observed target cells in sickle cell anaemia. Smith (1943) pointed out that the target appearance in macrocytes is very

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HAEMATOLOGY (Continued).

suggestive of Mediterranean Anaemia. Target Cells form a very prominent feature of the disease, but they are not observed in all cases of the disease or the trait. They were most numerous in severe cases of the trait. Plates VIII and IX show how numerous they can be. It is possible that they are less numerous in the severe forms of the disease owing to the marked anisocytosis and poikilocytosis, which occurs in such cases.

The etiology and function of the target cell has been the subject of discussion and it is of importance, since it clarifies some of the points regarding the etiology of Mediterranean Anaemia. Bohrod (1941) considered that they were evidence of blood regeneration, but Valentine and Neel (1945) disagreed with this. Barrett (1938) considered the shape of target cells, and concluded that they were bowl shaped. Such a cell is flatter than a normal erythrocyte. This is a change in the opposite direction to spherocytosis and this explains the change in the erythrocyte fragility which occurs. This will be discussed later. Target cells have been produced in vitro by suspending normal erythrocytes in plasma or serum rendered hypertonic. Further, target cells from cases of sickle cell anaemia may be converted to cells of normal appearance by suspending them in the patient's own plasma rendered hypotonic by dilution with distilled water. Valentine and Neel (1945) concluded from these experiments that the target cell is one whose envelope is large in relation to its contents.

I did not know of the observations just recorded at the time my investigations were being carried out, else I could have repeated similar experiments with blood from cases of Mediterranean Anaemia. I did, however, on four occasions examine blood from partially haemolysed specimens when estimating erythrocyte fragility. Blood films were made from the erythrocytes not haemolysed by 0.30 gm.% saline - the solution in which haemolysis is normally complete. I had anticipated finding a large number of target cells in such solutions, but was surprised to find them exceptional. This is, of course, in keeping with the observations of Valentine and Neel. The full significance of this was not appreciated at the time. The fact was recorded merely as an observation and experiments on a proper scientific basis were not carried out.

Reticulocytes. An increase was observed in all cases of the disease, and this varied in extent. Some cases of the trait showed an increase in reticulocytes, but the incidence was less than that recorded by Smith (1943). This may be due to inadequacy of technique, but his incidence of reticulocytosis in the trait is higher than demonstrated by Wintrobe et al. (1940). Punctate basophilia and polychromasia were also prominent features of the disease. Their significance is the same as that of the reticulocytosis.

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HAEMATOLOGY (Continued).

Detection of the trait.

The changes described, while severe in the case of the disease, may only be slight in the case of the trait. Difficulty was encountered in a number of cases in deciding from the blood film whether or not the trait was present. The problem was sometimes solved by the recognition of target cells, but the difficulty could be very real when these were absent. Smith (1946) stated that members of the family of a case of Mediterranean Anaemia should not be regarded as haematologically normal until many slides had been examined. This practice was observed. Fortunately, as the erythrocyte fragility was abnormal in all cases of the disease and the trait, there was a check of the interpretation of the blood film, and error was avoided.

Fragility of the Red Cells.

The fragility test is probably the most important single procedure in the diagnosis of Mediterranean Anaemia, and its importance in diagnosing the trait has already been stressed. The method used for estimating fragility was based on Creed's technique (1938), and percentage haemolysis was done in each case. Analysis of figures obtained in cases of the disease and the trait have shown them to be similar in each case, and the graphs drawn illustrate the general tendency. In most cases haemolysis started at the normal level of 0.45- 0.42 gm.NaCl.%, though in some cases it did not start until 0.39 or 0.36 NaCl. gm.%. The end point was variable and did not bear any relationship to the degree of anaemia. The greatest decrease in the fragility was in case 10, Louis Loizou, with an end point of 0.09 gm. NaCl.%. Here, however, resistance had been increased by splenectomy. The literature does record cases in which haemolysis has not been complete even in distilled water, Wintrobe (1946).

In any one family it was noted that cases of the disease had a fragility as low - decreased - or lower than the lowest fragility of any of the members of the family. In cases in which the fragility was repeated it was found to be a constant factor. In the two cases followed from birth the fragility remained constant, although the blood picture changed from that of the trait to that of the disease. Records I obtained of case 3, Maritsa Ioannou, and case 10, Louis Loizou, after his splenectomy, showed no change in his fragility over a period of years.

Baty et al. (1932) and Cooley and Lee (1932) noticed that a pale gelatinous layer remained at the bottom of the test tubes when haemolysis had occurred. This observation was confirmed on all the cases of the disease here recorded. It was also noted in cases of the trait.

The haemolysis curves follow the sigmoid pattern of normal haemolysis, but with certain differences.

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The curve often starts within the normal limits and remains within that range up to 30-40 % haemolysis. It then gradually deviates to show increased resistance. At 70-80 % this deviation becomes much more marked and haemolysis of the last 10% often extends over quite a broad range.

The explanation of this decreased fragility is based on corpuscular shape. Haden (1934) studied the blood of various animals and found that the shape of the red cells varied from relatively flat or slightly biconcave discs to almost perfect spheres. He showed that the more spherical the corpuscle the greater the fragility to saline solutions. Castle and Daland (1937) showed by direct microscopic examination that normal haemolysis is preceded by a phase in which the red cells assume a spherical shape, and that the susceptibility of the red cell to haemolysis can be forecast mathematically from a knowledge of its shape. This observation supports the work of Valentine and Neel (1945), which showed how target cells could be converted into apparently normal cells. The increased resistance of the red cells in Mediterranean Anaemia can be explained, therefore, on the basis of flattening of the red cell. The important ratio is that of diameter to thickness. If this ratio increases then the fragility decreases. As the target cell is a flattened bowl-shaped cell, this explains why it is highly resistant to haemolysis.

The Rhesus factor.

No reports have yet appeared in the literature regarding the part played by the Rhesus factor in Mediterranean Anaemia. Only limited opportunities presented for studying this aspect. In the fifth family both parents with the trait were found to be Rhesus positive, as were cases 6, 7 and 8 with the disease. One brother and two sisters with the trait were Rhesus positive, and one sister without the disease or trait was also Rhesus positive. In the ninth family both the patient and her mother were Rhesus positive. From these few observations it would appear that the Rhesus factor plays no part in the causation of Mediterranean Anaemia. From the behaviour of the disease one would not expect any relationship to the Rhesus factor, since there is no clinical similarity to haemolytic disease of the newborn. In that condition the first child is seldom affected, but there is an increasing incidence and severity in subsequent offspring. Further, if the first month is survived the infant is normal haematologically. By way of contrast rank in the family bears no relation to the development of Mediterranean Anaemia. This was stated by McIntosh and Wood (1942) and it is well borne out by this present study. Further, the condition develops at least months after birth, at a time when any Rhesus incompatibility should have worn off.

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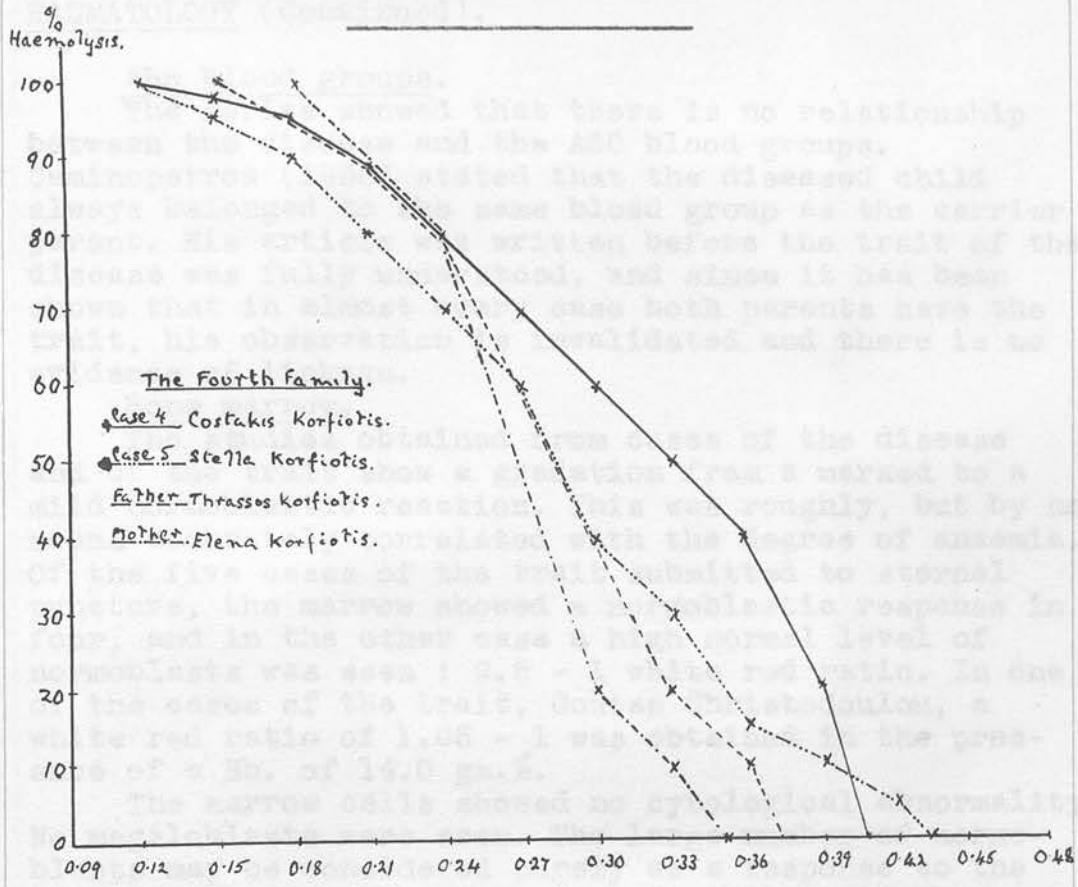
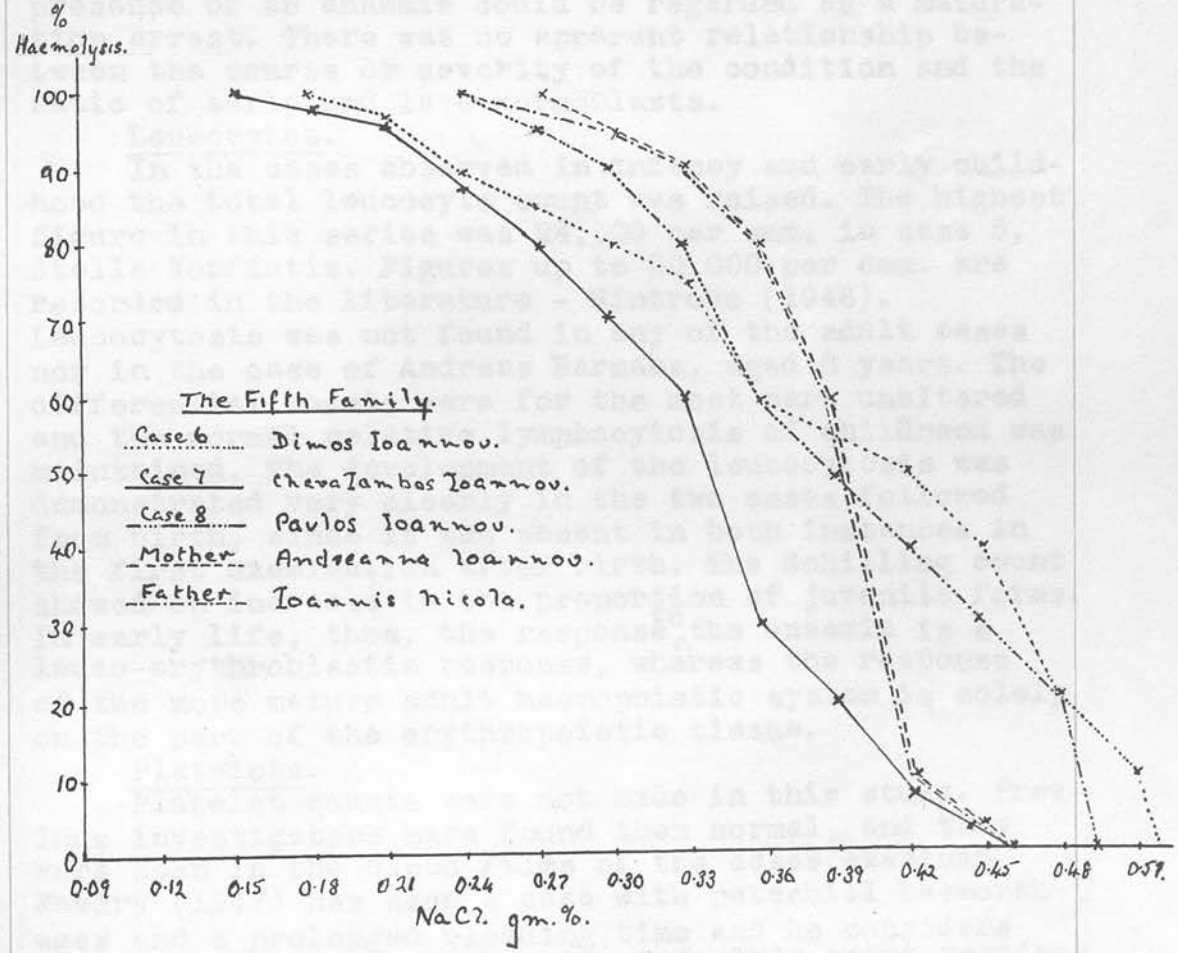
HAEMOLYSIS CURVES.





HAEMATOLOGY (Continued).

ABO blood groups.

The series showed that there is no relationship between the disease and the ABO blood groups. Caminopetros (1938) stated that the diseased child always belonged to the same blood group as the carrier parent. His article was written before the trait of the disease was fully understood, and since it has been shown that in almost every case both parents have the trait, his observation is invalidated and there is no evidence of linkage.

Bone marrow.

The studies obtained from cases of the disease and of the trait show a gradation from a marked to a mild normoblastic reaction. This was roughly, but by no means accurately correlated with the degree of anaemia. Of the five cases of the trait submitted to sternal puncture, the marrow showed a normoblastic response in four, and in the other case a high normal level of normoblasts was seen : 2.6 - 1 white red ratio. In one of the cases of the trait, Costas Christodoulou, a white red ratio of 1.05 - 1 was obtained in the presence of a Hb. of 14.0 gm.%.

The marrow cells showed no cytological abnormality. No megaloblasts were seen. The large number of normoblasts may be considered purely as a response to the anaemia. It resembles that seen in idiopathic hypochromic anaemia. The large number of normoblasts in the presence of an anaemia could be regarded as a maturation arrest. There was no apparent relationship between the course or severity of the condition and the ratio of early and late normoblasts.

Leucocytes.

In the cases observed in infancy and early childhood the total leucocyte count was raised. The highest figure in this series was 24,500 per cmm, in case 5, Stella Korfiotis. Figures up to 30,000 per cmm. are recorded in the literature - Wintrobe (1946). Leucocytosis was not found in any of the adult cases nor in the case of Andreas Barnaba, aged 8 years. The differential counts were for the most part unaltered and the normal relative lymphocytosis of childhood was maintained. The development of the leucocytosis was demonstrated very clearly in the two cases followed from birth, since it was absent in both instances in the first examination after birth. The Schilling count showed an increase in the proportion of juvenile forms. In early life, then, the response to the anaemia is a leuco-erythroblastic response, whereas the response of the more mature adult haemopoietic system is solely on the part of the erythropoietic tissue.

Platelets.

Platelet counts were not made in this study. Previous investigators have found them normal, and they were seen in the blood films of the cases examined. Fawdry (1947) has seen a case with petechial haemorrhages and a prolonged bleeding time and he considers that the platelets may be affected. This point requires further study.

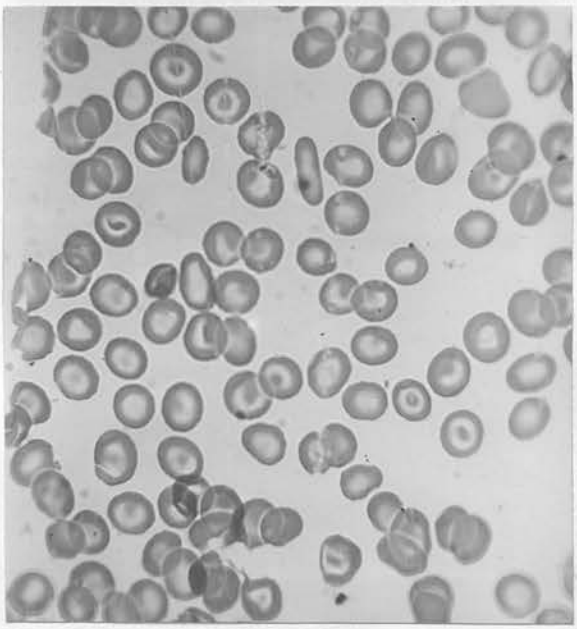


Plate VIII.

