THE ANAEMIAS OF PREGNANCY

ASPECTS PERTAINING TO THE INCIDENCE AND PATHOGENESIS OF THE ANAEMIAS OF PREGNANCY ENCOUNTERED IN SOUTH AUSTRALIA

A THESIS

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Indeed such inadequacies as we have seemed to find in empiricism have been discovered by strict adherence to a doctrine by which empiricist philosophy has been inspired: that all human knowledge is uncertain, inexact and partial. To this doctrine we have not found any limitation whatever.

Bertrand Russell (1948).

"Human knowledge, its scope and limits".

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The regulations of the University of Adelaide for the degree of Doctor of Medicine require:

(1) A declaration that the thesis is the writer's own composition. This declaration may be found immediately following the list of contents.

(2) An indication of where the writer considers the thesis to advance medical knowledge or practice. This subject is contained in the Conclusion on page 109.

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DECLARATION OF THE ORIGINALITY OF THE STUDY AND THE PERSONAL COMPOSITION OF THIS THESIS.

The organisation of this survey, and the haematological investigation and treatment of the patients therein, has been the sole responsibility of the Author. I wish to state that all the experimental work outlined in this thesis has either been conducted by myself, or by Miss B. A. Crompton who has been working under my direction throughout the latter part of these investigations. The actual composition of this thesis is entirely my own.

INTRODUCTION.

An Outline of the Study Undertaken.

Owing to the emergence of unforeseen problems during the study, some modifications to its character were necessary during the three years over which it has extended. The purpose of this introduction is to outline the study as it was originally conceived, and to indicate briefly the problems which arose and the modifications which were required during its course.

The definition of anaemia of pregnancy is of necessity somewhat arbitrary, and it depends upon the average haemoglobin values found in the community. These are largely dependent upon the dietetic habits, living standard and environmental conditions of the population involved.

It is not surprising, therefore, to find that the values accepted as normal and anaemic respectively have varied considerably over the years. With the higher living standards of today, and the advancement of medicine, there is a general tendency to raise the lower limit of the haemoglobin level which is acceptable as normal in pregnancy. Scott and Govan (1949), working in Glasgow, found that 20.1% of their patients had haemoglobin values of less than 8.9 g. of haemoglobin per 100 ml. of blood at some stage during their pregnancy. They accepted from 8.9 g. to 9.9 g. of haemoglobin per 100 ml. of blood as low normal values.

By contrast, in a survey of the haemoglobin values of 177 randomly selected patients at the Queen Victoria Maternity Hospital Adelaide, the lowest haemoglobin encountered was 9.0 g. per 100 ml.

This survey of the anaemias of pregnancy was commenced at the Queen Victoria Maternity Hospital in March, 1958. The objects, at the outset, were to determine what proportion of patients with haemoglobin values below 11.0 g. per 100 ml. of blood were iron deficient, and what

proportion of patients resistant to iron therapy were of a frankly megaloblastic, or had an incipient megaloblastic, type of anaemia. This was initiated by the number of reports of a high incidence of megaloblastic anaemia in the British Isles, and also by the possibility that some patients might be suffering from an arrest of haemopoiesis before the development of a frank megaloblastic change in the bone marrow.

In Adelaide, a percentage scale was adopted in the past for the assessment of haemoglobin values, in which 100% corresponds to 15.3 g. of haemoglobin per 100 ml. of blood. From the point of view of the haematologist interested in comparative values, it is recognised that the percentage scale has serious disadvantages; but as this system is in current clinical use, it is necessary to deal in terms of this scale for local usage. Haemoglobin values are estimated using the oxyhaemoglobin method with a Unicam S. P. 300 absorptiometer, which has been calibrated from iron estimated haemoglobin standards. The optical density is then checked against a scale on which 11.0 g. of haemoglobin per 100 ml. of blood corresponds to 72%, and 10.7 g. of haemoglobin per 100 ml. of blood corresponds to 70%.

For the purpose of this investigation, the haemoglobin level of 10.7 g. per 100 ml. of blood (70%) was chosen as the arbitrary limit for the following reasons:

1. It would include all patients with haemoglobin values of less than 11.0 g. per 100 ml. of blood.
2. The figure of 70% provided an easy criterion for all members of the staff to recall, and would minimise confusion as to the level at which patients were referred for special investigation.
3. The numbers of patients referred would not be too excessive.
4. It was believed that this level would include all patients with

frank megaloblastic forms of anaemia.

5. The majority of obstetricians regard this level of haemoglobin as the minimum at which the risks of delivery are acceptable; and patients with haemoglobins below this value at the 39th week of gestation, are usually transfused before delivery.

It was arranged that all patients with haemoglobins of 10.7 g. per 100 ml. of blood, or less, should be referred to the Medical Complications Clinic organised by Dr. R. A. Durston at the Queen Victoria Maternity Hospital. It was hoped to obtain, as near as possible, a complete coverage of patients from all the clinics.

No folic acid or vitamin B_{12} was administered except through the channels provided by this clinic; and throughout the thirty-eight months during which these investigations have been carried out, only four patients have had folic acid given to them outside the survey.

The scheme of treatment and investigation commenced with the routine administration to all antenatal patients of 15 gr.(0.9 g.) of ferrous gluconate per day. Routine haemoglobins and haematocrit estimations were carried out on the patient's first attendance at the antenatal clinic; and subsequently at 28 weeks and 34 weeks of gestation.

All patients with haemoglobin values of less than 11.0 g. per 100 ml. at the first visit were given a trial therapy of oral ferrous gluconate (1.8 g. daily). Only in exceptional cases of severe anaemia, with evidence of severe iron deficiency, or failure of response to oral iron, was parenteral iron therapy commenced before the 28th week of pregnancy. In all cases where the haemoglobin level was below 11.0 g. per 100 ml., a red cell count and a packed cell volume was carried out with calculation of the red cell indices. The reticulocyte count was estimated, using the method of incubation in brilliant cresyl blue (Dacie, 1956). The white cell count was

estimated, and a differential count of the white cells carried out on a stained blood smear, together with an examination of the red cell morphology.

The majority of the patients entered the survey between the 28th and the 32nd weeks of pregnancy, as calculated from the expected date of delivery. Each patient had a loose leaf card compiled (Appendix 1) on which was entered most of the relevant haematological data, and from which it was possible to assess her progress, and to summarise the investigations.

In the initial phase of this investigation, all patients who were anaemic at 30 to 32 weeks gestation were treated with iron-dextran (Imferon, Bengers Ltd.).

In this initial phase of the investigation, lasting twelve months, it was found that the anaemias fell into three main groups, which can be classified as follows:

1. Normocytic or microcytic cases responding to parenteral iron therapy.
2. Normocytic or macrocytic cases not responding to parenteral iron therapy:
    - i. Responding to Folic Acid and B_{12}
    - ii. Not responding to Folic Acid or B_{12}:
        - (a) of toxic aetiology
        - (b) of unknown aetiology.
3. Microcytic cases showing no response to parenteral iron therapy.

The first case investigated in the survey fell into group 3, and it was soon appreciated that in these cases I was probably dealing with the thalassaemia trait. Owing to the relative infrequency of gross folic acid deficiency in this preliminary evaluation, and the relatively large numbers of iron refractory microcytic anaemias occurring in the Greek, Italian and

Cypriot patients, the emphasis of the investigation had, of necessity, to be shifted from the original objectives to the investigation and diagnosis of thalassaemia minor.

This group, in many respects, resembled severe iron deficiency anaemias when regarded from the haematological aspect; and in the second phase of twelve months, intensive efforts were commenced in an attempt to discriminate between the iron deficiency cases and the thalassaemia minor group at an early stage, and to obviate any necessity for parenteral iron therapy in these cases.