Blood film of Savvou Frandesco - Mother of Case No. 1. This film shows the typical features of the trait. Mild anisocytosis and poikilocytosis, many target cells and a few ovalocytes are seen. (x 850)

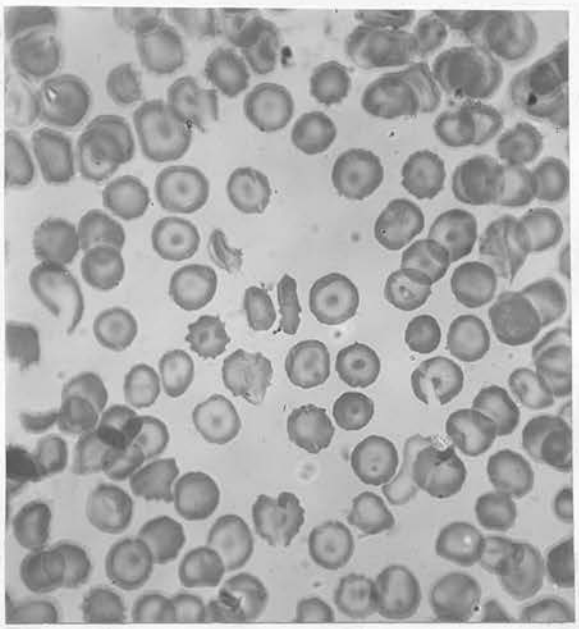


Plate IX.
Blood film of Case No. 5. Stella Korfiotis, age 3 days. This film showed the typical features of the trait. Mild anisocytosis and poikilocytosis, target cells and ovalocytes are seen. There is a macrocytic target cell near the top left corner. (x850)

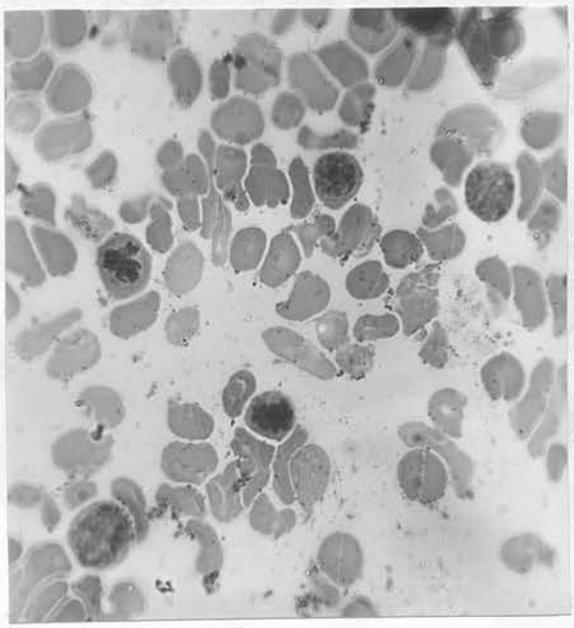


Plate X.
Blood film of Case No.5. Stella Korfiotis, aged 85 days. Anisocytosis and poikilocytosis have increased and normoblasts have appeared in the peripheral blood.(x850)

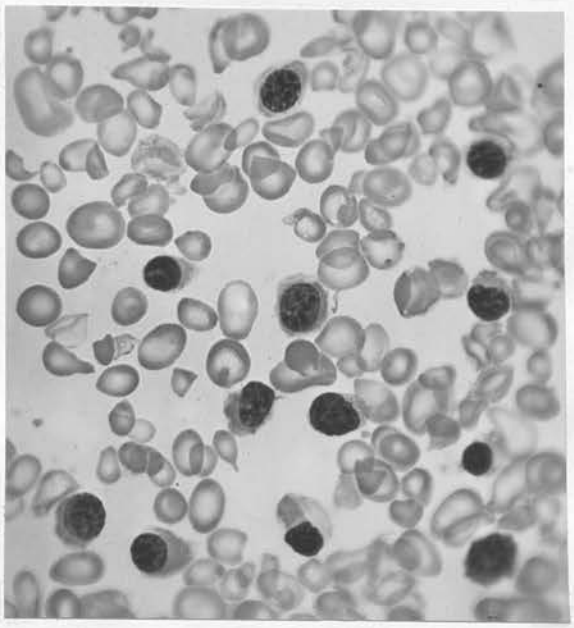


Plate XI.
Blood film of Case No.5. Stella Korfiotis, aged 116 days. The changes already described have increased in intensity. Early normoblasts are now very numerous. A marked degree of hypochromia is apparent. (x850)

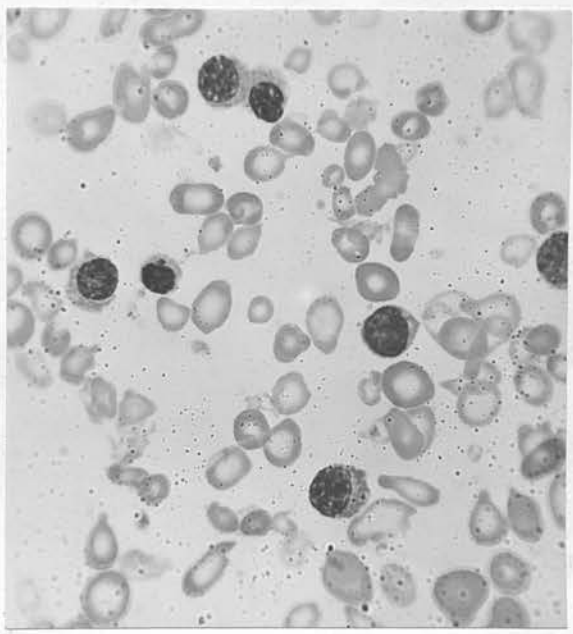


Plate XII.
Blood film of Case No.5. Stella Korfiotis, aged 158 days. The changes have advanced further. The hypochromia and thinness of the cells is most pronounced. (x850)

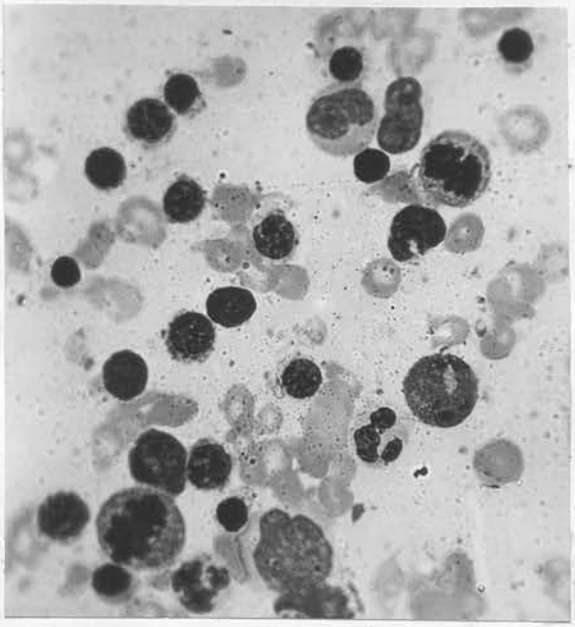


Plate XIII.

Marrow film of CaseNo. 5. Stella Korfiotis, age 158 days. The picture shows the marked normoblastic response, which was seen. (x850)

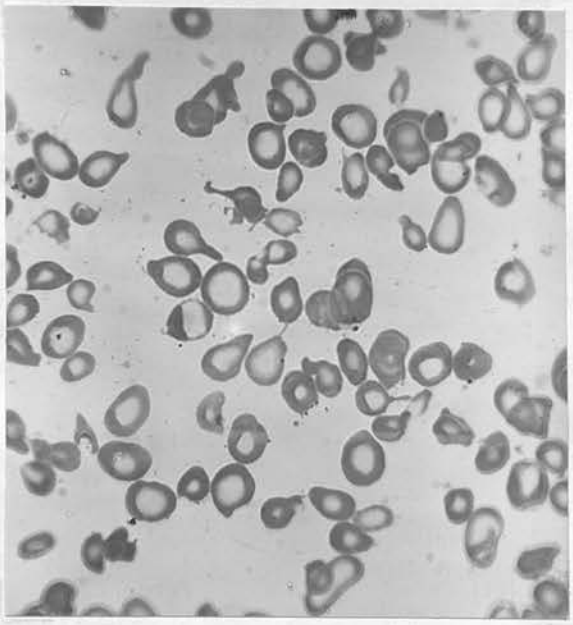


Plate XIV.
Blood film of Case No.6. Dimos Ioannou.
This film shows the characteristics of a mild case of the disease. Severe anisocytosis and poikilocytosis are seen and there is hypochromia. Many microcytes are seen, but no target cells in this picture. No normoblasts are seen, and they were in fact scanty. (x850)

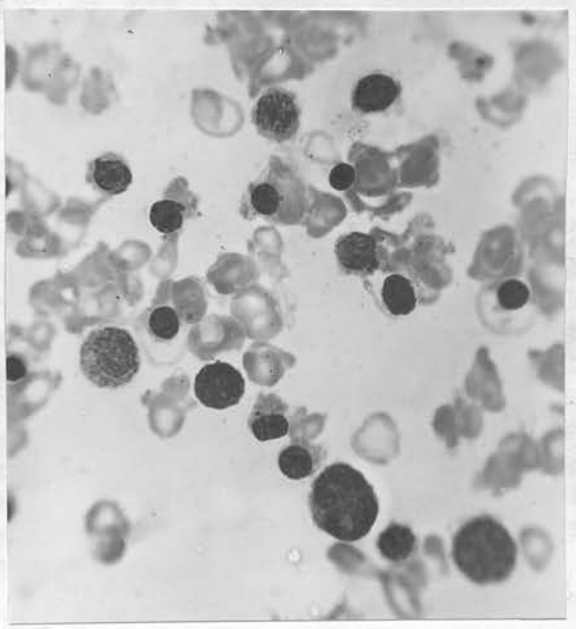


Plate XV.
Marrow film of Case No. 6. Dimos Ioannou.
The picture illustrates the normoblastic response which was found. (x850)

THE RADIOLOGICAL APPEARANCES.

CASE REPORTS.

CASE NO. 2. MARIE CHRISTODOULOU.

Skull. The frontal bone showed thickening of the diploe, gradually increasing upwards from the glabella to a maximum at the centre of the bone, and then diminishing to reach normal thickness at the coronal suture. The horizontal plate of the frontal bone was also involved. The inner and outer tables of the frontal bones were thinned, particularly the outer table. No radial striations were yet apparent, but there was an early granular appearance in the frontal bone. General osteoporosis was present. Mandible and Maxilla were also affected.

Tubular bones. Wrist, hand and knee were X-rayed. Epiphyseal development was normal for the age. General osteoporosis was present, and the changes were equal in degree in the bones examined. Broadening of the medullary cavities and cortical thinning were present, giving the metacarpals and phalanges a rectangular appearance. This change was most marked in the metacarpals. Coarse reticulation with vacuolated areas were seen in all the bones examined.

Christodoulou Michaelides, father of the patient, showed the trait of the disease. His skull, hands and wrists revealed no radiological abnormality.

CASE NO. 3. MARITSA IOANNOU.

Skull. General osteoporosis was present. Thickening of the diploe involved the frontal, parietal and occipital bones. Thickness of1 cm. was maintained upwards for 5 cm. and then diminished to the coronal suture. The parietal bone thickened to a maximum at its centre, and then diminished to the lambdoidal suture. Similar changes occurred in the occipital bone. Inner and outer tables were thinned, the latter being reduced to a mere line. No radial striations were present, but there was granularity of the upper parts of the frontal and parietal bones. Osteoporosis and trabeculation were seen in the mandible and maxilla.

Chest. Ribs showed marked osteoporosis with coarse reticulation along the whole of their length. The heart was enlarged to the left with a prominent pulmonary conus.

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CASE 3. (Continued)

Clavicles showed broadening of the medullary cavities, thinning of the cortex and coarse reticulation throughout their length.

Scapulae. Changes were confined to the acromions, coracoid processes and glenoid regions, and consisted of osteoporosis and coarse trabeculation.

Sacrum. There was marked osteoporosis with coarse trabeculation, which attempted to maintain the normal architectural framework.

Iliac bones. Marked osteoporosis was present with irregular trabeculation. This formed no general pattern, but was thickest near the lower end of the sacro-iliac joint.

Lumbar vertebrae showed coarse irregular trabeculation.

Tubular bones. There was generalised osteoporosis, cortical thinning and broadening of the medullary cavities. This however did not cause general broadening of the shafts of the bones, though localised enlargement of the distal end of the tibia was present. The right humerus was bent at the proximal end of its shaft as though it had been fractured, though no such history was obtained. Coarse reticulation was seen in the carpus and tarsus. The ends of all the tubular bones showed coarse reticulation and small vacuolated areas. In addition, horizontal trabeculae were seen at the proximal ends of the humerus, radius and ulna, and the distal ends of the humerus, tibia and fibula. Irregular trabeculae were present in the metacarpals, metatarsals and phalanges. The changes in the tubular bones were of fairly even distribution, though the changes in the feet were slightly more severe than in the hands.

CASE NO. 4. COSTAKIS KORFIOTIS.

Skull. Slight osteoporosis was present. The frontal bone showed marked diploic thickening, increasing in degree upwards from the glabella to become maximal at the centre of the bone, and then diminishing to normal thickness at the coronal suture. The horizontal plate of the frontal bone was also involved. Inner and outer tables of the frontal bones were thinned, particularly the outer table. There was no radial striation or reticulation.

Chest. The bony framework showed general osteoporosis with coarse trabeculation in the ribs, clavicles and scapulae. The heart was enlarged both to the right and left.

p.t.o.

CASE 4. (Continued)

Pelvis. General osteoporosis was present. There was irregular striation in the iliac bones radiating outwards from the sacro-iliac joint.

Tubular bones. General osteoporosis was present, and changes were evenly distributed. There was broadening of the medullary cavities with thinning of the cortex. This gave a rectangular appearance to the metacarpals, metatarsals and phalanges. Localised broadening was noted at the distal ends of the femora and radii. Reticulation was present at the ends of the bones and involved the epiphyses, which were of normal development in respect of age. Small vacuolated areas were seen in the metaphyseal regions and diminished in number towards the centre of the diaphyses. A few scattered trabeculae were also present near the ends of the bones.

CASE NO. 6. DIMOS IOANNOU.

Skull. Thickening of the diploe was seen in the frontal and parietal bones, and extended back as far as the occiput. The horizontal plate was also involved. Inner and outer tables were thinned, particularly the latter. Thickening was maximal, 1.3 cm., over the upper part of the frontal bone, but there was no acute narrowing at the coronal suture. No radial striation was noted, but there was a granular appearance in the frontal bone and upper part of the parietal bone; osteoporosis was also present in the same area. Osteoporosis and trabeculation were seen in the maxilla and mandible.

Chest. Ribs showed slight trabeculation at their anterior and posterior ends. There was slight cardiac enlargement both to right and left.

Lower lumbar vertebrae and sacrum. There was marked osteoporosis with reticulation.

Iliac bones. Irregular striations extended outwards over the medial part of the bones from a denser area near the lower ends of the sacro-iliac joints.

Clavicles. Osteoporosis and trabeculation involved the lateral halves of the bones.

Scapulae. Osteoporosis and trabeculation were present only in the acromial processes.

Tubular bones. Early reticulation and vacuolation were seen at the proximal ends of humerus, tibia and fibula, and distal ends of radius, ulna, femur and tibia. Transverse striae were seen at the proximal end of the ulna. Metacarpals, metatarsals and phalanges were spared.

This case showed an irregular distribution of osseous changes: skull changes were marked, yet the hands and feet showed no changes, despite changes in other long bones.

CASE NO. 7. CHARALAMBOS IOANNOU.

Skull. The frontal bone showed diploic thickening, which increased as it extended upwards from the frontal sinus, to become maximal - 0.5 cm. - at the centre of the bone. It then diminished and reached normal thickness at the c oronal suture. The horizontal plate of the frontal bone was also involved. Slight thickening of the parietal diploe was present at the vertex. Thinning of the inner and outer tables was present, the latter being the more affected. Radial striation was not present, but there was a coarse granularity of the upper parts of the frontal and parietal bones. Trabeculation was seen in the maxilla and mandible. Osteoporosis was confined to the areas which showed these changes.

Chest. The ribs showed osteoporosis and reticulation at their anterior and posterior ends. Cardiac dilatation was present both to right and left.

Clavicles. Osteoporosis and trabeculation involved their whole length.

Scapulae. Osteoporosis and trabeculation were confined to the areas of the scapulae above the spine, and to the acromial processes.

Sacrum. There was osteoporosis and irregular striation, conforming in general to the natural architectural pattern.

Iliac bones. Irregular trabeculation extended outwards from a dense network at the lower part of the sacro-iliac joints.

Lower lumbar vertebrae. Osteoporosis and coarse trabeculation were present.

Tubular bones. Changes were distributed in an irregular manner. The distal end of the humerus showed decalcification and irregular trabeculation. The proximal end of the ulna showed decalcification with vacuolated areas and irregular trabeculae. Early reticulation was seen at the proximal and distal ends of the tibia and fibula. The other long bones were within the limits of normal. It was noted that the changes were maximal at the elbow joint, whereas the hands and feet were spared.

CASE NO. 8. PAVLOS IOANNOU.

Skull. There was thickening of the diploe of the frontal, parietal and occipital bones. The horizontal plate of the frontal bone was involved. Thickening was maximal over the centre of the frontal bone, and diminished gradually through the parietal bone to reach normal thickness at the lambdoid suture. There was thickening again in the occipital bone. There was no acute narrowing at the coronal suture. The inner

p.t.o.