Pre-treatment serum iron values were taken, and the results assessed in conjunction with the response to iron therapy and the pre-treatment red cell indices.

Owing to the fact that the time available for treatment of the anaemia is limited during pregnancy, and that the results of the serum iron values were not immediately available, it was usually necessary to initiate parenteral iron therapy pending the receipt of the results of this estimation. It was found that some patients showed an apparent response to parenteral iron therapy in spite of a serum iron level which was within the normal range. This resulted in an increased necessity for the evolution of a reliable method for the discrimination between the iron deficiency and the thalassaemia cases.

Paper electrophoresis, using a barbiturate buffer, was tried but the separation of the A_2 haemoglobin component was neither satisfactory nor constant. Starch gel electrophoresis was used with an 0.25 m. barbiturate buffer, as modified by Dr. Curtain; and good separation of the haemoglobin A_2 component was obtained. No satisfactory method of quantitation could be found, however, and evaluation could only be made by visual inspection.

During the last eighteen months of this investigation, a method using a tris-hydroxy-methyl-amino-methane buffer with paper electrophoresis, and quantitated with brom-phenol blue dye, has been evolved, and its accuracy determined. *

It was then possible to initiate a survey of the A_2 haemoglobin levels of all the Greek and Italian patients attending the Queen Victoria Maternity Hospital, this extended over a period of fourteen months, and the results were correlated with the other laboratory findings. Further work under the same scheme has been carried out in an attempt to determine the specificity of raised haemoglobin A_2 values in other forms of anaemia, and in non-pregnant patients regarded as thalassaemia minor cases. During the last phase of this survey of anaemia, the administration of parenteral iron to all cases regarded as thalassaemia minor was discontinued, as it was felt that further iron therapy was contra-indicated in these patients.

An additional complication was introduced by the work of Haddow and Horning (1960) on the sarcomatous effects of iron-dextran in mice, and the subsequent editorials in the British Medical Journal. Following a review of the relevant literature, and some discussions with Professors H. N. Robson and G. M. Wilson, it was felt that the case was not proven. The use of iron-dextran, therefore, has been continued, but on a more limited scale, in cases in which there was evidence of iron deficiency.

A further subsidiary study on the blood volumes and red cell masses of a series of patients was necessitated by the observations of Lund and Sisson (1958), who suggested that, owing to the effects of haemodilution in pregnancy, the peripheral red cell values were grossly inaccurate,

* The evolution and evaluation of this method, and the subsequent survey of the Greek and Italian patients in which it has been used, has been greatly assisted by a grant from the National Health and Medical Research Council of Australia, which commenced in January, 1960. With the aid of this grant a graduate biochemist, Miss B. A. Crompton, has been employed.

and that an assessment of the haematological status of the patient could only be made on measurements of the red cell mass. This observation would be of fundamental importance to much of the haematological research in pregnancy, not only in this investigation, but in many others which have preceded it throughout the years.

This thesis is therefore concerned with a number of aspects pertaining to the study of anaemias of pregnancy. It is proposed to deal with each aspect separately.

In the historical review, the salient features in the development of present day concepts of the anaemias of pregnancy is dealt with; and whilst an exhaustive review of all the literature is beyond the scope of this thesis, an attempt has been made to cover all the more important contributions to the subject in so far as they form a background to these investigations. More specific data from the literature will be dealt with in the relevant sections.

The body of the thesis deals with each aspect as an integral part, and the whole is brought together within the summary. It is hoped that this treatment of the subject will lead to clarity and an avoidance of repetition and overlapping.

HISTORICAL REVIEW.

The history of the anaemias of pregnancy has been dominated in the literature by two main themes; the more dramatic of these being the fascination exerted on many physicians by the megaloblastic anaemias of pregnancy and the evolution of the present day therapeutic approach. This has been fostered by its potentially fatal possibilities, and by the frequent dramatic suddeness of onset.

The second subject which has in the past and present excited much controversy and interest has been the "physiological anaemia" of pregnancy.

The Megaloblastic Anaemias of Pregnancy.

Walter Channing, in 1842, is generally credited with having first recognised cases of severe anaemia in pregnancy which were probably megaloblastic in origin. (Quoted by Osler, 1919).

Gusserow, in a description of five cases, is credited by Sir William Osler with having drawn the attention of the profession to the seriousness of this condition in pregnancy.

From the time of Addison's description of pernicious anaemia, in 1855, there was considerable confusion as to whether there was a separate form occurring in pregnancy.

Sir William Osler, in his inimitable manner, reviewed the anaemias of pregnancy in 1919. He classified them into four groups, as follows:

1. Anaemia due to haemorrhage; either acute, at delivery, or gradual, due to repeated small haemorrhages as in abortion.
2. The severe anaemias which are often accompanied by vomiting.
3. Post-partum anaemias: and in this connection, he credits a

Dr. Palmar Howard with having insisted that these are not identical with true Addisonian Anaemia, as a large percentage of cases recover.

4. Anaemia associated with post-partum sepsis.

This last category had caused considerable confusion in the past, owing to the features of pyrexia, vomiting and splenomegaly which it shared with pernicious anaemia of pregnancy.

Sir William went on to state that he agreed with Dr. Howard that pernicious anaemia of pregnancy was quite distinct from Addisonian Pernicious Anaemia, and that the prognosis was much better than was generally thought at that time. Of the seven cases which he had personally seen in Montreal and Philadelphia, all had recovered, and moreover, the recovery had been permanent. He also made the interesting observation that the number of cases appeared to have increased during World War 1, and he thought that underfeeding may have led to this increase. His concepts were not universally accepted; and Pepper (1921), in reviewing the literature, doubted whether pernicious anaemia of pregnancy was a separate entity.

The condition of pernicious anaemia of pregnancy was only rarely recognised, and Bardy (quoted by Studdiford, 1934) in a thesis written in 1924, reviewed the literature of Europe and America over the previous thirty-eight years, and was only able to find sixty-eight cases.

The next major step forward was made by Minot and Murphy in 1926. They treated forty-five cases of Addisonian Pernicious Anaemia with a diet including cooked calf and beef liver, which produced remissions in all their cases. This finding was of course soon applied to cases of anaemia in pregnancy, and Evans (1929), reported two cases: one of which responded to minced liver, and the other to parenteral injections of liver extract.

Following their brilliant work on the intrinsic and extrinsic

factors, Castle and Strauss turned their attention to the pregnancy anaemia; and in 1933, reported two cases who responded to liver extract, and two cases who showed some response to oral beef-steak and gastric juice. They concluded that the mechanism was probably similar to that of Addisonian Pernicious Anaemia, except that the defect in the intrinsic factor was only of a temporary nature, conditioned by pregnancy. They also postulated that there might be some lack of extrinsic factor in the diet.

In 1935, Davies said that the treatment for macrocytic anaemias of pregnancy should be the same as for the non-pregnant pernicious anaemias, but that in some cases intramuscular therapy might also be required.

Although megaloblastosis in pernicious anaemia of pregnancy had been previously described, Heilbrun (1936) appears to have been the first author to have used the finding on megaloblastosis of the bone marrow as a criterion of diagnosis in the pernicious anaemia of pregnancy.