CASE NO. 8. (Continued)

and outer tables of the affected bones were thin, particularly the outer tables. No radial striation was present, but a coarse granularity and osteoporosis affected the upper part of the skull. Trabeculation was seen in the maxilla and mandible.

Chest. The ribs were normal. The heart was enlarged both to the right and left.

Clavicles. Osteoporosis and reticulation were present only in their lateral halves.

Scapulae. Osteoporosis and reticulation were present only in the acromial processes.

Sacrum. Osteoporosis was noted with reticulation following the normal architecture.

Iliac bones. Irregular trabeculation was present emerging from a dense network near the lower ends of the sacro-iliac joints.

Tubular bones. The degree of involvement was irregular. The distal end of the humerus and proximal ends of the radius and ulna showed osteoporosis and coarse trabeculation with some longitudinal striae. A few coarse trabeculae were present in the metacarpals, otherwise the hands and wrists were normal. The proximal and distal ends of the femur, tibia, and fibula all showed slight osteoporosis with early coarse reticulation and vacuolation. Metatarsals showed coarse reticulation at their proximal ends. The changes in the long bones were most marked at the elbow joint. Elsewhere they were minimal.

CHARILAOUS IOANNOU, brother of Dimos, Charalambos and Pavlos showed the trait of the disease. His skull, elbow, wrist and hand showed no radiological abnormality.

CASE NO. 9. ANDREAS BARNABA.

Skull. General osteoporosis was present. Thickening of the diploe involved all the flat bones. It was most marked over the centre of each flat bone, and diminished towards the coronal suture. The frontal, parietal, and occipital bones were involved in decreasing order of severity. The horizontal plate of the frontal bone was also affected. The inner and outer tables were thinned, particularly the outer, which was only visible as a thin line at the vertex. Elsewhere it could not be recognised. The inner table gradually thickened as it passed back from the frontal to the occipital bone. Striations were present extending radially outwards from the inner table, giving an appearance of hair standing on end. Coarse reticulation extended throughout the skull involving, in addition to the bones already mentioned, the base of the skull, the maxilla and mandible.

p.t.o.

CASE NO. 9. (Continued)

Chest. Coarse reticulation was seen along the whole length of the ribs. The heart was enlarged to the left.

Clavicles. Osteoporosis, cortical thinning and coarse reticulation were generalised.

Scapulae. Coarse, irregular reticulation and osteoporosis were present throughout both bones.

Iliac bones. There was coarse reticulation radiating outwards irregularly from a dense network round the sacro-iliac joints. Osteoporosis was present.

Sacrum. There was osteoporosis and coarse reticulation show ing some resemblance to normal architecture.

Lumbar and cervical vertebrae. Osteoporosis and coarse reticulation were present.

Tubular bones. Epiphyseal development was normal for the age. Changes were uniformly distributed and involved epiphyses, carpus and tarsus as well as the other bones. Osteoporosis, cortical thinning and broadening of the medullary cavities were present. This gave a rectangular appearance to the metacarpals and metatarsals. Reticulation was present in all the long bones, and was coarser in the diaphyses than epiphyses. Vacuolated areas also became apparent towards the centres of the bones. Horizontal striae were also seen at the proximal ends of the humerus, radius and ulna, tibia and fibula, and distal end of the humerus. Some transverse striae were seen in the metacarpals and metatarsals.

CASE NO. 11. STELLAKIS DIKAIOS.

Skull. There was moderate diploic thickening of the frontal bone. This was most marked at the orbital ridge and gradually diminished to normal at the coronal suture. The outer table was diminished in thickness. There were no radial striations or granularity.

Hands and feet. Osteoporosis, cortical thinning and broadening of the medullary cavities were present. There was early reticulation.

CASE NO. 13. PARASKEVOU CHARALAMBOS.

Skull. Diploic thickening affected the frontal, parietal, and occipital bones. Frontal bone thickening, which extended also into the horizontal plate of the frontal bone, was maximal at the orbital ridge and diminished to normal at the coronal suture. The parietal bone thickening was maximal at the centre of the bone, and diminished to normal at the coronal and lambdoid sutures. Similar changes occurred in the occiput. Thinning of the inner and outer tables was present, particularly the outer. No radial striations were seen. There was an early granular appearance in the affected bones.

p.t.o.

CASE NO. 13. (Continued)

Chest. Ribs showed osteoporosis and early trabeculation extending along the w hole length of the bones. The heart was enlarged both to the right and left.

Clavicles. Osteoporosis, cortical thinning and trabeculation were generalised.

Scapulae. Osteoporosis and trabeculation were present throughout the bones.

Iliac bones. Coarse reticulation was present radiating outwards in an irregular manner from the sacro-iliac joints.

Tubular bones. Epiphyses were normal for the age. All the bones, including the tarsus, carpus and epiphyses, were uniformly affected. Thinning of the cortex and broadening of the medullary cavities were present. This produced a generalised thickening of the shafts of the long bones, except the femur, tibia and fibula, and gave the metacarpals and metatarsals a rectangular appearance. There was a localised broadening of the proximal end of the humerus, distal ends of the femur and tibia. The changes consisted of osteoporosis, and coarse reticulation at the bone ends. Vacuolated areas were present in the diaphyseal regions. In the shorter tubular bones, these changes involved their whole length.

Lance Corporal CHARALAMBOS IOANNIDES.

As already reported, this apparently healthy Cypriot soldier showed a severe degree of the trait of the disease, with a normal haemoglobin level. No radiological abnormality was seen in his skull, hand and wrist or pelvis.

C O M M E N T A R Y.

Owing to the fact that Mediterranean Anaemia does not occur in the British Isles, except in unusual circumstances, the British literature contains but few references to the radiological appearances. Bywaters (1938) described a case in an English girl, born of English parents, indistinguishable from Mediterranean Anaemia. This case showed radiological changes in the skull, metacarpals, metatarsals and clavicles, but no changes in the femur, tibia, radius or ulna. Flynn (1943) described a case seen in America, and reviewed the literature. Moncrieff and Whitby (1934) described a case seen in London: a child born of Greek parents.

p.t.o.

COMMENTARY (Continued)

A case was described by Bush (1937), but Teall (1942) suggested that though the case was one of a chronic haemolytic anaemia, it was not a case of Mediterranean Anaemia. The experience of this disease gained from this investigation confirms this view. Teall himself described the appearances from an American case. Fawdry (1944) referred to the skull changes. The condition has been described very fully in the American literature. Karshner (1928), Vogt and Diamond (1930), Mandeville (1930), Baty et al. (1932), Koch and Shapiro (1932), Borzell (1933), Feingold (1933), and Grinnan (1936) all describe the appearances in different cases. Caffey (1937) gives an account of twentyone cases followed for various periods, showing the development of the radiological changes.

Distribution of the bone changes.

The cases studied emphasise the extensive nature of the changes in this disease. Though bony changes bear no direct relation to the degree or duration of the disease, severe anaemic cases surviving to adult life are likely to show changes involving the whole skeleton. Maritsa Ioannou, Case No. 3, is an example of this. Changes in the skull, particularly in the frontal bone, have always been quoted as the typical bony lesion, and have frequently been described when changes elsewhere have been neglected. Skull changes were seen in all nine cases, but bore no relation to the degree or duration of the disease, nor to the extent of the other bone changes. Caffey (1937) pointed out that the skull is not a reliable criterion of the presence of the disease, nor an index of the degree of involvement of the rest of the skeleton. His observation is thus confirmed. Apart from showing no relationship to changes in the skull, changes in the tubular bones themselves were of variable degree. Cases Nos. 3,4,9 and 13 showed generalised involvement, but cases Nos. 6, 7, and 8 showed an irregular distribution. It may be significant that these three cases were brothers. Caffey (1937) was impressed with the consistency of the changes in the metacarpals in all types of cases and at all stages of the disease. Dimos and Charalambos Ioannou, Cases Nos. 6 and 7, showed normal metacarpals and phalanges despite changes elsewhere. One cannot but feel that no specific changes are a reliable index of the disease and variation may be considerable.

Age of onset of bone changes.

It is unfortunate that no opportunity was presented to radiograph the two cases followed from birth: Stella Korfiotis, Case No. 5, and Ioannoula Dikaios, Case No. 12.

p.t.o.

Koch and Shapiro (1932) have recognised skull changes at seven months, and Caffey (1937) tubular bone changes at four months. Since all the other cases presented were older, no further contribution can be made to this aspect of the condition.

Changes in individual bones.

The Skull. The frontal squamosa is the region principally involved, but the changes may also be seen in some cases in the horizontal plate of the frontal bone, the parietal and occipital bones, maxilla and mandible. The frontal squamosa and horizontal plate show thickening of the diploe. Caffey (1937) states that these are the earliest changes seen in all cases. The thickening may be maximal at the orbital ridge and then gradually diminish to the coronal suture, as in Cases No. 11 and 13. The thickening may increase to become maximal at the centre of the frontal bone as in Cases 2, 4, 6, 7, 8 and 9. In Case No. 3 the original thickness was maintained for a short distance before diminution occurred. In all cases diminution in thickness occurs as the coronal suture is reached, and in most cases the thickness at this and other suture lines is normal. When the diploe of the parietal and occipital bones is thickened, this change is maximal at the centre of these bones, and thinning occurs towards each suture line, more particularly the coronal suture. The marked depression at the coronal suture in the case of Andreas Barnaba, Case No. 9, was quite obvious clinically, as shown by his photograph. In none of the cases described was the parietal bone involved in advance of the frontal bone, nor the occipital bone in advance of the parietal. It seems clear that the order of bone involvement is first the frontal bone, then the parietal bone and finally the occipital bone.

Thinning of both tables occurs, particularly the outer. In severe cases the outer table may not be recognisable, or it may only be shown as a thin line. Case No. 9 illustrates this. The bones involved show a coarse granularity in young subjects, which appears to have developed into a 'honey comb mesh' appearance in older cases. This is seen best in the frontal bones and upper part of the parietal bones. Radial striation is a less frequent feature than granularity or 'honey comb mesh'. It is an appearance of hair standing on end, radiating out from the inner table. I would suggest that it is merely a view of the 'honey comb mesh', taken with the trabeculae vertical. This change was only seen in Case No. 9, but it seems fortuitous that it occurred only once in this series. Dawdry (1944) agreed that though the appearance was distinctive when seen, it was by no means constant. Osteoporosis was invariably seen in the bones involved.

p.t.o.

COMMENTARY (Continued)

Flat bones. Osteoporosis with increased reticulation is seen in varying degree and distribution in the different cases. In the iliac bones this reticulation extends outwards irregularly from a denser area near the sacro-iliac joint; it does not follow a definite pattern. The sacrum, however, shows some attempt to follow normal bony architecture. Radiographs of the pelvis were taken in seven cases and the changes just described were a constant feature in them all. The scapulae were affected to a variable extent. In the severer cases the whole bone was involved: Cases Nos. 3, 4, 9 and 13. In the milder cases changes were more limited. The acromion process was constantly involved: Cases Nos. 6, 7, and 8; and in Case No. 7 the area of the scapula above the spine was affected.

Chest. The ribs showed similar changes in varying degree. No changes were seen in Case No. 8, a mild case. Cases Nos. 6 and 7 showed changes only at the anterior and posterior ends of the ribs. In the severer cases the whole length of the ribs was affected. Cardiac enlargement was found in all the cases. It has been recognised that this finding is constant and varies depending on the degree of the anaemia. It is thus a secondary change. Cooley, Witwer and Lee (1927) and Hunter (1946) have referred to this.

The tubular bones. There is involvement of the tubular bones of varying degree and distribution. In the severe cases marked changes of roughly uniform degree were found uniformly distributed: Cases Nos. 3,4, 9 and 13. In milder cases changes showed an irregular distribution: Cases Nos. 6, 7 and 8. Further, changes in the skull do not parallel changes in the tubular bones. This was shown clearly in Case No. 6.

There is widening of the medullary cavities with cortical thinning. In the short tubular bones such as the metacarpals this gives a rectangular appearance. In the cases described this change was seen only in the cases radiographed in childhood. The broadening of the medullary cavities was more marked in the upper limb bones than in the lower. This may be on account of the greater length of the lower limb bones. Caffey (1937) described a case in which the rectangular appearance of the short tubular bones persisted until death at ten years nine months. In the adult cases presented, there was no evidence to show whether this appearance had been present in earlier years or not. The severity of the anaemia in the case of Maritsa Ioannou, Case No. 3, make one think that at least in this instance such was the case.

As a general rule expansion of the shafts was uniform, though localised expansions at the ends of shafts were found in certain instances. Caffey (1937) appears to be the only writer to describe this change. He stated that this finding was uncommon, but when it occurred, it was most frequent at the distal end of the femur.

p.t.o.

COMMENTARY (Continued)

Case No. 3 showed enlargement of the distal end of the tibia; Case No. 4 the distal ends of femur and radius; Case No. 13 the proximal end of humerus and the distal ends of femur and tibia.

Osteoporosis is noted wherever there is bone involvement. It is probably the result of loss of compact bone due to cortical thinning. In some of the cases reported the medullary surface of the thinned cortex was irregular, as if it had been eaten away by the expanding marrow. In view of these changes, it is surprising that pathological fractures are rare. In the fourteen cases reported no history of fracture was obtained in any case. Radiograph of the right humerus of Maritsa Ioannou, Case No. 3, suggested an old healed fracture of the neck.

Epiphyseal development was normal for age in all cases. Cypriots as a race are short of stature, and the cases conformed to this racial pattern. There was no case of dwarfism. Caffey (1937) stated that there was delay both in growth and maturation of the skeleton. He attributed this to the effect of the chronic anaemia. The cases with severe anaemia die in infancy, and the later effect on growth is thus not seen. Maritsa Ioannou, Case No. 3, was a severe case, yet did not show any dwarfism. Costakis Korfiotis, Case No. 4, and Paraskevou Charalambos, Case No. 13, were both severe cases in late infancy, but though they showed effects of their anaemia in other ways, there was no retardation of skeletal development.

The architecture of the tubular bones is profoundly altered. In early cases ininfancy there is a fine reticulation affecting particularly the ends of the bones, but in the case of the short tubular bones, the whole length of the bones is involved. In the older cases the reticulation is coarser. It is also noted that in the epiphysis and adjacent part of the metaphysis the reticulation is fine, and that as the centre of the diaphysis is reached, the reticulation becomes coarser. Also small vacuolated areas make their appearance and they may extend up the diaphysis beyond the limits of the reticulation. In the short tubular bones the changes may affect the whole length of the bones, but in the longer bones, the changes stop short before the centre of the diaphysis is reached. Vacuolation shows up very clearly in the humerus and metacarpals, but any bone may show this appearance. When reticulation is marked, irregular striae appear and in general run in a horizontal direction, though they may be oblique. In adult cases the reticulation is very coarse and would be better called trabeculation. Irregular striation is often prominent. Growth is now complete and changes are found only at the ends of the bones.

p.t.o.

COMMENTARY (Continued)

Caffey (1937) described a case in which with increasing age, there was an increase in bone density, and a disappearance of the coarse reticulation previously present. This was due to an increase of trabecular bone filling the marrow cavity, and tightening the mesh of the trabecular pattern, thus causing a moderate osteosclerosis. Cortical bone, however, remained atrophic, and the rectangular outline persisted unchanged. The case of the English child reported by Bywaters (1928) also showed increased density in the skull. This appearance was not seen, however, in any of the cases in this series.

Correlation of radiological and pathological changes. In the series of cases reported only one case died while the investigation was being carried out. Unfortunately pe rmission for post mortem examination was not obtained. Whipple and Bradford (1932) gave a detailed account of the post mortem findings on one of their cases, and it is possible to correlate these changes with the appearances seen radiographically.

The histological appearance of ossification in the epiphyseal cartilage is normal. This is what would be expected, since growth is normal, and it would not be, if ossification were affected. There is thinning of bone trabeculae, and at the same time proliferation of delicate new bone. There is no evidence of osteoclastic activity or of inflammatory reaction. This is surprising in view of the vacuolated appearance. It would seem, therefore, that bone is laid down normally, and that the changes already described take place subsequently. The radiological changes are in keeping with this hypothesis. In the regions were bone has been recently laid down, i.e. the epiphysis and metaphysis, changes are minimal. Further down the diaphysis, where the bone has been laid down longer, the changes are more advanced. In adults the centre of the diaphysis is normal. It is possible that the hyperplastic marrow has not extended down as far as the centre of the diaphysis, since its activity is always most marked at the ends of the tubular bones, and in normal individuals does not extend beyond the bone ends. Caffey (1937) reported dilatation of the medullary cavities as the earliest lesion in the tubular bones, together with atrophy of cortical and cancellar bone. These changes are seen several months before reticulation becomes apparent. This suggests that medullary expansion to accommodate the hyperplastic marrow is the primary change. Cortical thinning and osteoporosis follow as a pressure atrophy. This explanation is not entirely satisfactory, since it is difficult, as Whipple and Bradford (1932) point out, to explain this type of bony reaction on the basis of simple hyperplasia and pressure atrophy.

p.t.o.