Ungley (1938) first drew attention to the fact that pernicious anaemia of pregnancy did not always respond to liver extract. He also made the interesting observation that although the mean corpuscular volume was increased in these cases, the mean cell diameter, as judged by the Price Jones Curves, was within normal limits. Angier, with a large team of co-workers (1945), synthesized Folic acid and showed that it was identical with the L. Casei factor isolated from liver, and that this compound would promote growth and haemoglobin formation in chickens. This was quickly followed by clinical trials in megaloblastic anaemias of pregnancy by Moore et al. (1945). They found that a good therapeutic response was obtained in pernicious anaemia of pregnancy, whereas the response in Addisonian Pernicious Anaemia was only sub-optimal. This inevitably raised the question as to whether the pathogenesis was the same

in the two conditions. This point of view was supported by Spies (1946) who also drew attention to the fact that, unlike Addisonian Pernicious Anaemia, many of the pregnancy cases failed to respond to liver extract, but had responded to yeast extract or proteolyzed liver. He postulated that there must be additional factors in these preparations not found in the liver extract.

Sheila Callender, in 1944, reviewed a series of twenty-five cases of megaloblastic anaemia of pregnancy, and insisted on the establishment of a megaloblastic bone marrow change as a criterion for diagnosis of this condition. It is of interest to note, however, that in one of her cases only a few typical megaloblasts were present in the bone marrow smears.

In more recent years, the use of folic acid in the megaloblastic anaemias occurring in Europe and America has become almost universal. The general opinion is that this drug will almost invariably produce a response in these temperate countries: (Davidson, Girdwood and Clark, 1948; Scott, 1954; Davidson, 1952; Girdwood, 1956; Ungley, 1950; Ungley and Thompson, 1950; Badenoch et al., 1955).

Holly (1958) believed that the addition of ascorbic acid was also necessary in order to convert the folic acid into the citrovorum factor.

Occasional cases have been described in the temperate climates which responded to Vitamin B_{12}. (Badenoch et al., 1955; Tacchi, 1958). Killander (1958) described three cases; but one of these not only had relapses in subsequent pregnancies, but had additional relapses at ten and twelve months post-partum respectively: and the diagnosis of a malabsorption syndrome does not appear to have been excluded. Adams (1956) described ten cases in South Africa responding to Vitamin B_{12}, and fourteen cases responding to folic acid. He thought that there might be several forms of

megaloblastic anaemias of pregnancy, as judged by the variation in response.

It is fairly generally agreed that many cases of megaloblastic anaemia occurring in the tropics will respond to B_{12} therapy: Patel and Kocher (1950a); Patel and Kocher (1950b); Das Gupta (1953); Tasker (1956). Some of these cases will also respond to folic acid: Das Gupta (1949); Das Gupta (1953); Tasker (1956).

A feature of many of these tropical forms appears to be the high mortality rate with which it is associated. Das Gupta (1949) had five fatalities in his reported series of forty-five cases.

Many of these tropical forms are also complicated by concomitant parasitic infections. Of five cases, described by Patel and Kocher (1950b), one had entamoeba histolyca in the faeces, one had giardia lamblia, and one had both malaria and faecal infection by ankylostoma duodenale.

It seems fairly clear that many of these cases have what Das Gupta (1949) described as a dimorphic anaemia with iron deficiency, and do not respond well to folic acid; some cases responding to B_{12} do not show a megaloblastic marrow: Das Gupta (1953). This finding introduces additional complications into the diagnosis and terminology of these cases, because Das Gupta (1949) has rightly deplored the use of the term "pernicious anaemia of pregnancy". If some of these cases are not megaloblastic, a further title is removed, and the term "macrocytic anaemia of pregnancy", suggested by Das Gupta, will also embrace many cases not related to deficiency of the specific haematinic factors. The designation of these cases becomes even more confused; and it is not helped by the fact that the investigation of megaloblastic anaemias of pregnancy is almost invariably handicapped by the urgency of each individual situation, as has been pointed out by Badenoch et al. (1955).

The most helpful line for the elucidation of these problems

appears to be the evolution of the biochemical and micro-biological techniques for investigation: Chanarin, Anderson and Mollin (1958) described a technique for the assessment of folic acid absorption, in which the patients are first saturated with folic acid, and the serum levels then assayed after an oral dose of folic acid. They found that in twenty-three pregnant women, the absorption of folic acid was reduced below the normal level, but that there was no difference between the absorption of folic acid in the normal pregnant women, and in the seven cases with megaloblastic anaemia of pregnancy.

Chanarin et al. in 1959, used a combination of the technique previously described, with a folic acid clearance test, in which the rate of folic acid removal from the plasma was used as an index to the state of unsaturation of the tissues. They found that some degree of tissue unsaturation was a fiarly usual accompaniment to pregnancy, and that this was increased in those patients who had a megaloblastic bone marrow; they also found an increased degree of unsaturation in women with twin pregnancies. In addition, Chanarin, Dacie and Mollin (1959) showed that in many cases of non pregnant haemolytic anaemia there is some degree of unsaturation; and they suggest that the demand in these cases is greater than normal, and cannot be supplied without depleting the body stores.

As they have demonstrated a universal, relative deficiency of absorption exists in pregnancy, and as there must be an increased demand for haemopoietic factors due to the expansion of the red cell mass occurring in pregnancy, it would not be surprising to find the folic acid demands outstripping the body stores in some cases, particularly if there is a poor dietetic intake of this substance.

Iron Deficiency and the "Physiological" Anaemias of Pregnancy.

The second aspect, which has in both the past and present excited much controversy and interest, has been iron deficiency and the so called "physiological" anaemia of pregnancy.

Willcocks, in 1881, established that there was a fall in the number of red blood cells during pregnancy. Until that time, there appears to have been some dispute as to whether this in fact occurred. He divided the anaemias into two forms: one of which was chlorosis, with a well marked impoverishment of the individual corpuscles; and the other form was one in which the corpuscles were rich in haemoglobin, but reduced in numbers. He attributed this latter form to the considerable enlargement of the vascular state which occurs in pregnancy.

Oliver (1894) described two fatal cases which, in retrospect, have many of the clinical features and a course suggestive of megaloblastic anaemias of pregnancy. In his discussion, he suggested that these may have been due to the ordinary anaemia or hydraemia of pregnancy.

Impetus was given to this theory when Dieckmann and Wegner, in 1934, established that the blood volume was expanded by an average of 23% during pregnancy; this has gained many supporters in the literature. Moore (1930) concluded that the mean level for three hundred normal pregnant cases of 11.7 g. of haemoglobin per 100 ml. of blood, was lower than the normal non pregnant value. Strauss and Castle, in 1932, commented that marked degrees of physiological anaemia resembled the anaemias associated with poor diet and blood loss; but they concluded that physiological anaemia is probably due to hydraemia, and is not a true anaemia. Scott and Govan (1949), Fullarton, Mair and Unsworth (1944), Kothari and Bhende (1950) and Whitby and Britten (1957) have accepted values above 10.4 g. (70% Haldane) as physiological. Taylor and Torpin (1951) do not

consider values below 11.0 g. per 100 ml. of blood as physiological. In recent years, there have been a number of challenges to the concept of physiological anaemia. Davidson, Donaldon, Lindsey and Roscoe, in 1944, showed that there was a progressive fall in the haemoglobin during pregnancy, but that the mean haemoglobin value had risen from 11.3 g. per 100 ml., which they had found in a group of "normal" patients under a similar survey in 1942, to a mean value of 12.7 g. per 100 ml. in 1944. Those who suffered the exigencies of wartime rationing in Britain will appreciate what a remarkable tribute this is, to the dietetic balance achieved under the prevailing conditions.

Benstead and Theobald (1952) found that the physiological anaemia was associated with a low mean corpuscular haemoglobin concentration and that normal haemoglobin values could be maintained throughout pregnancy by the administration of ferrous sulphate. Davis and Jennison (1954) were of the opinion that continuous oral iron therapy, from the 16th week of pregnancy, would prevent the development of physiological anaemia. They showed that patients with haemoglobin values of 11.9 to 13.0 g. per 100 ml. when first seen, showed a mean rise of 1.4 g. between the 32nd week of gestation and term, when given oral iron. The comparable rise in the control group was only 0.3 g. per 100 ml. of blood.

Fisher and Biggs (1955) also found that they could eliminate any fall in haemoglobin values during pregnancy by the administration of iron; and they were of the opinion that any fall in haemoglobin was due to iron deficiency. Gerritsen and Walker (1954) in a most interesting observation on the Bantu, (in whom it is well known there is a very high iron intake, Gerritsen and Walker, 1954) found that the haemoglobin level in pregnant Bantu is maintained at 13.7 g. per 100 ml. throughout pregnancy. They

compared this to the normal, non pregnant value of 13.9 g. per 100 ml. in the female Bantu. As the authors point out, it is rather difficult to compare these values with the values found by other workers, as the population in question lives at an altitude of 5,000 feet.

Verloop, Blokhuis and Bos (1959) also showed that in a group of patients with a mean haemoglobin value of 11.4 g. between the 4th and the 6th months, they could produce, by iron therapy, a rise of 0.8 g. in the haemoglobin by the 8th month; as contrasted with a fall of 0.6 g. in a similar group of untreated patients. Furthermore, they showed that some difference in haemoglobin value was continued in a follow up during the post-natal period.

Sturgeon (1959) in a trial of intravenous iron and oral iron during pregnancy, found that there was a slight fall in haemoglobin concentration to 12.1 g., which was maintained until term, in spite of the iron therapy. This was a mean fall of 0.7 g. compared with the values found at the six month post-partum. He was able to show, however, that there was a bigger fall, to 11.4 g. per 100 ml., in the untreated patients at term.

Hagberg and Lundstrom (1955) gave intravenous saccharated iron to seventeen healthy, pregnant patients; and found that this prevented the sideropenia which is otherwise a constant feature of pregnancy. Moreover, they found that in these patients, the haematocrit values at the end of pregnancy closely approached those of healthy, non-parous women.

Holly (1955) who has carried out a considerable amount of work in this field, also considers that haemoglobin values of 10.0 g. per 100 ml. are too low to be accepted as normal in pregnancy. He is of the opinion, that iron deficiency exists in nearly all haemoglobin falls below the level of 12.0 g. per 100 ml. of blood; and he has found that only 33% of patients

maintain this level without iron, whereas 80% maintain it with oral iron - of which he suggests a minimum trial period of 90 days. In 1957(a), however, using radioactive iron uptake into the erythrocytes, he demonstrated that there did appear to be some defect in utilisation of iron in the pregnant patient. He has used oral cobalt as a stimulant in an attempt to potentiate erythropoiesis, and he was able to show that a significantly greater number, (90%), of patients maintained a minimum value of 12.0 g. haemoglobin per 100 ml. of blood. (Holly 1957b).

The mode of action of cobalt in the stimulation of erythropoiesis is not known; in addition, some patients on this drug show toxic manifestations, and as yet, no other workers appear to have adopted this form of therapy in pregnancy.

Holly (1955) has pointed out that if the haemoglobin level falls because of the increasing plasma volume, it is only to be expected that the bone marrow will respond to the relative anoxaemia. (Hurtado, Merino, Delgado, 1945).

This concept appears to be reasonable, and although not universally accepted it does appear to be more acceptable to present day workers in this field. More attention has therefore been given, of late, to the occurrence of mild degrees of iron deficiency in pregnancy and the relative diagnostic values of serum iron, and free erythrocyte protoporphyrin and copper values in pregnant and non pregnant states. It is now a common procedure to administer oral iron as a matter of routine in pregnancy, and to administer parenteral iron to those patients who are severely anaemic, or who report for treatment late in their pregnancy, or who appear to be refractory to oral iron therapy.

The iron requirements for the developing foetus and for the increasing red cell mass have been emphasised by many writers; including

Kerr and Davidson (1958); Bland, Goldstein and First (1930); McGeorge (1935), and Sisson and Lund (1957).

Holly (1960) has estimated the pregnancy iron requirements as 375 mg. for foetal development and 50-100 mg. for the placenta; an increase in haemoglobin mass, of approximately 130 g., requires 442 mg. of iron for this purpose, which brings the total to at least 900 mg. for pregnancy. Coleman, Stevens and Finch (1955) estimated the extra requirements of the pregnant women as 500 mg. of iron during the pregnancy, and 150 mg. during lactation. Holly (1960) estimates the normal storage of iron in the non pregnant state at approximately 1,000 mg., and in his opinion few women commence their pregnancy with normal iron reserves. It is obvious that if there is any deficiency of iron storage at the onset of pregnancy, some degree of deficiency of iron is likely during that pregnancy. The average rate of absorption from a normal diet is no more than 1.5 to 2.0 mg. (Holly 1960). It is unlikely that this will compensate for any marked storage deficiency during pregnancy. Oral iron, if taken and absorbed, may compensate; but, as has been pointed out by Fisher and Biggs (1955) one of the main difficulties is that patients who feel well during the early stages of pregnancy cannot be relied upon to take the oral preparations. In addition, there may be some degree of failure of absorption during pregnancy: Scott and Govan (1949) investigated 900 cases of anaemia of pregnancy; and of these they had found that 14.3% of the simple iron deficient anaemias failed to respond to oral iron, but they did respond to saccharated iron given parenterally. They concluded that there was some failure to absorption of iron present in these cases.

Davies (1935) found that his patients were more prone to the development of anaemia when they were achlorhydric. Scott and Govan (1951)

noted that many of the patients who were refractory to oral iron, but who responded to parenteral iron, were achlorhydric. They also found that this was not corrected by the addition of hydrochloric acid given orally. The serum iron levels were low, and there was no question of saturation of the plasma iron binding capacity impeding absorption. They suggested the possibility of the transformation of the iron into a non-absorbable compound. Ungley (1938) also found that in iron deficiency anaemias of pregnancy associated with achlorhydria, the absorption of iron is not improved by the addition of hydrochloric acid to the therapy.

In view of the importance of iron therapy to iron deficient patients during pregnancy, it is not surprising that considerable attention has been paid to the parenteral administration of various types of iron. In 1930, Cappell, working with rats and mice, demonstrated that saccharated iron was taken up by the reticulo endothelial system in a manner similar to that found in the disposal of excess iron liberated from intravascular haemolysis and suggested that this compound could be used for the treatment of iron deficiency in the human.

Many workers have used saccharated iron, but one of the drawbacks has been the occurrence of vaso-vagal types of attacks. Scott and Govan (1951) found that between 1% and 2% of their patients reacted in this manner.

Cappell et al. (1954) and Scott and Govan (1954) simultaneously produced the first reports of the use of intramuscular iron dextran in a grand total of sixty-five patients; they found that there were no reactions, and that a good response to therapy was obtained. Nicholson and Assali (1952) and Mulla (1958) have reported a total of forty-five treated patients who had no reaction to these compounds; and they stated that a response can be obtained in patients with initial haemoglobin levels within

the range sometimes accepted as physiological in pregnancy. They found that, in general, the response was inversely proportional to the initial haemoglobin level. Jennison and Ellis (1954) found a rise of 0.27 g. of haemoglobin per 100 ml. of blood for each 100 mg. of iron dextran given. The equivalent figures for Scott (1956b) and Schwartz, Greenwald and Tendler (1958) were 0.3 g. of haemoglobin and 0.32 g. of haemoglobin per 100 mg. of iron respectively. It can be seen that in the more severely anaemic cases there is unanimity of opinion on the degree of response to be expected.

Hagedorn (1957) found that there were no toxic effects in ten patients; but Scott (1956) in a series of six hundred treated patients, experienced three reactions, which included one asthmatic attack and one vaso-vagal type of attack. She suggested that it was inadvisable to treat patients with asthma or allergic skin rashes. She also found, in some patients, a delayed response which she attributed to the rapid increase in the blood volume. On an average dose of 500-600 mg., she found that occasional relapses occurred, particularly in the obese patients.