COMMENTARY (Continued)

At post mortem examination the inner and outer tables of the skull were found to be separated and in large measure obliterated. The bone was made up of a sponge like marrow in which there were rather heavy bone trabeculae arranged at right angles to the dura. The marrow was deep red with a chocolate tint. The bone was soft and could be cut with a scalpel. This description satisfactorily explains the radiological appearances. The sponge like marrow gives the 'honey comb' appearance, and this confirms the suggestion made that the appearance of radial striation is seen when the trabeculae are photographed in the vertical position. Bone marrow was shown to grow through the thin remains of the cortex into the periosteum and to split it. This explains the apparent loss of the outer table of the skull in some cases.

The ribs, sternum and vertebrae showed the same changes as did the calvarium. Whipple and Bradford (1932) noted that the rib changes were most marked at their anterior and posterior ends. This was also found to be the case radiologically in the milder cases.

Specificity of the bone lesions. It is clear from the literature that the changes are in no way specific for Mediterranean Anaemia. They have been reported, though as a less frequent occurrence, in other chronic haemolytic anaemias such as Sickle Cell Anaemia, by Vogt and Diamond (1930) and Caffey (1937); Acholuric Jaundice by Friedman (1928) and Caffey (1937). Why these changes should be peculiar to haemolytic anaemias and should not occur in other chronic anaemias has not been explained. Nor is it clear why the changes should be so much more severe in Mediterranean Anaemia than the other haemolytic anaemias.

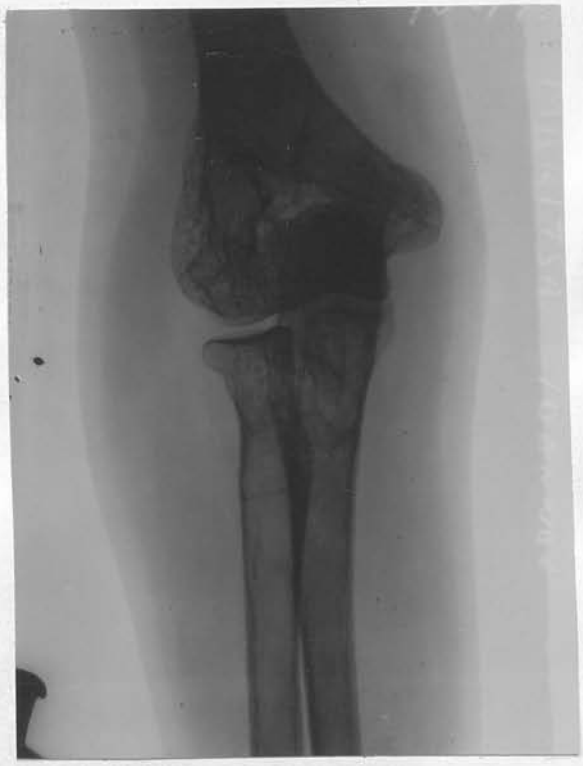


Plate XVI.
Case No.3. Maritsa Ioannou.
X-ray of elbow.

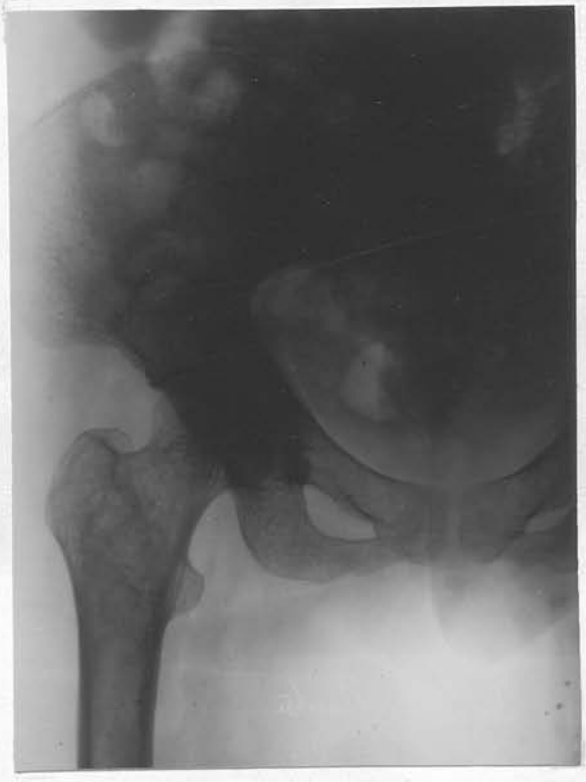


Plate XVII.
Case No. 3. Maritsa Ioannou.
X-ray of pelvis.

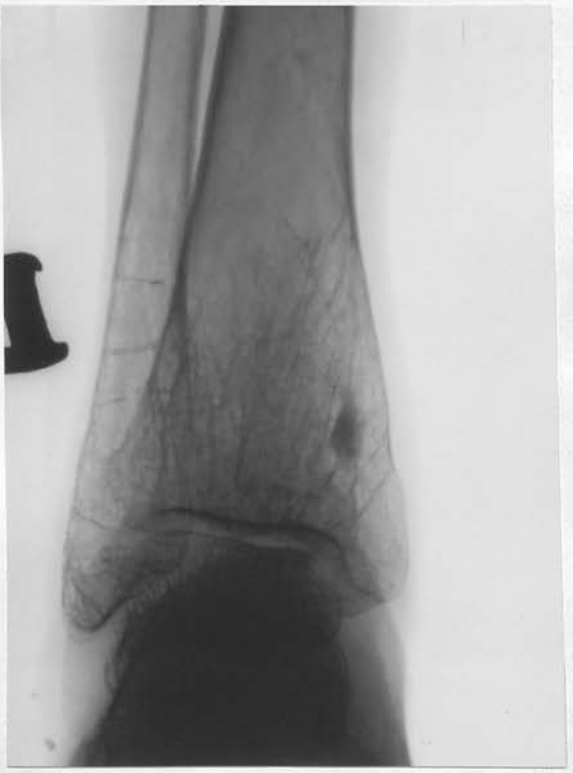


Plate XVIII.
Case No. 3. Maritsa Ioannou.
X-ray of ankle.

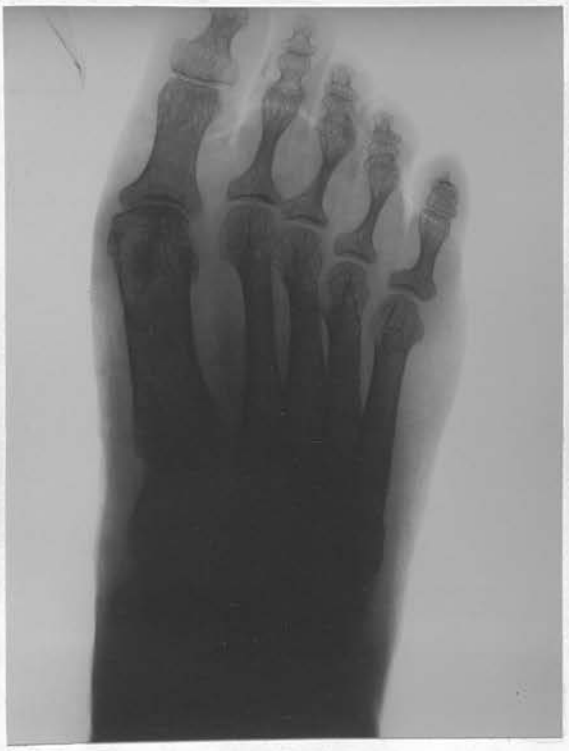


Plate XIX.
Case No. 3. Maritsa Ioannou.
X-ray of foot.

Plate XX.
Case No. 9. Andreas Barnaba.
X-ray of skull.

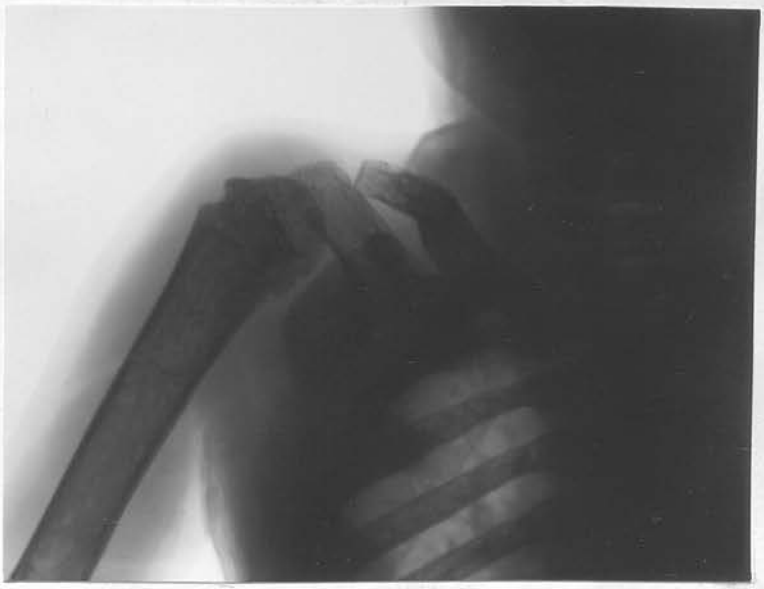


Plate XXI.
Case No. 9. Andreas Barnaba.
X-ray of shoulder.

Plate XXII.
Case No. 13. Paraskevou Charalambos.
X-ray of forearm.

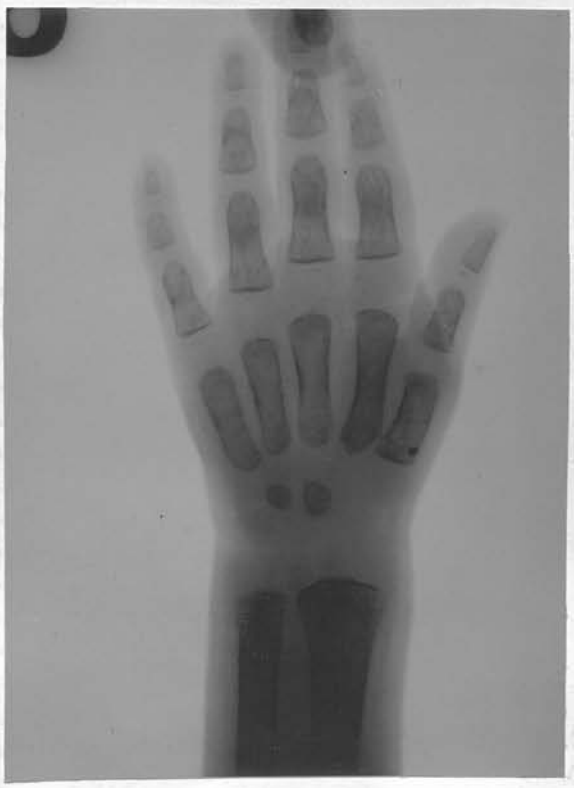


Plate XXIII.
Case No. 13. Paraskevou Charalambos.
X-ray of hand and wrist.

THE INCIDENCE OF THE TRAIT.

In view of the frequency of the trait in the families with cases of the disease, it was obviously necessary to determine the incidence of the trait in the general Cypriot population. From the description of the trait already given, it will be clear that the essential features are: 1. the diminished fragility of the R.B.Cs. in saline - 0.30% saline being the critical value for complete haemolysis. 2. the changes in the blood film - anisocytosis and poikilocytosis, usually target cells, and often ovalocytes and microcytes. Determination of the fragility and examination of the blood film was therefore made on a hundred Cypriots. These were chosen at random. A large number of them were healthy soldiers, and thus males predominate in the analysis. Soldiers detained on the surgical side of the military hospital were among those examined, but care was taken not to include those suffering from any general disease. The cases were those awaiting procedures such as hernia repairs. The results were as follows:

No.	Sex.	Race.	Hb. gm.%.	Fragility. %. saline.	Blood film. AP.T.O.M.	Result.
1.	M.	G.	14.5	0.39-0.21	+ + + +	Trait.
2.	M.	G.		0.42-0.30	- - - -	Normal.
3.	M.	G.		0.42-0.27	+ + + +	Trait.
4.	M.	G.		0.39-0.21	+ + + +	Trait.
5.	M.	G.		0.45-0.33	- - - -	Normal
6.	M.	G.		0.45-0.33	- - - -	Normal.
7.	M.	G.	15.2	0.39-0.30	- - - -	Normal.
8.	M.	G.	13.7	0.36-0.15	+ + + +	Trait.
9.	M.	T.	14.8	0.45-0.33	- - - -	Normal.
10.	M.	T.	14.7	0.48-0.33	- - - -	Normal.
11.	M.	T.	15.0	0.45-0.27	- - - -	Normal.
12.	M.	G.	13.4	0.45-0.24	+ + + +	Trait.
13.	M.	G.	13.1	0.36-0.21	+ + + +	Trait.
14.	M.	T.	13.7	0.45-0.33	- - - -	Normal.
15.	M.	G.	13.6	0.39-0.24	+ + + +	Trait.
16.	M.	T.	11.7	0.36-0.24	+ + + +	Trait.
17.	F.	G.	13.1	0.45-0.24	+ + + +	Trait.
18.	M.	A.	13.0	0.39-0.21	+ + + +	Trait.
19.	M.	T.	14.0	0.42-0.24	+ + + +	Trait.
20.	F.	G.	14.8	0.48-0.27	- - - -	Normal.
21.	M.	T.	14.0	0.45-0.30	- - - -	Normal.
22.	F.	G.	14.5	0.45-0.30	- - - -	Normal.
23.	M.	G.	14.7	0.39-0.30	- - - -	Normal.
24.	M.	G.	14.0	0.45-0.33	- - - -	Normal.
25.	M.	G.	13.4	0.48-0.33	- - - -	Normal.
26.	M.	G.	14.8	0.45-0.30	- - - -	Normal.
27.	M.	G.	14.8	0.45-0.30	- - - -	Normal.
28.	M.	T.	14.0	0.39-0.24	+ + + +	Trait.
29.	M.	G.	14.8	0.42-0.27	- - - -	Normal.
30.	M.	T.	14.0	0.45-0.24	+ + + +	Trait.

p.t.o.

INCIDENCE OF THE TRAIT (Continued).

No.	Sex.	Race.	Hb. gm.%.	Fragility. %.saline.	Blood film. AP.	T.	O.	M.	Result.
31.	M.	G.	13.4	0.42-0.24	+	+	+	+	Trait.
32.	M.	G.	14.7	0.51-0.39	-	-	-	-	Normal.
33.	M.	T.	13.4	0.45-0.30	-	-	-	-	Normal.
34.	M.	G.	12.8	0.51-0.33	-	-	-	-	Normal.
35.	M.	T.	14.0	0.45-0.33	-	-	-	-	Normal.
36.	M.	T.	13.1	0.45-0.33	-	-	-	-	Normal.
37.	M.	G.	14.0	0.45-0.27	-	-	-	-	Normal.
38.	M.	G.	13.7	0.45-0.30	-	-	-	-	Normal.
39.	M.	G.	14.0	0.48-0.33	-	-	-	-	Normal.
40.	M.	G.	13.4	0.48-0.33	-	-	-	-	Normal.
41.	M.	G.	13.8	0.48-0.33	-	-	-	-	Normal.
42.	M.	G.	14.0	0.51-0.36	-	-	-	-	Normal.
43.	M.	G.	15.2	0.51-0.33	-	-	-	-	Normal.
44.	M.	G.	14.7	0.51-0.33	-	-	-	-	Normal.
45.	M.	G.	13.8	0.48-0.33	-	-	-	-	Normal.
46.	M.	T.	13.6	0.45-0.24	+	+	+	+	Trait.
47.	M.	G.	13.4	0.45-0.24	+	+	+	+	Trait.
48.	M.	G.	14.0	0.42-0.21	+	+	+	+	Trait.
49.	M.	G.	14.8	0.45-0.33	-	-	-	-	Normal.
50.	M.	T.	16.2	0.48-0.36	-	-	-	-	Normal.
51.	M.	G.	12.0	0.39-0.24	+	+	+	+	Trait.
52.	M.	G.	13.4	0.45-0.33	-	-	-	-	Normal.
53.	M.	T.	13.3	0.45-0.33	-	-	-	-	Normal.
54.	M.	G.	12.1	0.48-0.33	-	-	-	-	Normal.
55.	M.	T.	13.3	0.48-0.36	-	-	-	-	Normal.
56.	M.	G.	13.3	0.48-0.36	-	-	-	-	Normal.
57.	M.	G.	13.3	0.48-0.30	-	-	-	-	Normal.
58.	M.	T.	13.3	0.45-0.33	-	-	-	-	Normal.
59.	M.	G.	12.7	0.39-0.24	+	+	+	+	Trait.
60.	M.	G.	17.1	0.42-0.33	-	-	-	-	Normal.
61.	M.	G.	15.9	0.42-0.30	-	-	-	-	Normal.
62.	M.	G.	14.6	0.48-0.30	-	-	-	-	Normal.
63.	M.	G.	13.3	0.48-0.24	+	+	+	-	Trait.
64.	M.	G.	13.3	0.39-0.24	+	+	+	+	Trait.
65.	M.	G.	13.3	0.45-0.30	-	-	-	-	Normal.
66.	M.	G.	13.3	0.45-0.30	-	-	-	-	Normal.
67.	M.	G.	14.6	0.45-0.33	-	-	-	-	Normal.
68.	M.	G.	12.7	0.45-0.30	-	-	-	-	Normal.
69.	M.	G.	14.0	0.45-0.33	-	-	-	-	Normal.
70.	M.	G.	15.2	0.48-0.33	-	-	-	-	Normal.
71.	M.	T.	15.2	0.48-0.33	-	-	-	-	Normal.
72.	M.	T.	14.0	0.48-0.33	-	-	-	-	Normal.
73.	M.	G.	13.3	0.45-0.27	+	+	+	+	Trait.
74.	M.	G.	12.7	0.45-0.27	-	-	-	-	Normal.
75.	M.	G.	14.6	0.51-0.27	+	+	+	-	Trait.
76.	M.	T.	15.9	0.48-0.33	-	-	-	-	Normal.
77.	M.	G.	12.7	0.48-0.30	-	-	-	-	Normal.
78.	M.	G.	14.0	0.45-0.30	-	-	-	-	Normal.
79.	M.	G.	10.8	0.48-0.30	-	-	-	-	Normal.
80.	M.	G.	15.2	0.48-0.33	-	-	-	-	Normal.

p.t.o.