Whilst working with iron dextran in rats, Richmond (1959) found that sarcomata developed at the site of injection after a time lapse of over nine months. Haddow and Horning (1960) repeated this work; and they found that they could produce sarcomata in mice, and also in one out of fifty hampsters. No tumours were produced in guinea pigs or rabbits similarly treated. An editorial in the British Medical Journal of March 12th, 1960 commenting on these findings and condemning the use of iron dextran, produced a controversy which resulted in a spate of letters and publications. Goldberg (1960) pointed out that the dosage used in these experiments was, at a minimum, seventy times greater than the total clinical dose for these animals would have been were they treated on a

comparative weight basis; and that when a lower dosage was used, as in both some of Richmonds animals and in an extensive series of animals that Goldberg himself had treated, no increase in tumour rate was found. He attributed the increased tumour rate to the swamping of the body's defence mechanisms; and he pointed out that similar effects could also be obtained by repeated trauma, or by repeated injections of a wide variety of agents including hypertonic saline and glucose.

An independent committee of pharmacologists, haematologists and physicians then considered the available data, and stated in The Lancet, of July 16th, 1960 that they were of the opinion that the use of iron dextran in the recommended clinical dosage carries a negligible risk in respect of carcinogenicity, and is probably less hazardous in other respects than intravenous preparations and blood transfusions. At the time of writing, no convincing case of sarcoma attributable to this drug has been reported.

Although the majority of obstetricians are unwilling to deliver an anaemic patient without transfusion, because of the risks of haemorrhage, surprisingly little information is available on the effects of anaemia on the patient in labour. Reid and Mackintoch (1937) found that the duration of labour was unaffected by a pre-existing anaemic state, but that there was an adverse influence on the stillbirth, neonatal and infant mortality rates. They thought that these findings might be due to the associated poor social circumstances of the patients. Woodruff (1951) in cases with severe anaemia in Africans, which he thought to be due chiefly to protein deficiencies, also found a high neonatal mortality rate; he thought that there was a tendency to premature labour in these cases. Taylor and Torpin (1951) found that there was an increase in the duration of labour of 20.3%, in primigravida, and of 22.9%, in multiparous women, in whom the

haemoglobin was below 11.0 g. per 100 ml.

Sturgeon (1959) found that the administration of iron to the mother had no effect on the iron nutrition of the child up to the age of eighteen months. Lapan and Friedman (1958) found that administration of iron to the mothers, resulted in higher haemoglobin and serum iron levels in the cord blood of the infants. Sisson and Lund (1957) were of the opinion that there was no difference in the haemoglobin or haematocrit levels in the children of iron treated women; but that evaluation of the total red cell mass did show some increase in the iron treated series.

SUMMARY OF SECTION I.

EVALUATION OF THE HAEMATOCRIT VALUES AND RED CELL MASS AS CRITERIA OF ANAEMIA IN PREGNANCY.

The variations in red cell mass with respect to the peripheral haemoglobin and haematocrit values, as reported by Lund and Sisson, have been shown to be due to the inaccuracy of assessing this measurement in terms of the body weight; and not, as they suggested, to absolute variations in the red cell mass.

The surface area and the ideal weight have been used for the assessment of the red cell mass, and these parameters appear to be preferable to the body weight.

Evidence is produced to suggest that the lean body weight would be the best parameter.

It is suggested that, at the present time, the peripheral haemoglobin and haematocrit values remain the best indicators of the haematological status of the patient; although there is some evidence that relative changes in the plasma volume do introduce some minor variations in the peripheral red cell indices.

SECTION I.

EVALUATION OF THE HAEMATOCRIT VALUES AND RED CELL MASS AS CRITERIA OF ANAEMIA IN PREGNANCY.

In 1958, Lund and Sisson reported that they had estimated the peripheral haemoglobin and the total haemoglobin mass in a number of pregnant patients, and found that in patients with similar values for the peripheral haemoglobin or haematocrit, there was a very wide divergence in the haemoglobin mass when calculated in terms of grams of haemoglobin per Kg. body weight. In some cases, this variation was in the order of 100% increase in the haemoglobin mass when compared to patients with a similar haematocrit. They accounted for these changes by suggesting that the patients with a low haemoglobin mass, and relatively high peripheral haemoglobins, were hypovolemic; and that those patients with a high haemoglobin mass and relatively low peripheral haemoglobin values were hypervolemic. They suggested that the peripheral haemoglobin and haematocrit values were unreliable indices of the haematological status of patients in pregnancy. They thought that the possibility of anaemia in pregnancy could only be evaluated in terms of the haemoglobin mass per Kg. body weight.

This concept was a reversion to the idea of the physiological anaemia of pregnancy, and if correct, would mean that much of the work done by various workers in this field was nullified. It was, therefore, of importance that this hypothesis should be tested.

Measurement of Blood Volume.

The Evans blue dye method with the dye extraction technique of Tornberg (1958) was used for these estimations of the red cell mass.

A batch of Evans blue dye was made up, containing 37.5 mg. of

dye in 25 ml. of saline. This strength was chosen because it would be less than 50% of the dosage at which tissue staining might take place: (Mollison, 1956). This strength of dye also meant that estimations could then be repeated, if required, on the same patient within a short interval.

Syringes were calibrated to deliver approximately 20 ml., by diamond marking a ring on the barrel and plunger. Final calibration was then carried out by delivering distilled water through the syringe, and weighing the amount discharged. The actual volume delivered was then incorporated into the final calculations.

This technique was felt to be preferable to aspirating a known quantity into the syringe, as it was found that the fine lines could be approximated with a high degree of precision; it was, of course, essential that all air bubbles be eliminated from the syringe.

Estimations were carried out on patients who had been requested to confine themselves to a light, non-fat breakfast, and to exclude milk from any fluids drunk during the morning. All the tests were carried out between the hours of 9.30a.m. and 10.30 a.m., and the patients lay down to rest for a minimum of fifteen minutes before the dye injection. The period required for complete mixing of the dye in the circulation of the pregnant patient, is known to be slightly longer than that for the non pregnant patient; but it was considered that a lapse of fifteen minutes would be sufficient and would avoid excessive loss of the dye. A correction factor of 100/97.5 was incorporated into the calculation to correct the loss during the fifteen minute time interval, (Mollison, 1956).

It is fairly generally agreed that the use of direct absorptiometric estimation of the dye in the patient's plasma is prone to large errors due to the presence of lipaemia and variable degrees of haemolysis in the specimens, (Gregerson and Rawson, 1959); and extraction methods of

varying types have been developed to overcome these objections. The method of Tornberg depends upon the release of the dye from its albumin linkage, and the precipitation of the protein by a detergent, followed by extraction of the dye into acetone. It was found that particular care was necessary to avoid evaporation of the acetone during centrifugation, and this was accomplished by using rubber bungs with fold over tops made for syringe withdrawal ampoules.

The actual technique followed was to add 1 ml. of plasma to 0.5 ml. of 1% "septin" (benzethonium chloride) and then to shake; 3 ml. of acetone is then added, and the solution again shaken. The tubes are then centrifuged at 3,000 r.p.m. for ten minutes; and the optical density of the clear supernatant is read at 620 mμ, in a Unicam S.P. 600 spectrophotometer. The resultant optical density is then compared with 1:101 standard made by diluting 0.02 of the dye solution in 2 ml. of the patient's pre-injection plasma. Blanks were also set up, and the results deducted, from the optical density of the specimens.

Haematocrit values were estimated by duplicate determinations in a governed haematocrit centrifuge, which was spun for 55 minutes. The mean value was taken, and the results obtained were corrected for trapped plasma.

Calculations were made using the body haematocrit/peripheral haematocrit ratios given by Caton et al. (1951). These authors, using a combination of the Evans blue dye technique for measuring plasma volume and the Cr. 51 technique for measuring red cell mass, have estimated the body haematocrit, and shown that the above ratio varies in pregnancy from 0.923 at twenty-three to twenty-nine weeks gestation to 0.960 at thirty-four to forty weeks; they also gave the intermediate values. Personal enquiry from these authors elicited the fact that corrections for trapped

plasma had not been made in their calculations. Their figures were, therefore, recalculated using their published data, with the figures for the haematocrit corrected for trapped plasma, in place of the uncorrected values which they had reported. This correction only made a small variation in their results, but it was considered desirable that this should be carried out, as all our figures incorporated this correction factor.

The haematocrit ratio, for the non pregnant cases quoted by Caton et al., of 0.90, is slightly below those of 0.91 accepted by Mollison; and the correction for trapped plasma would bring the two sets of figures into near agreement. It would be anticipated that some rise in the body haematocrit/peripheral haematocrit ratio would be encountered with the general dilatation of the blood vessels during pregnancy.

After this correction had been made, values for the haematocrit ratios were 0.947 and 0.984, at twenty-three to twenty-nine weeks and thirty-four to forty weeks of gestation, respectively. The appropriate peripheral haematocrit ratios were then used for the relevant period of pregnancy in each case.

The formulae used in the calculations was as follows:-

$$\text{Plasma Volume} = \text{Plasma Volume Injected} \times \frac{\text{O.D. Standard}}{\text{O.D. Test Plasma}} \times \text{Dilution of Standard}$$

$$\text{Red Cell Mass} = \text{Plasma Volume} \times \frac{\text{Corrected Venous Haematocrit}}{100 - \text{Corrected Venous Haematocrit}}$$

Detailed results for each case are given in Appendix 2.

It was found that when the haematocrit values were compared with the red cell mass, calculated in terms of ml. per Kg. body weight, there was a very wide scatter of points as Lund and Sisson had demonstrated. It was soon noticed, however, that patients of heavy build tended to have a

low red cell mass, per Kg. body weight, in relation to the haematocrit value obtained; and that patients of light build tended to have a relatively high red cell mass, per Kg. body weight, in relation to their peripheral haematocrit value. This finding is shown in Figure 1, in which the tendency can be seen.

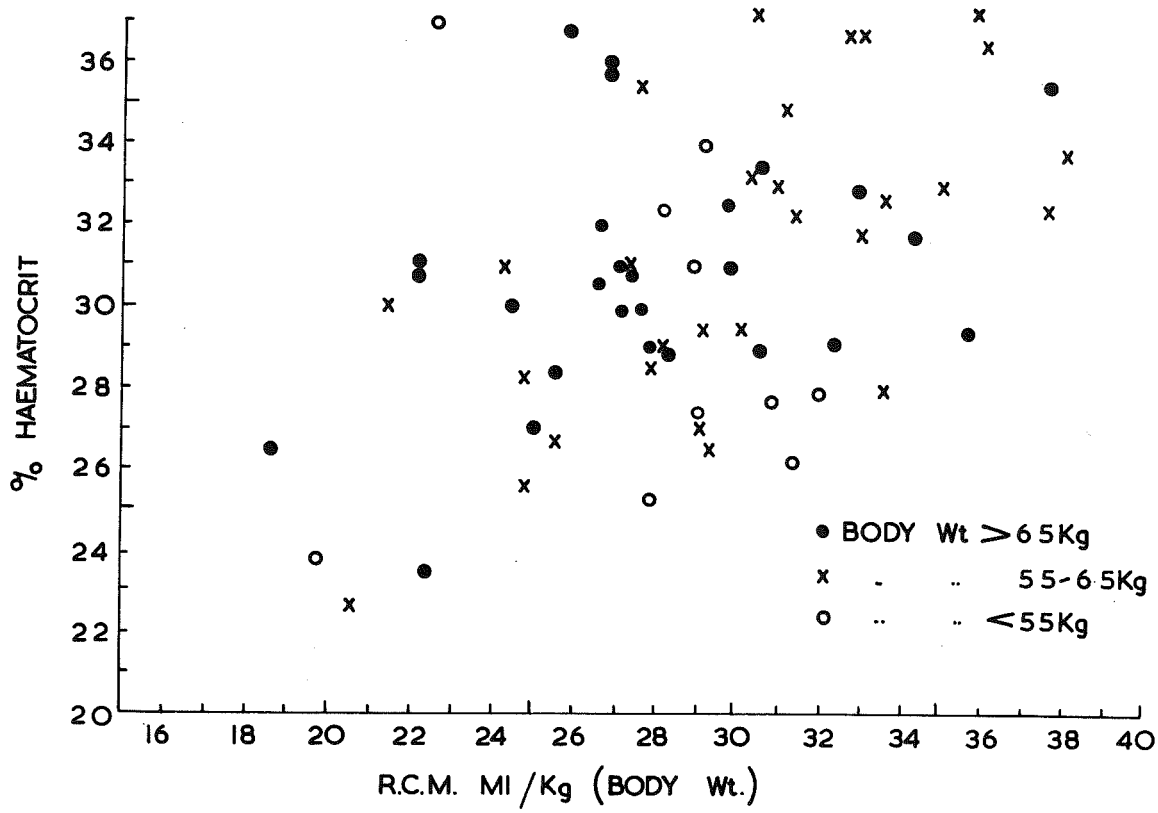
It is obvious, from histological appearances, that the volume of blood vessel capacity in adipose tissue is considerably smaller than that encountered in muscle and other organs of a more vascular nature, and it is therefore illogical to attempt to assess individual changes in terms of body weight. This aspect has been reviewed by Gregerson and Rawson (1959), who discussed the distribution of blood in the various organs; and by Keys and Brozek (1953), who were of the opinion that the lean body weight could be assessed with some degree of accuracy from skinfold measurements. Edwards and Whyte (1960) showed in twenty-five hospital patients, evaluation of the blood volume in terms of lean body mass - calculated from the skinfold thickness - gave only poor correlation.* They found that the surface area of the patients was a better index on which to base the assessments of the blood volume. They also obtained good correlation using the ideal weight for individual patients, taken from tables.

It is generally agreed that the best guide to the assessment of the individual blood volume measurement can be obtained by actual measurement of the lean body mass. This is usually carried out by the use of formulae involving either the antipyrine space alone, or measurement of both the antipyrine and thiocyanate spaces, which are indices for the assessment of the total body water and the extracellular fluid space respectively.

Muldowney (1957) estimated the antipyrine space and blood volume in a series of twenty-two normal male and female subjects; and he showed

* Due to the poor correlation of skinfold measurements with red cell mass.

FIGURE 1.



Illustrating scatter of results when the red cell mass is evaluated in terms of body weight, and then compared with the peripheral haematocrit values.
Note: Tendency for patients of heavy build to be grouped above and to the left of the centre.

that the wide scatter of values obtained, when the blood volume is plotted in terms of body weight, is eliminated when the blood volume is evaluated in terms of the lean body mass (Figure 2). He concluded that, for reasons which have already been discussed, the body weight was an inadequate parameter for the assessment of the blood volume.

At present, the measurement of the lean body weight in pregnancy appears to be an aspect which has been overlooked by workers in this field; and as it would be necessary to carry out trials, not only during pregnancy, but also at some stage after pregnancy in order to assess the fundamental significance of the formulae used, the preliminary work involved would need to be quite extensive.