INCIDENCE OF THE TRAIT (Continued).

No.	Sex.	Race.	Hb. gm.%.	Fragility. % saline.	Blood film. AP.	T.	O.	M.	Result.
81.	M.	G.	14.6	0.45-0.30	-	-	-	-	Normal.
82.	M.	G.	13.3	0.45-0.30	-	-	-	-	Normal.
83.	M.	G.	14.6	0.48-0.33	-	-	-	-	Normal.
84.	M.	G.	14.0	0.45-0.30	-	-	-	-	Normal.
85.	M.	G.	14.0	0.45-0.30	-	-	-	-	Normal.
86.	M.	G.	15.2	0.48-0.30	-	-	-	-	Normal.
87.	M.	G.	15.9	0.42-0.30	-	-	-	-	Normal.
88.	M.	G.	12.1	0.45-0.27	+	+	+	+	Trait.
89.	M.	T.	13.3	0.45-0.33	-	-	-	-	Normal.
90.	M.	G.	12.1	0.45-0.30	-	-	-	-	Normal.
91.	M.	G.	12.7	0.45-0.33	-	-	-	-	Normal.
92.	M.	G.	12.7	0.48-0.33	-	-	-	-	Normal.
93.	M.	G.	10.8	0.45-0.24	+	-	+	+	Trait.
94.	M.	G.	15.2	0.45-0.30	-	-	-	-	Normal.
95.	M.	G.	14.0	0.45-0.24	+	+	+	+	Trait.
96.	M.	G.	11.4	0.45-0.27	+	+	+	-	Trait.
97.	M.	G.	12.7	0.45-0.27	+	-	+	+	Trait.
98.	M.	G.	12.1	0.48-0.33	-	-	-	-	Normal.
99.	M.	G.	12.7	0.48-0.33	-	-	-	-	Normal.
100.	M.	G.	12.7	0.45-0.30	-	-	-	-	Normal.

Abbreviations.
M.-male. F.-female. G.-Greek Cypriot. A.-Armenian. T.-Turkish Cypriot. AP.-Anisocytosis and Poikilocytosis. T.-Target cells. O.-Ovalocytes. M.-Microcytes.

Analysis of Results.

	Greek.	Turk.	Armen.	Male.	Female.	Total.
Normal	56	16	0	70	2	72
Trait	22	5	1	27	1	28
Total	78	21	1	97	3	100

These figures show an incidence of the trait of 28% in the 100 persons examined. Both Greek and Turkish communities are affected. Clearly the number of people examined is too small to be statistically significant, but it does give an indication of the high incidence of the trait.

It will be noted that it was stated at the beginning of this section that 0.30% saline was taken as the critical point for complete haemolysis. In the cases examined, a few cases in which haemolysis was not complete until 0.27% saline have been passed as normal. Such cases always present difficulty, and careful examination of the blood film is necessary before coming to a decision. The problem is all the more difficult, since changes in such cases are minimal.

p.t.o.

INCIDENCE OF THE TRAIT (Continued).

The presence of target cells often decides the issue. This difficulty was stressed by Smith (1946) in a personal communication to me. He stated that in doubtful cases many slides must be examined before the individual can be regarded as haematologically normal. This advice was followed.

It was apparent quite early on in this investigation that a high proportion of the general population in Cyprus showed the trait. I pointed this out to Dr. Fawdry, and he had the opportunity to do a similar investigation while he was doing a malaria survey. He adopted the same technique, as described subsequently under the village survey, for checking the fragility in 0.30% saline, but he did not check the blood films. The survey was carried out on Limassol school children with the following results.

	Total.	Trait.	Normal.	Percentage.
Greek Males.	224.	41.	183.	18%.
Females.	193.	30.	163.	15%.
Total.	417.	71.	346.	17%.
Turk Males	62.	10.	52.	16%.
Females.	53.	14.	39.	27%.
Total.	115.	24.	91.	21%.
Grand total.	532.	95.	437.	18%.

The figures for Turks are not large enough to make the apparent discrepancy between male and female incidence significant, and probably as amongst the Greeks, the trait is of equal incidence in males and females. This approximately even distribution of the trait between males and females is in contrast to the observation of Fawdry (1947) that the fully developed disease occurs predominantly in males. He had observed more than 100 cases during a period of seven years, and had noted that male children were affected more often than female children in the proportion of 3 to 1.

Neel and Valentine (1945) investigated the incidence of the trait in individuals of Italian descent in Rochester, N.Y., between 1928 and 1942. They found 11 cases of the disease, giving one case per every 2,368 births. On the assumption that the disease is due to homozygosity for the inherited factor, and the trait due to heterozygosity, this would give an incidence of the trait of one per 25 individuals, with a mean variation of 1 per 18 to 34. This incidence is much lower than has been found in Cyprus, where the higher rate may be the result of the inbreeding that will have taken place in an island with a population of less than half a million. Damashek (1943) found changes in the blood film - target cells or ovalocytes in 8 out of 60 (13%) unselected Italians in Boston.

p.t.o.

INCIDENCE OF THE TRAIT (Continued).

Conclusions.

From the investigations carried out by Dr. Fawdry and myself, it is clear that the trait of Mediterranean Anaemia is present in about 20% of the Cypriot population - roughly equally distributed between Greek Cypriots and Turkish Cypriots and between males and females.

A VILLAGE SURVEY.

When the results of the analysis of the general populace became apparent, it was an obvious step to find out how the trait and disease manifested themselves in an actual village. It was decided to carry out a survey of a whole village.

The village of Alithinou was chosen. This is a village in the Troodos range of mountains, about 3,500 feet above sea level. Villages of this type are very isolated. Each one of them owns a bus, which keeps them in communication with civilisation. For the most part, however, the villagers stay at home looking after their farms. Their families have lived in the same village for generations, and so inbreeding has resulted. As it was very aptly put to me by one of the inhabitants of Alithinou, "The boys cannot now marry the girls because they are related to them." From the point of view of studying the hereditary nature of this disease, however, this was a great advantage.

Alithinou was chosen for the survey for several reasons. The fifth family which was examined in the series came from Alithinou. This therefore meant that the disease and trait were present in the village. This family had been very co-operative and were willing to help. The father of the family, Ioannis Nicola, was the village Mukhtar - the village chief. Through his co-operation it was possible to examine 65 out of the 80 inhabitants. All were Greek Cypriots.

The technique of the investigation was as follows: 500 cc. of 0.30% saline was prepared. 20 cc. of 1.6% sodium citrate was added to this solution. The citrate solution was isotonic with the saline. Control experiments with normal individuals and those already known to have the trait showed that it did not affect the fragility end points. The citrate was added to prevent any coagulation when the blood was added to the solution. In previous experiments this had been found occasionally to vitiate results. 1cc. of the solution was added to a small agglutination tube, and a drop of blood was added to this from a finger prick. At the same time two thin blood films were made and subsequently stained.

p.t.o.

A VILLAGE SURVEY (Continued).

The survey was made on 18th. Feb., 1947. The villagers were most helpful, though they did not understand the significance of the investigation. Certain villagers were not available, either on account of work, or through absence from the village. Fortunately it rained on the day of the survey, and most of the farmers had not gone out to the fields.

The results of the survey are tabulated below, and the inter-relationship of the villagers, together with the incidence of the trait among them, is shown subsequently on a diagram in the section dealing with the hereditary aspect of the disease.

No.	Sex.	Age.	F.	M.	Relatives.	0.30% NaCl. Frag.	Film. AP.	T.	O.	M.	Trait.
1.	M.	65	d.	d.	h. of 2.	-	-	-	-	-	No.
2.	F.	62	d.	d.	w. of 1.	+	+	-	+	+	Yes.
3.	M.	65	d.	d.	b. of 6,8.	+	+	+	+	+	Yes.
4.	F.	70	d.	d.	s. of 5.	+	+	+	+	-	Yes.
5.	F.	76	d.	d.	s. of 4.	+	+	+	+	+	Yes.
6.	M.	57	d.	d.	h.7.b.3,8.	+	+	+	+	+	Yes.
7.	F.	65	d.	d.	w. of 6.	+	+	-	+	+	Yes.
8.	M.	54	d.	d.	h.9.b.3,6	+	+	-	+	+	Yes.
9.	F.	50	d.	d.	w. of 8	+	+	-	+	+	Yes.
10.	F.	50	d.	d.	c. of 8	-	-	-	-	-	No.
11.	F.	40	d.	d.	w. of 12	-	-	-	-	-	No.
12.	M.	35	1.	2.	h.11.b.13,16.	+	+	-	+	-	Yes.
13.	F.	23	1.	2.	s.12,15,16.	-	-	-	-	-	No.
14.	M.	36	-	-	h. of 15.	-	-	-	-	-	No.
15.	F.	37	1.	2.	w.14.s.12,13.	-	-	-	-	-	No.
16.	M.	32	1.	2.	h.17.b.12,13,	not examined.					
17.	F.	48	3.	d.	w.16.s.18(19)	-	-	-	-	-	No.
18.	F.	38	3.	d.	s.17.(19).	-	-	-	-	-	No.
19.	M.	36	3.	d.	h.20.s.(17,18)	+	+	+	+	+	Yes.
20.	F.	35	d.	4.	w. of 19.	-	-	-	-	-	No.
21.	M.	56	d.	5.	h. of 22.	+	+	-	+	+	Yes.
22.	F.	50	d.	d.	w. of 21.	-	-	-	-	-	No.
23.	F.	25	-	-	w. of 24.	-	-	-	-	-	No.
24.	M.	31	8.	9.	h.23.b.25-28.	+	+	+	+	+	Dis.
25.	M.	26	8.	9.	b.24,26-28.	+	+	+	+	+	Yes.
26.	M.	13	8.	9.	b.24,25,27,28	+	+	+	+	+	Dis.
27.	M.	26	8.	9.	b.24-26,28.	+	+	+	+	+	Dis.
28.	F.	20	8.	9.	s.24-27.	+	+	+	+	-	Yes.
29.	M.	10	12.	11.	b.30-33.	+	+	+	-	+	Yes.
30.	F.	8	12.	11.	s.29,31-33.	-	-	-	-	-	No.
31.	F.	6	12.	11.	s.29,30.	-	-	-	-	-	No.
32.	F.	4	12.	11.	s.29-31,33.	+	+	-	+	+	Yes.
33.	M.	1	12.	11.	b.29-32.	+	+	+	+	+	Yes.
34.	M.	8	14.	15.	b. of 35.	-	-	-	-	-	No.
35.	F.	3	14.	15.	s. of 34.	-	-	-	-	-	No.
36.	F.	18	16.	17.	s. of 37.	-	-	-	-	-	No.
37.	F.	16	16.	17.	s. of 36.	-	-	-	-	-	No.
38.	F.	12	19.	20.	s. of 39.	-	-	-	-	-	No.
39.	M.	10	19.	20.	b. of 38.	-	-	-	-	-	No.
40.	M.	24	21.	22.	b.41-44.	-	-	-	-	-	No.

p.t.o.

No.	Sex.	Age.	F.	M.	Relatives.	0.30% NaCl. Frag.	Film. AP.	T.	O.	M.	Trait.
41.	F.	19	21.	22.	s.40,42-44.	-	-	-	-	-	No.
42.	F.	13	21.	22.	s.40,41,44.	-	-	-	-	-	No.
43.	M.	8	21.	22.	b.40-42,44.	+	+	-	+	+	Yes.
44.	F.	15	21.	22.	s.40-43.	+	+	+	+	+	Yes.
45.	F.	3	24.	23.	s. of 46.	+	+	-	+	+	Yes.
46.	F.	1	24.	23.	s. of 45.	+	+	-	+	+	Yes.
47.	M.	56	d.	d.	h. of 48.	+	+	-	-	+	Yes.
48.	F.	54	d.	d.	w. of 47.	+	+	-	+	+	Yes.
49.	F.	25	-	d.	w. of 50.	-	-	-	-	-	No.
50.	M.	28	47.	48.	h. of 49.	+	+	+	+	+	Yes.
51.	F.	60	d.	d.	s. of (48).	-	-	-	-	-	No.
52.	M.	60	-	-	h. of 53.	-	-	-	-	-	No.
53.	F.	55	d.	d.	w. of 52.	-	-	-	-	-	No.
54.	F.	25	52.	53.	s.55,56.	-	-	-	-	-	No.
55.	F.	18	52.	53.	s.54,65.	-	-	-	-	-	No.
56.	F.	14	52.	53.	s.54,55.	-	-	-	-	-	No.
57.	M.	42	-	-	h. of 58.	-	-	-	-	-	No.
58.	F.	42	-	-	w. of 57.	+	+	-	+	+	Yes.
59.	F.	22	57.	d.	s.60,61,(62-64)	-	-	-	-	-	No.
60.	M.	18	57.	d.	s.59,61, etc.	-	-	-	-	-	No.
61.	F.	13	57.	d.	s.59,60, etc.	-	-	-	-	-	No.
62.	M.	8	57.	58.	b.63,64,(59-61)	+	+	-	+	+	Yes.
63.	M.	7	57.	58.	b.62,64, etc.	+	+	+	+	+	Yes.
64.	F.	2	57.	58.	b.62,63, etc.	+	+	+	+	+	Yes.
65.	M.	65	d.	d.	-	-	-	-	-	-	No.
66.	F.	22	65.	-	-	+	+	+	+	+	Yes.

Abbreviations.

M.-mother.(in headline.) male. (under sex headline).
F.-father.(in headline). female.(under sex headline).
h.-husband. w.-wife. b.-brother. s.-sister. c.-cousin.
( )-step relative. d.-died.
AP.T.O.M.- as used in previous table.

Analysis of the survey.

	Males.	Females.	Total.
Normal.	9.	25.	34.
Trait.	13.	15.	28.
Disease.	3.	-	3.
Total.	25	40.	65.

It will be seen that about 50% of the people examined showed either the trait or the disease. Owing to the small numbers involved this high rate could be explained on normal statistical variation. It is, however, more likely to be due to the results of inbreeding in a community containing the trait. It would be interesting and instructive to observe the incidence of the trait in a large series of towns and villages, noting particularly the incidence in villages where inbreeding had occurred - i.e. in mountain villages.

The hereditary factors involved and brought to light in the survey will be discussed subsequently.

ETIOLOGY.

The Incidence of Mediterranean Anaemia in Cyprus.

The fourteen cases of the disease reported in this thesis were seen by me in a period of nine months. Other cases of the disease were seen, but investigation of them was not possible. Further cases were reported to me which I did not have the opportunity of studying. In general, I was in contact only with the area around Nicosia, and my knowledge of cases was confined to this region. The pathological laboratory of the Government Hospital, Nicosia, examined the blood of 15 cases of Mediterranean Anaemia in the course of the five months from March to July 1946. It is clear that far from being a rarity Mediterranean Anaemia is a very common disease in Cyprus. An estimate of the total number occurring in Cyprus at any one time must necessarily be a rough hazard. Adult cases may pass undetected, and many of the villages are not covered by medical supervision, which can be relied upon to diagnose the condition. Fawdry (1947), whose experience of the disease was obtained over a period of seven years spent in different parts of the island, considered that one case of the disease could be found in every two villages. This gives a total of about 400 in the island as a whole at any one time, and an incidence of one per thousand of the population. How he has computed the incidence in the towns such as Nicosia, Famagusta and Limassol, which contain a fair proportion of the population, is not clear. His figure of 400 does give an idea of the problem, however, and in this connection it is of interest to note that Wintrobe (1942) stated that less than a hundred cases had been recorded in the literature. The incidence of the trait is discussed in a separate section.

Regarding the distribution ofthe cases among the different races of the island, Fawdry (1947) noted that Greeks and Turks were equally affected. This is what would be expected from the incidence of the trait among these two communities. The fourteen cases recorded in this study were all Greek Cypriot, but this was purely fortuitous; I have seen cases among Turks. Fawdry never saw a case among the Armenian community; but it will be observed that an example of the trait was found in their number among the hundred Cypriots examined for the presence of the trait. Presumably the Armenians are not exempt. Fawdry further noted that males were more often affected than females in the proportion of three to one. This point will be considered in the section dealing with the hereditary aspect of the disease.

p.t.o.

ETIOLOGY (Continued).

The Cause of the Haemolysis in Mediterranean Anaemia.

While Mediterranean Anaemia shows the typical features of a haemolytic anaemia, it has not been stated in the literature whether the haemolysis is due to an abnormality of the erythrocyte, i.e. a dyshaemopoietic anaemia, or due to haemolysins in the plasma similar to those demonstrated by Chauffard and Vincent (1909) and Dameshek and Schwarz (1938) in the acquired type of acholuric jaundice. As in pernicious anaemia both increased haemolysis and dyshaemopoiesis occur. That the increased haemolysis is due to instability of the abnormal erythrocytes and not to any haemolysin in the plasma is shown by the following experiment. I am indebted to Fawdry for the details:

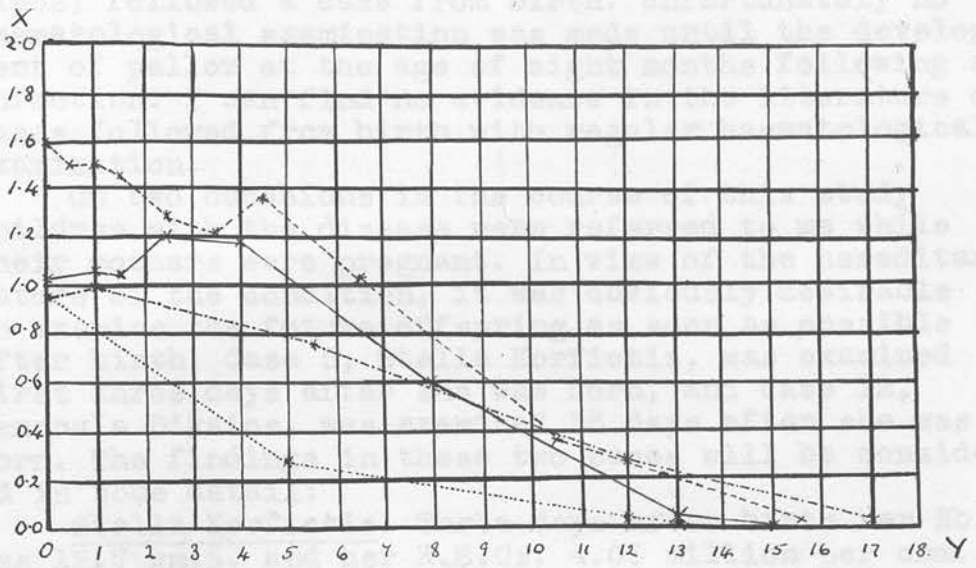
Cases of Mediterranean Anaemia of blood group A or B were transfused with blood from healthy Englishmen of blood group O, and the survival of their red cells was followed by the technique described by Ashley (1919). High titre group O serum was used to precipitate the recipient's cells, leaving the group O cells to be counted. The graphs showed that the cells survived up to about 100 days. The figures are of the same order as those of Callander, Powell and Witts (1945) for normal individuals. It may be concluded, therefore, that no abnormal haemolysins were present in the plasma. To one other child with Mediterranean Anaemia with a Hb. 3.6 gm.%. a transfusion of his father's blood was given, raising the Hb. level to 8.7 gm.%. The father showed the trait of the disease. In two weeks only the Hb. fell to 5.1 gm.%., during which time the child had daily fever and slight icterus. It was not possible to carry out differential R.B.C. counts, but it would seem that the abnormal red cells were destroyed abnormally rapidly. Further studies on this point are required. It is possible in this one case that some other cause for incompatibility may have operated. The result suggests, however, that even cells of the trait are more susceptible than normal to destruction in the circulation. Valentine and Neel (1944) point out the undesirability of transfusing cases of the disease with blood from their parents, a practice which has often been carried out.

The Development of the Disease.

The time of onset of the disease has been given as varying from shortly after birth up to the early years of life, usually the first two years of life. It has been shown in the section dealing with the presenting symptoms in the discussion of the symptomatology that the disease may be present long before the disease is recognised, and that the disease may in fact be present in adult life and yet not be recognised at all.

p.t.o.

Rate of destruction of one litre of normal group O blood by persons with Mediterranean Anaemia.



——— Melika Domus. Age 16 years. Group A.
------- Menachem Domus. Age 14 years. Group A.
-·-·-·- Alekos Euripedes. Age 27 years. Group B.
..... Phoebos Daniel. Age 3 years. Group A.

X Cells not agglutinated by Group O serum. Millions per c.mm.

Y Weeks since transfusion.

ETIOLOGY (Continued).

Age of onset as determined by symptoms is inaccurate and only haematological changes can be accepted in deciding the age of onset in any given case. Koch and Shapiro (1932) described a case which was recognised clinically at the age of three months. It was a severe case similar to case 4 in the series. Fawdry (1947) recognised six cases by this age. Whipple and Bradford (1932) followed a case from birth. Unfortunately no haematological examination was made until the development of pallor at the age of eight months following an infection. I can find no evidence in the literature of cases followed from birth with regular haematological examination.

On two occasions in the course of this study children with the disease were referred to me while their mothers were pregnant. In view of the hereditary nature of the condition, it was obviously desirable to examine the future offspring as soon as possible after birth. Case 5, Stella Korfiotis, was examined first three days after she was born, and case 12, Iannoula Dikaios, was examined 35 days after she was born. The findings in these two cases will be considered in some detail:

Stella Korfiotis. Three days after birth her Hb. was 12.7 gm.%. and her R.B.Cs. 4.05 million per cmm. This shows a definite anaemia, particularly in view of the normal figures for that age. A reticulocytosis of 8% was also present. The film showed the characteristic features of the trait - see plate IX. - there were no circulating normoblasts, and the changes were not as severe as would be found in the disease. At the age of 85 days, however, the Hb. had fallen to 6.4 gm.%. and the R.B.Cs. to 2.85 million per cmm. Circulating normoblasts were now present and the film was typical of the mild form of the disease - see plate X. It will be noted that despite the marked anisocytosis and poikilocytosis, the red cells are still quite well filled. Further observation at monthly intervals showed an increasing severity of the condition - see plates XI and XII - and she died from a respiratory infection at the age of 165 days. Estimation of her red cell fragility was done on each occasion that an examination was made, and it was found to be increased and at a constant level the whole time. Enlargement of the liver and spleen was noted at the age of 85 days.

Iannoula Dikaios. 35 days after birth her Hb. was 8.9 gm.%. and her R.B.Cs. 3.4 million per cmm. No circulating normoblasts were seen, and the film was characteristic of a severe degree of the trait. At the age of 71 days her Hb. had fallen to 7.1 gm.%. and her R.B.Cs. to 2.8 million per cmm. Circulating normoblasts were now present. A further fall in Hb. was noted on a

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ETIOLOGY(Continued).

subsequent occasion, and an increase in the number of circulating normoblasts. The red cell fragility was estimated on each occasion an examination was made. It was increased and was the same on each occasion. Enlargement of the spleen was noted at the age of 71 days.

These two cases show the development of the condition very clearly. It is impossible to date the actual onset of the disease, since the process is a gradual one. In both cases there was anaemia present at birth. The presence of circulating normoblasts as a criterion of the presence of the disease is inadequate here, since at the age of 35 days Iannoula Dikaios had a Hb. of only 8.9 gm.%., but no circulating normoblasts were seen. In both these cases it has been shown that a marked degree of anaemia was present at a time when the mothers of the infants did not regard their offspring as at all unwell.

The development of the anaemia in these two cases tempts one to put forwarda hypothesis regarding the factors operating behind the development. As will be shown in the next section, the most likely cause of the condition is failure in the synthesis of haemoglobin. This may be due to the absence of some agent acting as a catalyst. In order to produce such a gradation of anaemia from the mild to the severe cases, such an absence would have to be relative. There might, of course, be some inhibiting factor acting in various degrees on the catalyst. This variable defect would be the link with the mutated gene causing the disease, and would be the fundamental error in Mediterranean Anaemia. This theory will be elaborated further in the next section. If it is correct, one would expect the development of the disease in the first few months of life. The disease would not be fully developed at birth, since the deficient factor could be partially supplied by the mother, herself partially deficient by virtue of herself having the trait of the disease. As the supply of this substance from the mother is cut off at birth, anaemia will develop when the supply is used up. The infant's Hb. will fall to a level dependent on how far the infant can supply the factor in question from its own resources, and clearly this level will be maintained for the rest of the patient's life except in so far as it is affected by external influences such as infections, poor diet, nephritis, etc. This theory will have to be tested by following further cases from birth. Age of onset of the disease judged clinically is of no value; haematological investigation carried out regularly from birth is what is required.

p.t.o.

ETIOLOGY (Continued).

Carrying the theory further, cases of the trait born of mothers with the trait and normal fathers should show the trait at birth. If the father has the trait and the mother is normal, offspring with the trait should be normal at birth, since the normal mother can supply the missing factor, but the trait should develop in the course of the next few months. I have only elaborated this theory since I left Cyprus, and I have not had the opportunity to test it out in practice.

Theories of Etiology.

Before the hereditary nature of Mediterranean Anaemia was discovered, there were many theories as to its causation. Local superstition in Cyprus held that only breast-fed babies contracted the disease, and many still hold that view. The case of Stella Korfiotis disproved this hypothesis.

The Greek schools have considered chronic malaria to be the cause of the condition, as shown by the writings of Choremis and Spiliopulos (1936) and Nittis and Spiliopulos (1937). They invoke various factors on a broad basis by suggesting an inheritable functional inferiority of the haemopoietic system, that began with the parents or ancestors through the noxious effects of syphilis, malaria or tuberculosis. This congenital inferiority of the haemopoietic system renders it more favourable for the development of Mediterranean Anaemia through subsequent toxic, infectious or nutritional damage. They cite malaria as the principal etiological factor in Greece. This 'toxic, infectious or nutritional damage' could not be demonstrated in any way in the cases shown, though infection may have brought some of the cases to light. The success of these workers with quinine has not been confirmed by other workers. It was usual in Cyprus to find that cases had had repeated courses of quinine both orally and by injection without any benefit at all. With the falling incidence of malaria in Cyprus the possibility of confusing the two conditions is becoming less frequent.

The rhesus factor has been considered under symptomatology, and has been shown to play no part in the etiology of this condition.

Munford (1931) quoted by Flynn (1943) suggested an obscure infection as the cause, or more probably an inherited endocrine deficiency. Yaguda (1935) suggested exhaustion of some maternal hormone necessary for the stimulation of normal bone formation in the foetus. No evidence was given in support of these hypotheses.

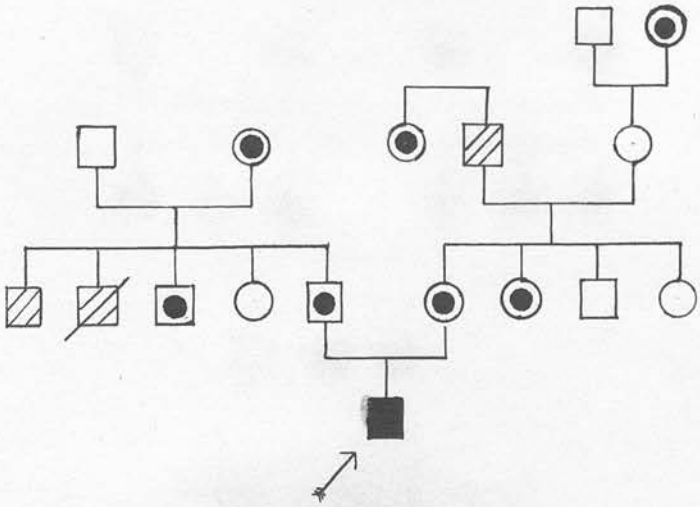
p.t.o.

ETIOLOGY (Continued).

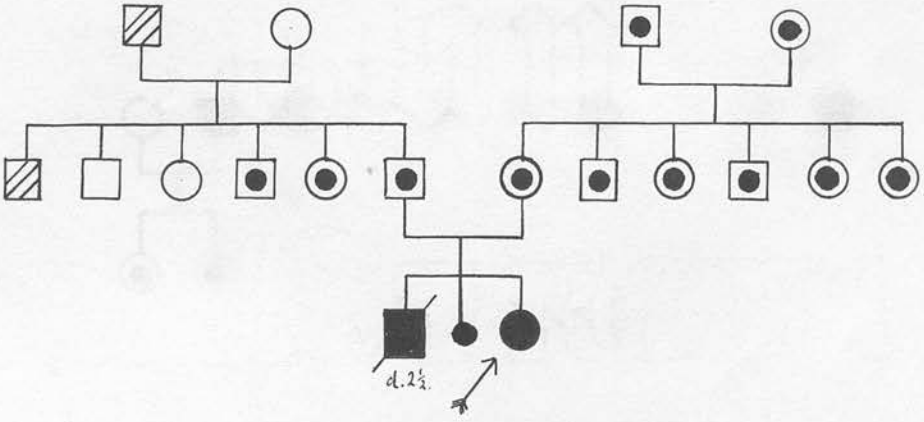
The hereditary nature of the disease will be discussed in a separate section, but it is pertinent to enquire here into the actual modus operandi of this hereditary factor. The most likely cause of the condition is some failure in haemoglobinisation of the red cell. The evidence in favour of this is strong. The red cell morphology resembles that found in iron deficiency anaemia. The red cell seems to be formed with an excessive membrane, but with little substance. There is no shortage of iron, since iron pigment is found in the tissues in large quantities, but rather a defect in its utilisation. Histologically pigment distribution is more that of haemochromatosis than that of a haemolytic anaemia. This was clearly shown by Whipple and Bradford (1936). This suggests some inborn error in metabolism. The marrow hyperplasia is probably an attempt to compensate for the faulty red cell formation. It will be noted that erythropoiesis proceeds normally to the normoblast stage. It is in the production of the final erythrocyte that deviation from normal takes place. This is the stage when haemoglobin is added to the red cell.

As has been suggested in the previous section, it is possible that there is some catalyst in the production of haemoglobin, which is either deficient or is inhibited in this condition. In the trait the factor can act only partially in the formation of haemoglobin, whereas in the disease its action is further limited, so that an anaemia develops to a degree depending on the availability of the factor concerned. Many problems of iron metabolism and the formation of haemoglobin require investigation. Since cases of Mediterranean Anaemia are seen in the Italian communities in America, it is to be hoped that studies with radio-active iron will be carried out.

THE FAMILY PEDIGREES.



THE FIRST FAMILY.



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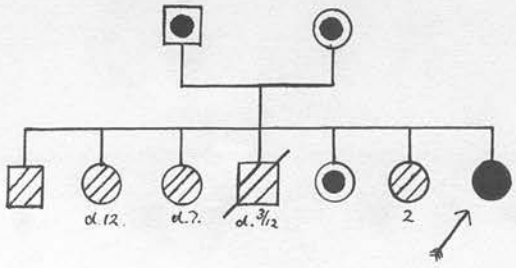
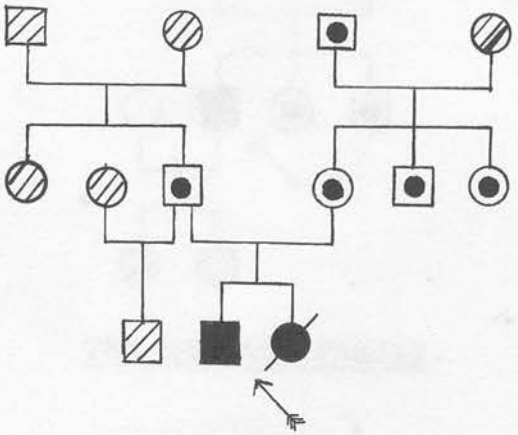
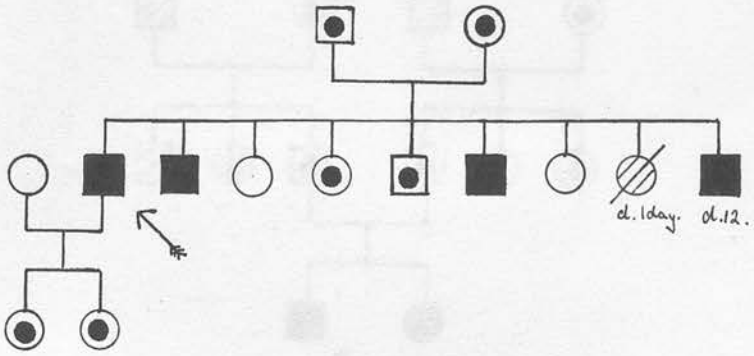


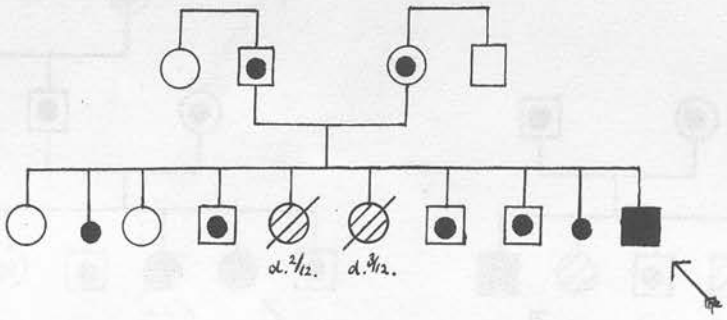
THE THIRD FAMILY.