For this reason, and also because the use of antipyrine for repeated estimations could entail some risk of aplasia of the marrow, it was considered that an extension of our project along these lines could not be undertaken. It was decided, therefore, to attempt a different approach to this problem.

Blood volume estimations and calculations of the red cell mass were carried out in anaemic patients, who were then treated with parenteral iron therapy. The red cell mass was again measured, after completion of the therapy.

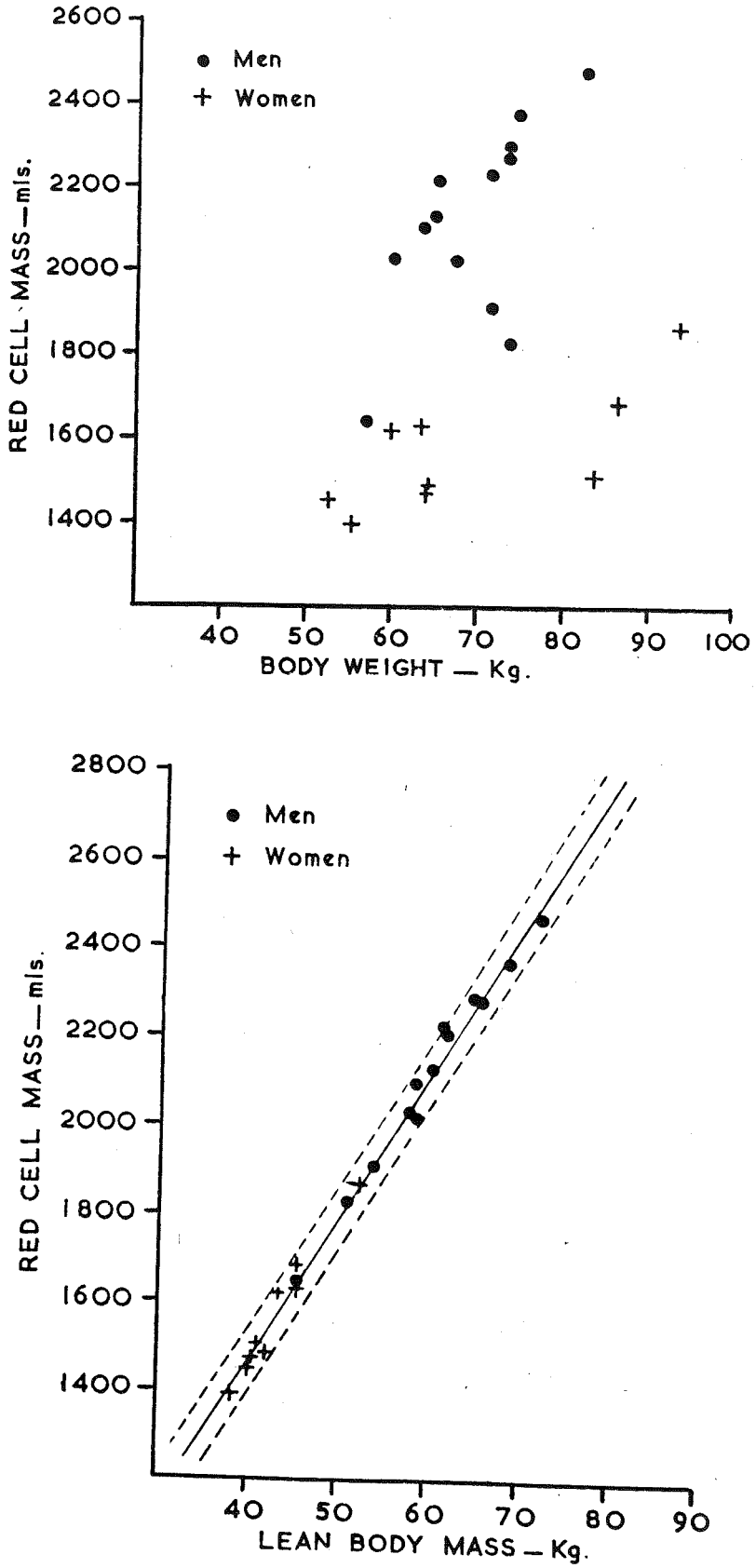
The figures obtained for the red cell mass were then evaluated in terms of total body weight, surface area, and ideal non pregnant weights taken from documenta Geigy (1956).

Body Weight.

The wide scatter of results obtained with this method has already been discussed, (Figure 1). The regression line calculated for these points has the formulae:

$$y = 0.56x + 20.45 \quad \text{and} \quad x = 0.64y + 8.95$$

FIGURE 2.



Taken from Muldowney (1957).

To show the comparative effects of using body weight and lean body mass as parameters on which to assess the red cell mass, in normal non-pregnant females and males.

The scatter of points from this line is represented by the correlation coefficient: $r = 0.48$

Surface Area.

This parameter gives a reduced scatter for the points in Figure 3, and moreover, the distribution of the heavy and light build patients has shown considerable mixing. The correlation coefficient for these values is increased: $r = 0.635$, and the formulae for the regression lines are: $y = 0.013x + 16.227$ and $x = 31.4y + 136.8$

Ideal Weight.

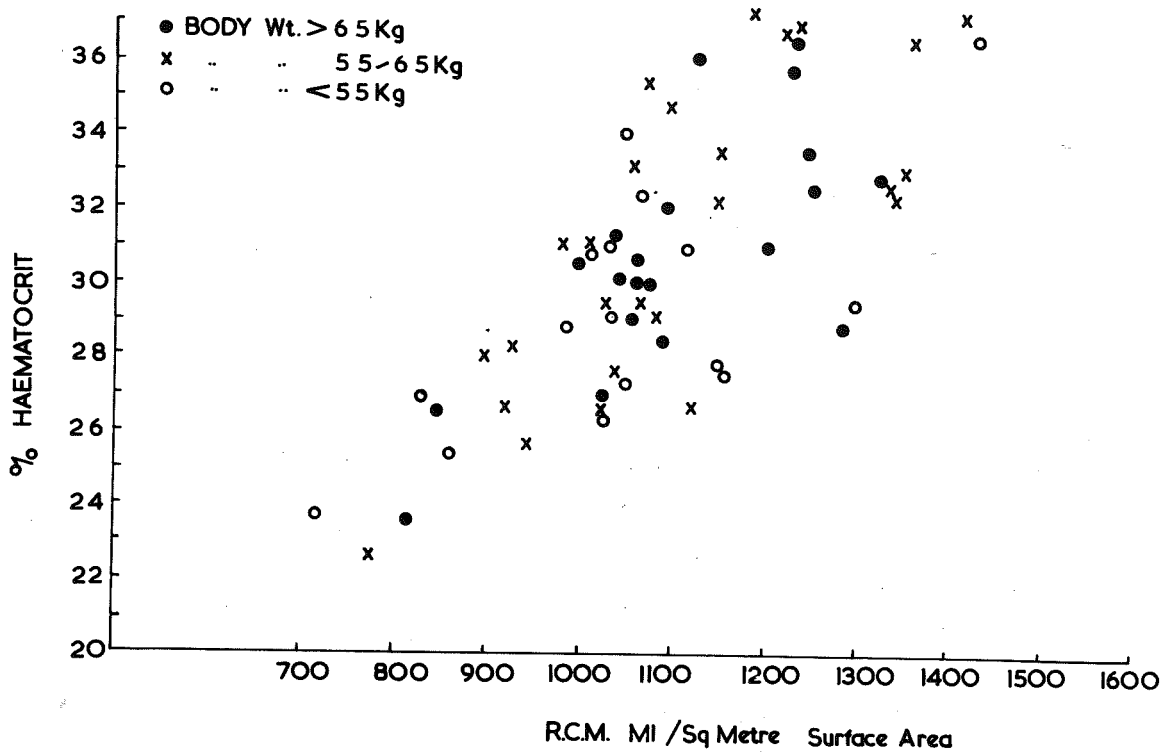
These values for the ideal weight, as reduced from tables of the patients' heights, would be expected to give some indication of the patients' non pregnant weights. Correlation has been attempted, using the pre-pregnancy weights which the patients gave from memory. The scatter of results obtained was so wide that it was obvious that the patients' memories were frequently at fault; and as a considerable number had very high values for the red cell mass, in terms of their pre-pregnancy weights, it would appear probable that some degree of understatement of this weight was not uncommon.