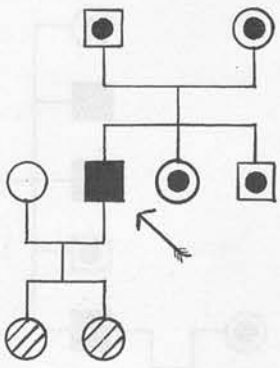
THE FOURTH FAMILY.



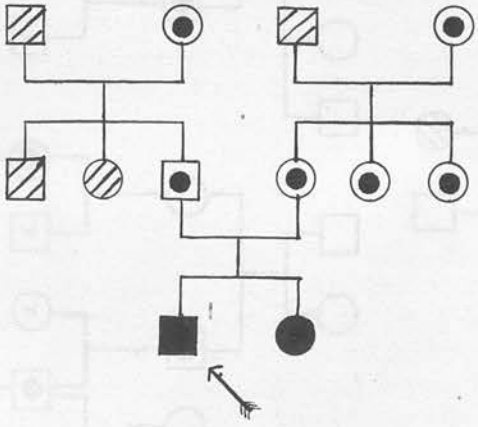
THE FIFTH FAMILY.



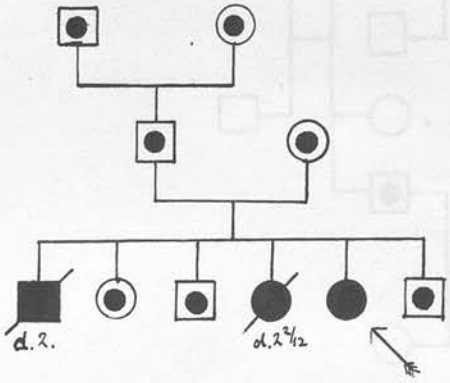
THE SIXTH FAMILY.



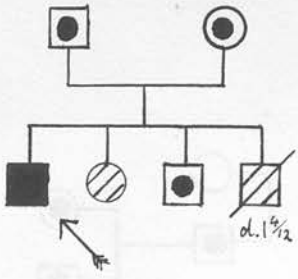
THE SEVENTH FAMILY.



THE EIGHTH FAMILY.

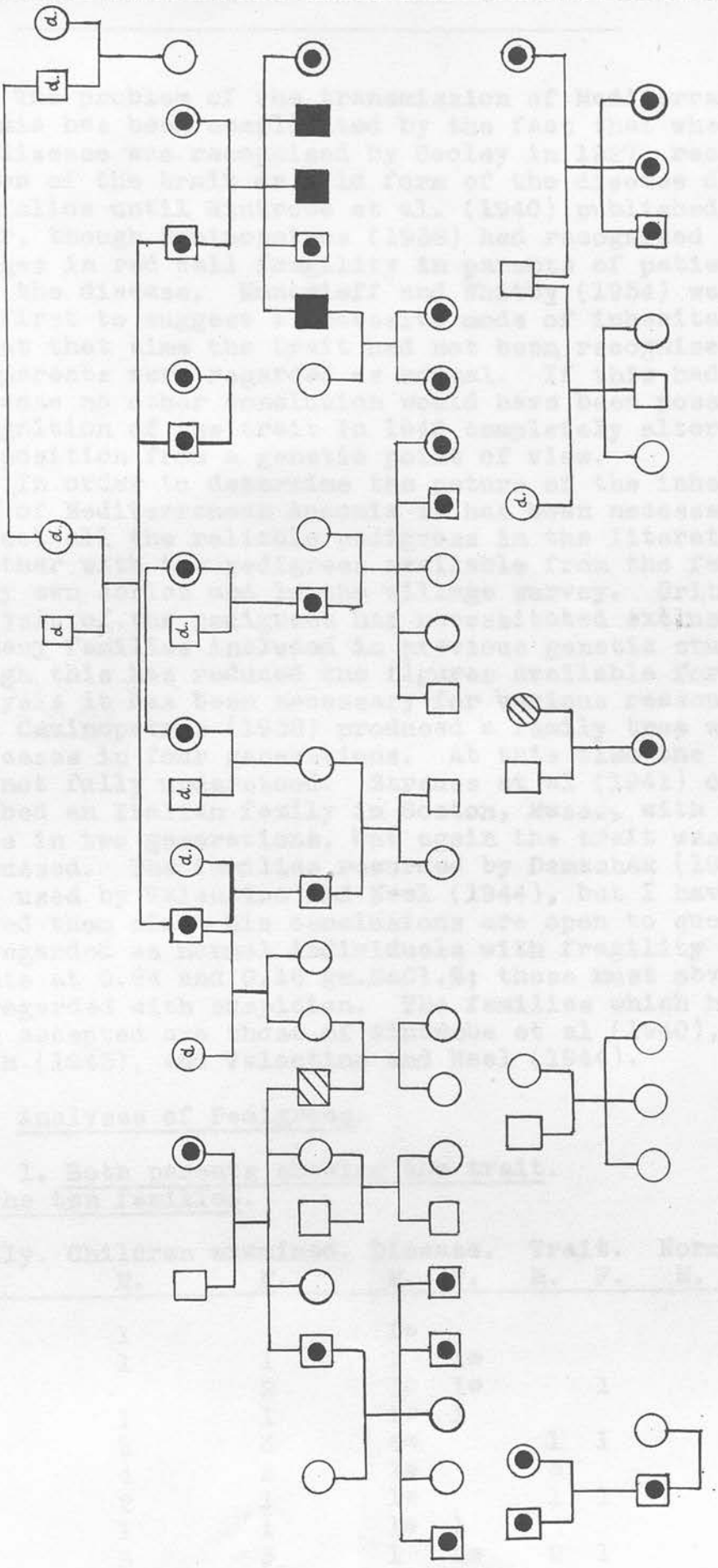


THE NINTH FAMILY.



THE TENTH FAMILY.

RELATIONSHIP OF THE INHABITANTS OF ALITHINOU SHOWING THE INCIDENCE OF MEDITERRANEAN ANAEMIA AND THE TRAIT.



The problem of the transmission of Mediterranean Anaemia has been complicated by the fact that whereas the disease was recognised by Cooley in 1927, recognition of the trait or mild form of the disease did not follow until Wintrobe et al. (1940) published their paper, though Caminopetros (1938) had recognised changes in red cell fragility in parents of patients with the disease. Moncrieff and Whitby (1934) were the first to suggest a recessive mode of inheritance, but at that time the trait had not been recognised, and parents were regarded as normal. If this had been the case no other conclusion would have been possible. Recognition of the trait in 1940 completely altered the position from a genetic point of view.

In order to determine the nature of the inheritance of Mediterranean Anaemia it has been necessary to collect all the reliable pedigrees in the literature together with the pedigrees available from the families in my own series and in the village survey. Critical analysis of the pedigrees has necessitated exclusion of many families included in previous genetic studies. Though this has reduced the figures available for analysis it has been necessary for various reasons. Thus Caminopetros (1938) produced a family tree with ten cases in four generations. At this time the trait was not fully understood. Strauss et al (1941) described an Italian family in Boston, Mass., with ten cases in two generations, but again the trait was not discussed. The families recorded by Damashek (1943) were used by Valentine and Neel (1944), but I have rejected them since his conclusions are open to question. He regarded as normal individuals with fragility end points at 0.24 and 0.16 gm.NaCl.%; these must obviously be regarded with suspicion. The families which have been accepted are those of Wintrobe et al (1940), Smith (1943), and Valentine and Neel (1944).

Analyses of Pedigrees.

1. Both parents showing the trait.

a. The ten families.

Family.	Children examined.		Disease.		Trait.		Normal.	
	M.	F.	M.	F.	M.	F.	M.	F.
1	1		1*					
2	1	1	1	1*				
3		2		1*		1		
4	1	1	1*	1				
5	5	3	4*		1	1		2
6	4	2	1*		3			2
7	2	1	1*		1	1		
8	1	1	1*	1				
9	3	3	1	2*	2	1		
10	2		1*		1			

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THE GENETICS OF MEDITERRANEAN ANAEMIA. (Continued)

Family.	Children examined. M.	F.	Disease. M.	F.	Trait. M.	F.	Normal. M.	F.
2ø	2	4			2	4		
9ø	1				1			
Total.	23	18	12	6	11	8		4
	41		18		19		4	
b. Alithinou.								
ø	1				1			
Total	24	18	12	6	12	8		4
	42		18		20		4	
c. Smith (1943).								
J.A.	3		2		1			
C.A.		1		1				
I.C.	1	2		1	1			1
A.DiB.	2		1		1			
J.F.	2	1	1		1			1
D.M.	3		2		1			
A.&N.P.	2(twins)		2					
J.P.	1	2	1	1		1		
S.S.	1	1	1	1				
Total	15	7	10	4	5	1		2
	22		14		6		2	
d. Valentine & Neel (1944).								
Y.	1	2		1	1	1		
Sc.	2	1	1		1	1		
Si.	1	4		1		2	1	1
Total	4	7	1	2	2	4	1	1
	11		3		6		2	
e. Wintrobe(1940).								
1.	2	2	1		1			2
Grand totals.								
Banton	24	18	12	6	12	8		4
Smith	15	7	10	4	5	1		2
Val.&Neel	4	7	1	2	2	4	1	1
Wintrobe	2	2	1		1			2
	45	34	24	12	20	13	1	9
	79		36		33		10	

Abbreviations: M. - Male. F. - Female.
* - Propositus.
ø - These families are the result of random selection. All the other families recorded by Banton were chosen because a known case of Mediterranean Anaemia was found in each one of them.

Criteria for selection of the above families.

In most instances both parents were examined and shown to have the trait. Since, as is shown later, the trait occurs in both parents of cases of the disease, it has been assumed that where there are cases of the disease, both parents must have the trait.

THE GENETICS OF MEDITERRANEAN ANAEMIA. (Continued).

2. Mating: Disease (male) X Normal (female).

a. The ten families.

Family No. 5. Two female children show the trait; no normals or cases of the disease.

3. Mating: Trait X Normal.

a. The ten families.

Family.	Sex of parent with trait.	Children. M.	Children. F.	Trait. M.	Trait. F.	Normal. M.	Normal. F.
1.	F.	2	1	2			1
1.	F.		1				1
	F.	2	2	2			2
1.	M.	1	3		2	1	1
2.	M.	3	2	2	1	1	1
	M.	4	5	2	3	2	2
b. Alithinou.							
	F.	1	2	1			2
	F.	1	2	1	2		
	F.	2	4	2	2		2
	M.	3	2	3			2
	M.	1	1			1	1
	M.	2	3	1	1	1	2
	M.	6	6	4	1	2	5
c. Smith (1943).							
A.B.	M.	1	1	1	1		
A.& J.S.	M.		1		1		
	M.	1	2	1	2		
F.De P.	F.	1	2	1	2		
M. G.	F.	1		1			
F. R.	F.	1	3	1	2		1
A.&J.S.	F.	2		2			
D. S.	F.	2		2			
	F.	7	5	7	4		1
d. Wintrobe (1940).							
	M.	1	2	1	1		1
e. Valentine & Neel. (1944).							
Y.	M.	3	5	1	4	2	1
Y.	F.	3	2	3	1		1
Sal.	F.	2	1	2	1		
	F.	5	3	5	2		1
Totals.							
Banton.	F.	2	2	2			2
	F.	2	4	2	2		2
Smith.	F.	7	5	7	4		1
Val.&Neel.	F.	5	3	5	2		1
	F.	16	14	16	8		6
		30		24		6	

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THE GENETICS OF MEDITERRANEAN ANAEMIA. (Continued).

Family.	Sex of parent with trait.	Children. M.	Children. F.	Trait. M.	Trait. F.	Normal. M.	Normal. F.
Totals.							
Banton.	M.	4	5	2	3	2	2
	M.	6	6	4	1	2	5
Smith.	M.	1	2	1	2		
Wintrobe.	M.	1	2	1	1		1
Val.& Neel.	M.	3	5	1	4	2	1
	M.	15	20	9	11	6	9
		35		20		15	
Grand total.							
	F.	16	14	16	8		6
	M.	15	20	9	11	6	9
		31	34	25	19	6	15
		65		44		21	

Criteria for selection of the above families.

1. Both parents examined: One found to have the trait and one found to be normal.
2. One parent examined and found to be normal, yet offspring present with the trait.
3. Exclusion of families with one unknown parent and one with the trait, since the unknown parent might have the trait.

The parents of affected offspring.

In all the ten families of my series it is noted that both parents of a case of the disease manifest the trait themselves. In the six families in the series recorded by Smith (1943), in which both parents were examined, the trait was again present in them both in all the six cases. This observation can also be noted in two families of Valentine & Neel (1944), one family of Wintrobe (1940), and four families of Damashek (1943). Fawdry (1947) had observed this feature in many cases since his attention had been drawn to it by Smith's paper (1943). He had not recorded the number of cases observed, but he gave me details of one such case. He had further noted that when both parents of a case were examined, he had invariably found both to show the trait. He had noted no exceptions to this rule. There are, therefore, 24 families in which this observation has been recorded, and many more in which it has been observed but not recorded. Smith (1943) refers to one exception he had observed in incomplete studies of one unreported family. It is clear then that as a general rule both parents of an affected offspring show the trait, though rare exceptions may occur. The reason for such exceptions will be discussed later.

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THE GENETICS OF MEDITERRANEAN ANAEMIA. (Continued).

Offspring of various matings.

Where both parents show the trait, it is seen from the analysis that offspring may show either the disease or the trait or they may be normal. An excess of males with the disease or trait, and an excess of normal females is noted and will be referred to later. Matings of normal individuals with those showing the trait were found to give rise to offspring who were either normal or showed the trait. This occurred irrespective of which parent showed the trait. In only one mating of a father with the disease and a normal mother were the offspring observed. In this case both children showed the trait. Since the disease is usually fatal in childhood, it is unusual to find cases of the disease having children. In the series recorded it was found on two occasions: Louis Loizou and Dimos Ioannou.

Relationship to other hereditary factors.

ABO blood groups and colour of eyes were recorded in all the family studies. No relationship to the inheritance of either of these phenotypes was observed. In the few cases in which the rhesus factor was determined no relationship to its inheritance was found. Caminopetros (1938) stated that the affected offspring always belonged to the same blood group as the carrier parent. The trait of the disease was not fully understood when his article was written, and as it is now clear that both parents have the trait, his observation ceases to be apposite. Inheritance of the trait, as opposed to the disease, also does not show any evidence of linkage with the inheritance of the ABO blood groups or colour of the eyes.

Sex incidence.

Analysis of the ten families, including authentic cases of the disease who had died and were not examined by me, show 12 males and 6 females to be affected. Including the figures quoted from other workers, the disease has been found to affect 24 males and 12 females. This predominance in males has also been noted by Fawdry (1947). Though he did not quote his figures he found the disease to affect males three times more often than females. Why this should occur is not clear. Sex predominance is very common throughout medicine, and apart from its occurrence in such conditions as Haemophilia, which is sex linked, the explanation is usually obscure. There is no evidence of sex linkage in this condition.

Two possible explanations have been put forward. Firstly, the gene may be more lethal to the female than to the male and cause intrauterine death. If this were so, it would not affect the normal offspring. One would therefore expect an equal number of normal male and female offspring occurring among the siblings of cases of the disease. Secondly, the penetrance of the

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gene may be less complete in the female than in the male. In this case, as a result of failure of penetrance, a number of female offspring should appear as normal instead of showing the trait. Analysis of the figures quoted shows one normal male and nine normal female siblings of the cases of the disease. Though the figures are small, they favour the theory of incomplete genetic penetrance in the female. One would also expect an excess of cases of the trait in males. In homozygous matings 20 males to 13 females showed the trait, and in heterozygous matings 25 males to 19 females were affected. In the heterozygous matings also there were 15 normal females to 6 normal males. These figures are less striking, and once again only small numbers are involved. In Fawdry's survey of 532 school children, quoted elsewhere in this thesis, no significant sex preponderance of the trait was observed. Clearly the problem is complex and that larger figures must be obtained before any conclusions can be drawn.

Theories of inheritance.

Three theories have been advanced. These have been analysed by Valentine and Neel (1944) and they require further consideration.

1. Mediterranean Anaemia is the result of homozygosity for a factor which when heterozygous gives rise to the trait. The factor itself is inherited as a mendelian dominant. If the heterozygote were regarded as normal, the disease would be inherited as a mendelian recessive. This had been suggested before the trait was recognised by Moncrieff and Whitby (1934), Caminopetros (1938) and Damashek (1943).

2. Cooley (1941) and Smith (1943) suggested that the severe and mild conditions have the same genetic basis and are due to a dominant factor which is variably expressed. In one person heterozygous for the factor a severe degree of anaemia may develop, whereas in another there may be only slight changes. The difference is presumably due to the effect of environmental and genetic modifiers.

3. McIntosh and Wood (1942) proposed that the disease is caused by the simultaneous presence of two nonallelomorphic dominant factors, one inherited from each parent.

As has been stated all recorded cases of the disease show both parents affected with the trait, when these have been examined. This is not in keeping with the second hypothesis, since only one parent need be affected to produce the condition. Since matings between 'affected x normal' would be much commoner than between 'affected x affected', the bulk of the cases would be expected to occur as a result of the former type of mating. In McIntosh and Wood's theory two

p.t.o.

different dominant factors are postulated. Since both parents show the same type of abnormality, one is required to accept the improbable suggestion that two different genotypes produce the same phenotype. The first theory is the only one which will fit the facts.

If one accepts the first theory, then certain proportions of offspring with the disease and with the trait are to be expected. In matings where both parents are heterozygous for the factor, i.e. showing the trait, then the proportion of cases with the disease to cases with the trait to normals should be 1 : 2 : 1. In the analyses given, the ratio is 36 : 33 : 10. In matings between a heterozygote and a normal offspring should show the trait or be normal in equal proportions. In the analyses the ratio is 44 : 21. These figures are not suitable for simple analysis. There are distorting factors which must be taken into consideration. Families with cases of the disease were analysed because these cases presented. This clearly gives a higher proportion of cases of the disease than normal. In the study by Valentine and Neel (1944) Hogben's method (1933) was used to correct for this error. This, however, is not entirely satisfactory, and I am advised that with figures as small as these and with such variable distorting factors detailed statistical analysis would not give any conclusive results. It is more important to consider each family analysed and see if any fail to satisfy the conditions laid down for a given hypothesis. By this standard all the families recorded satisfy the first hypothesis.

Variation in severity of the disease and trait.

As has been shown already both the disease and the trait show a wide variation in intensity. Valentine and Neel (1944) considered that the blood picture fell into two fairly well defined groups - the mild case or trait and the severe case. Only relatively few cases were found in between these two extremes. From the experience of this study and from the widening experience of cases reported in the literature, it seems that such a distinction is not valid. It would be useful, if one could accept such a hypothesis, since that would fit in well with the theory of heterozygous and homozygous responsibility for the trait and for the disease. It is much more likely, however, that the variation is due either to variable penetrance of the gene or to modifiers, both genetic and environmental. In practice no specific environmental factors could be recognised. In Cyprus the disease was found to affect rich and poor, town and country dweller alike, and no relationship to any other disease was recognised. It seems likely that the genetic modifiers play the more important role.

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THE GENETICS OF MEDITERRANEAN ANAEMIA. (Continued).

This theory offers an explanation of why Smith (1943) found a case of the disease in which only one parent showed the trait. Since the differentiation between the trait and the disease is arbitrary, it is quite possible for genetic modifiers to act in such a way that the factor responsible might produce a severe enough effect even in the heterozygous state to give rise to the disease. It will also explain why cases in the same family usually appear to show the same degree of severity, since the genetic modifiers would be the same in each family.

Expected incidence of Mediterranean Anaemia.

It has been shown that approximately 20% of the population of Cyprus show the trait of the disease; in other words, one in five of the population is affected. The chance of mating between two such affected persons is therefore one in twentyfive. On the theory of inheritance just expounded one would expect one in four offspring of such a mating to give rise to the disease. This gives an incidence of one in a hundred births. This, however, does not take account of the effect of genetic penetrance and modifiers, nor of intrauterine death of the fertilised ovum. It is also possible that fertilisation itself is affected. It is impossible to compare this figure of one in a hundred with Fawdry's (1947) suggested incidence of the disease of one per thousand in the general population. The result is further distorted by the fact that the severe disease is fatal in early infancy or childhood, and therefore the proportion of the general population affected by the disease is still further reduced. Any calculations on this basis could only be made after an extensive analysis of the island to find the true incidence of the trait and the disease, together with accurate observations on the age at death of cases of the disease, and the normal expectancy of life of the Cypriot.

Race limited disease.

Mediterranean Anaemia is found in a limited region of the Mediterranean seaboard. Greece, Italy, Sicily and Sardinia all show a number of cases as well as the high incidence now recorded in Cyprus. Cases have been reported however from Turkey, Syria and even as far west as Spain. Cases have been recorded in other countries when parents with the trait have emigrated. The majority of such cases have been recorded in America, but some have been seen in England: Moncrieff and Whitby (1934) and Rubie (1948). Apart from this, sporadic cases have been recorded as far afield as India, China and South America. In these

p.t.o.

cases there was no evidence of the parents being remotely connected with the Mediterranean. The case recorded by Bywaters (1938) in an English child is an example of this.

The theory of genetic mutation explains these observations satisfactorily. A genetic mutation occurs and if breeding is continued in a localised community then the mutation persists and establishes itself in that community. As Cooley (1941) has pointed out, there is no such thing as a race limited disease. The spread of hereditary disease is limited only by the isolation of the community in which it is found. In the case of Mediterranean Anaemia the mutation probably occurred several centuries ago in Greece, and the disease has accordingly spread to the neighbouring countries. Cooley (1941) considered that the Mediterranean races show a predisposition to genetic mutations giving rise to blood dyscrasias. The sporadic cases recorded far afield from the Mediterranean are thus the result of a fresh mutation, identical with the one which probably originated in Greece. If fortuitously such mutations were given the opportunity to establish themselves, then fresh foci of the disease would occur.

Conclusions.

From the data analysed it is clear that Mediterranean Anaemia does not follow exactly any given Mendelian pattern. This, however, is typical of mammalian inheritance in general. The explanation which fits the facts most closely is to postulate a mutation of a autosomal gene. This abnormal factor is inherited as a Mendelian dominant. In the heterozygous state it gives rise to the trait. When the mutation occurs in its allelomorph, i.e. in the homozygous state, the disease is produced. Variation in severity of either the disease or the trait is due essentially to the effect of genetic modifiers. These conclusions are similar to those reached by Gates (1946). It should be added that it is exceptional in human genetics to find a condition in which a trait can be recognised with ease and with certainty.

DIAGNOSIS.

The clinical picture described is so characteristic that there is no difficulty in diagnosing the condition when it presents. In Cyprus two conditions have to be differentiated, since they give rise to anaemia and splenomegaly. These diseases are chronic malaria and kala-azar. Both can be differentiated from Mediterranean Anaemia by haematological examination. Sickle cell anaemia, which occurs in the negro races, is not seen in Cyprus. Haden and Evans (1937), however, reported a case from Sicily showing target cells as well as sickle cells. This condition has much in common with Mediterranean Anaemia, but the sickle cells serve to differentiate it.

TREATMENT.

No attempt was made to treat the cases described in the series from the point of view of their anaemia, though symptomatic treatment was given for minor complaints. This was partly because there was not adequate time to observe the effect of any specific line of therapy, but essentially because most known therapeutic agents have been tried and found wanting. Wintrobe (1946) states that this is the one form of hypochromic microcytic anaemia which does not respond to iron therapy. Iron, supplemented by copper, manganese, Vitamin B_6, and pentnucleotide have all been tried. The gamut of endocrine preparations, thyroid, oestrogens, etc. have also proved to be of no avail. Liver has no effect. There are no reports of the effect of folic acid, but a response to this therapy is not to be expected since this agent is effective only when a megaloblastic marrow is found, and this never occurs in Mediterranean Anaemia. Since malaria was cited by the Greek physicians as the cause of the disease, quinine therapy has been well tried and has had its advocates:(Nittis and Spiliopulos, 1937; Choremus and Spiliopulos, 1938; Grillo, 1939). Radiotherapy to the spleen and spray irradiation to the haemopoietic system to prevent continued escape of immature red cells into the peripheral blood stream have be en thoroughly investigated by various workers: (Koch and Shapiro, 1932; Nusbaum, 1931; Hunter, 1936). The consensus of opinion is that no benefit resulted from these measures.

Hopes of success were entertained from splenectomy. Case 10, Louis Loizou, had his spleen removed, but the condition remained and there was little change in his

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TREATMENT (Continued).

Hb. level. An increase in circulating normoblasts was noted - this is the usual response in these cases to splenectomy. While one would not expect a cure from splenectomy, one would anticipate some alleviation of the condition. The abnormal red cells are destroyed more rapidly than normal in the circulation, and after removal of one of the main sites of red cell breakdown, one would expect that the red cells would survive longer, and the Hb. level be raised accordingly. This is what occurs in acholuric jaundice. The results of splenectomy have been uniformly disappointing, and there is no justification for the operation. It has, in fact, been generally abandoned. Why the disease does not show improvement after splenectomy is not clear. It will be remembered, however, that the distribution of the iron pigment was not that found in cases of haemolytic anaemia, but rather that seen in haemochromatosis. The significance of this is not known, but it is possible there may be some correlation with the failure of splenectomy.

Blood transfusion is the only means available for alleviating the condition. In view of the temporary nature of the response, this was not attempted in the series, though I saw cases transfused at the Government Hospital, Nicosia. Fawdry (1947) had considerable experience of transfusion, and from the few cases I saw transfused I concur with his observations. The anaemic child in a few hours is transformed from a helpless gasping creature into a more or less normal individual able to work and play with pleasure, but in the course of a few weeks he slips back into his original condition. The answer to the problem, therefore, as far as relief of symptoms is concerned, is to keep the child's blood in a condition approximating to normal, by transfusion repeated at about two monthly intervals for life. Repeated transfusions bring severe reactions; veins become thrombosed and intratibial marrow drips must be used. Compatible donors become harder to find. Clearly it is doubtful if a life of semi-invalidism, punctuated by repeated transfusions is better than no life at all. Occasionally, however, improvement may be maintained following transfusion, but whether this is post hoc or propter hoc cannot be stated. Fawdry tells of one child in a moribund condition with a Hb. 2.2 gm.%., who required transfusions at three monthly intervals, her Hb. falling to this low level after each one. After her third transfusion her improvement was maintained for almost a year, her Hb. ranging between 5.8 and 7.3 gm.%. during this time. The effect of maintaining a child's blood at approximately normal strength over a period of years has not yet been determined.

COURSE AND PROGNOSIS.

Since the cases in this series were observed over a period of only nine months, and the records are confined in most instances to one examination, no conclusions can be drawn regarding the course of the disease. One case only was 'complete' in that it was followed from birth to death. Quoted figures for case 3, Maritsa Ioannou, and case 10, Louis Loizou, showed that variation in the Hb. level only takes place in slight degree. This conforms with previous observations. The disease is not punctuated by haemolytic crises such as occur in acholuric jaundice. Occasionally, however, spontaneous improvement may take place. Fawdry (1947) told me of an adult aged 20 years, who when first seen in 1940 was only 4' 8¾" in height, and was profoundly disabled. In 1946 he had grown five inches, his abdominal protruberance was scarcely noticeable, and his general disability was very slight.

In a disease showing such variations in intensity as have been demonstrated, the prognosis is naturally very variable. In the severest cases death occurs at an early age, as in case 5, Stella Korfiotis. As in this case the cause of death is usually an intercurrent infection to which the severely anaemic child succumbs. At the other end of the scale cases can occur without the patient being aware of the disease, and showing no limitation of activity. Case 10, Dimos Ioannou, was an example of this type. Though these mild cases do not suffer any apparent disability, it is likely that they have a diminished resistance to infections, and re-act poorly to those conditions which normally cause anaemia, e.g. chronic nephritis. Thus the prognosis depends largely on whether the haemoglobin level is adequate to maintain resistance against the infections to which the individual is subjected. The ability of the impaired haemopoietic system to respond to particular calls made upon it for an increased response must also be borne in mind.

Baty et al. (1932) pointed out that when the disease is discovered in the first year of life death often occurs within six months. As has already been stated, the diagnosis of the disease in the first year of life is dependent on so many factors, that this statement does no more than emphasise the high mortality of the severe cases. Most of the children in whom the disease is recognised in the first two years of life die before puberty. Fawdry (1947) considered that if the Hb. level is 5.8 gm.%. or higher at the age of five, the child had a good chance of surviving to adult life.

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COURSE AND PROGNOSIS (Continued).

When a case of Mediterranean Anaemia has occurred in a family, the parents require a prognosis regarding any future offspring. In view of the hereditary nature of the condition, as complete a family history as possible should be obtained, and as many members as are available should be examined for the trait.. It is essential to determine to what extent the parents show the trait, and to examine any other children for the trait or the disease. It has been suggested in the section on symptomatology that when the disease is found in a family, its degree of expression remains roughly the same, though exceptions to this rule do occur. If this is true, one has some idea of the prospects of the disease in any further affected children. As in nearly all cases the parents will be heterozygous for the affected gene, the chances of producing an offspring homozygous for this particular gene are one in four. While individual families do not clearly follow any given Mendelian pattern, it is quite likely that such parents will have children showing only the trait or even being haematologically normal.

In view of the high incidence of the trait, this problem of inheritance of the disease is of great importance to Cyprus. The affected gene is sublethal only in the homozygous state & the race itself is in no danger of extinction. It would be desirable to warn couples showing the trait of the possible consequences to their offspring, should they marry, particularly if they both are severe cases of the trait. It is unlikely in Cyprus that the prospect would dissuade any such couple from marrying.

SUMMARY.

Ten families were investigated showing cases of Mediterranean Anaemia. The clinical features of the cases were described and haematological investigations were carried out on the relatives to demonstrate the trait of the disease. Three examples of the trait were recorded in detail.

Symptomatology was discussed. The variation in severity of the condition was stressed. Pallor and fever were analysed as presenting symptoms and it was pointed out that they form a fallacious index of the age of onset of the condition. It was shown that most of the symptoms were the result of the anaemia. The facies of the patients was discussed and the term mongoloid was criticised. Two new observations: Ulceration of the legs and premature closure of the fontanelle were described. The occurrence of symptomless cases was also stressed. It was shown that the severity of the condition is roughly the same in affected siblings.

The principal features of the blood changes in the disease and in the trait were described. The disease was separated from the trait by the arbitrary, and not entirely satisfactory, distinction of whether circulating normoblasts were present or not. The significance of the anisocytosis and poikilocytosis was discussed. The morphology of target cells and other abnormal forms was considered. The decreased erythrocyte fragility was correlated with the flattening of the red cells and with the presence of target cells. The essentials required for the diagnosis of the trait were stated to be anisocytosis and poikilocytosis and decreased red cell fragility. Target cells, though usually present, were not essential. Bone marrow studies revealed a normoblastic response of varying degree. In early childhood a leuco-erythroblastic response was noted.

The radiological appearances of the skeleton in nine of the cases were described. It was shown that the essential change was loss of bone substance at the expense of a proliferating marrow. The whole skeleton might be involved, though there was a predilection for certain areas. Changes in the frontal bone and in the metacarpals were among the most frequent recorded. Osteoporosis, diploic thickening, widening of bone shafts, reticulation and striation were shown to be the main changes. How these changes affected individual bones and how they varied with age was also demonstrated. The severity of the changes was roughly proportional to the degree of anaemia and the age of the patient. The radiological changes were correlated with the pathological findings. It was pointed out that the changes were not specific for Mediterranean Anaemia, but might be found in other haemolytic anaemias.

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SUMMARY. (Continued.)

The incidence of the trait of the disease in Cyprus was investigated. The results of examining 100 Cypriot adults and 532 school children were recorded. It was concluded that about 20% of the population showed the trait. There was no significant difference between the incidence in the sexes and between the Greek and Turkish Cypriots.

A survey of an isolated mountain village was carried out. It demonstrated clearly the hereditary nature of the trait and the disease and how the incidence of these abnormalities could be increased as a result of inbreeding.

Etiology was discussed. The incidence of Mediterranean Anaemia in Cyprus was investigated and the suggestion was made that about 400 cases existed at any one time in the island. An experiment was quoted to show that the haemolysis which takes place in the disease is the result of dyshaemopoiesis and is not due to haemolysins in the serum. The development of the disease from birth was illustrated by two cases, and suggestions were made regarding the underlying mechanisms. Theories of etiology were advanced, and it was suggested that the most likely cause of the disease was an inborn error in metabolism, resulting in failure of haemoglobinisation of the red cell.

The genetic aspect of the disease was analysed, and results of other workers were quoted. It was shown that cases of the disease invariably have parents who both show the trait. No relationship to the ABO blood groups, colour of eyes, or rhesus factor could be demonstrated. The sex incidence of the disease showed a preponderance among males, but the reason for this is not clear. Theories of inheritance were discussed and it was concluded that the most likely explanation was an abnormality of an auotsomal gene, inherited as a Mendelian dominant, which in the heterozygous state gives rise to the trait and in the homozygous state gives rise to the disease. The whole picture, however, is influenced by genetic modifiers.

The essential problems in diagnosis were stressed.

The failure of known therapeutic measures was described; blood transfusion being the only procedure which alleviates the condition. This is the one form of hypochromic microcytic anaemia which does not respond to iron therapy.

The course and prognosis of the disease were outlined. It was shown that as a general rule haemoglobin levels remained fairly constant in each case, and that the prognosis depended on whether that level was adequate to meet the demands, such as infections, which were made upon it. The outlook for future offspring in an affected family was considered in the light of the Mendelian inheritance already described.

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STUDIES ON THE MATURATION OF SEX GLANDS IN ANIMALS

with special reference to:

COLCHICINE EFFECTS AND INDUCED POLYPLOID CELLS IN THE TESTES OF MICE

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