The correlation obtained with the ideal weight (Figure 4), ($r = 0.605$), was similar to that obtained with the surface area, and once again considerable interchange of points was observed, from one side of the regression lines to the other.

Variations in Red Cell Mass with Iron Therapy.

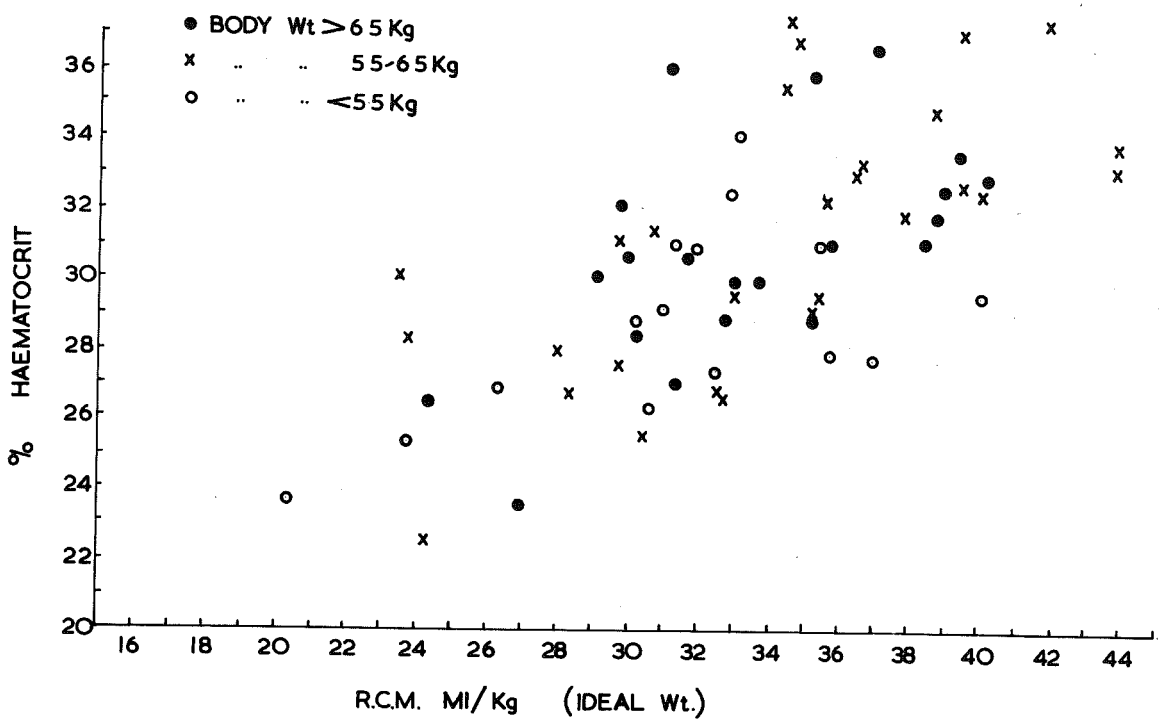
It was thought that the residual scatter in the points, when the red cell mass was evaluated in terms of the surface area and ideal weight, was probably attributable to the inaccuracy of these parameters. If this were the case, and the state of relative hydraemia of the patient was not a major factor, it was theorised that increases in the haematocrit

FIGURE 3.



Illustrating the reduced scatter of the results when the red cell mass is assessed in terms of body surface area.

FIGURE 4.



Illustrating the scatter in the results, when the red cell mass is assessed in terms of the ideal weight for the individual patient.

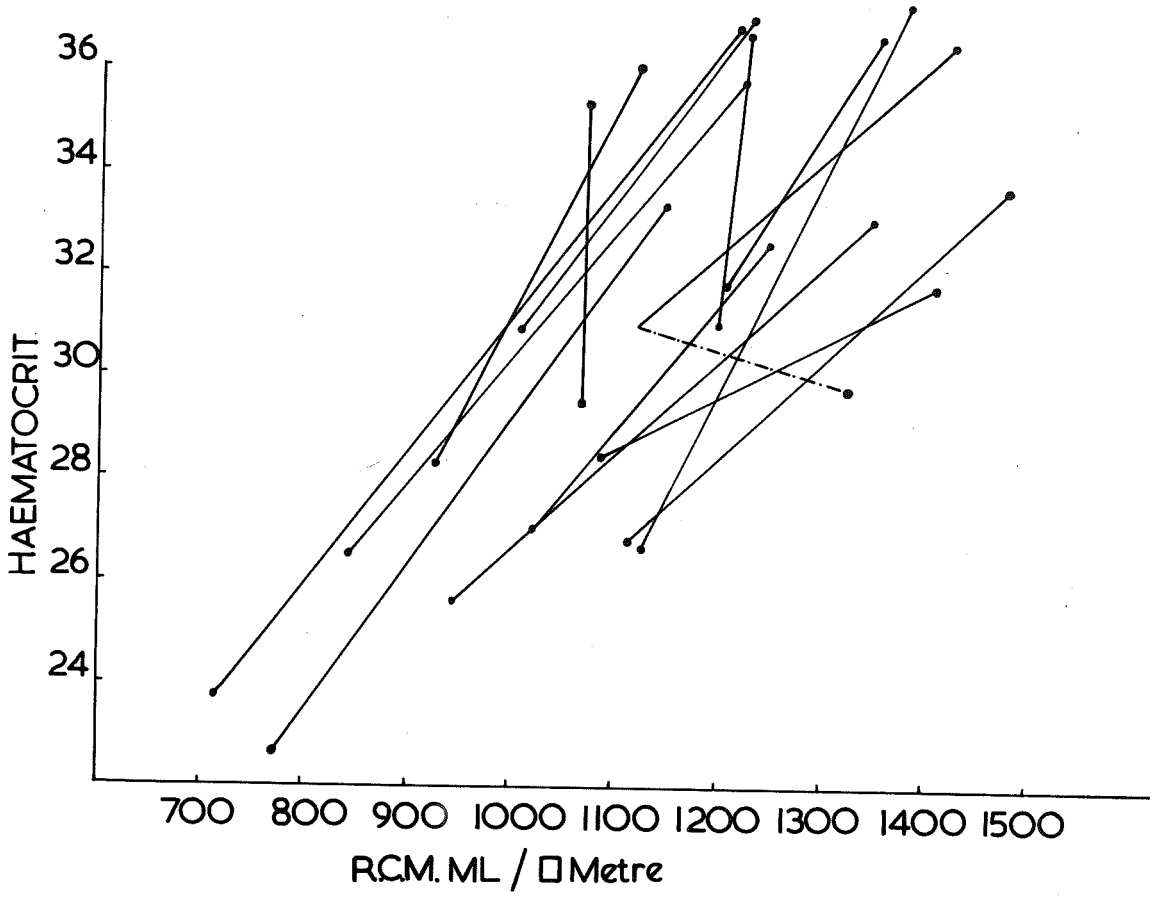
and red cell mass would probably be proportional in individual patients. By joining the points obtained before, and those obtained after, parenteral iron therapy, a series of parallel lines should then be obtained.

This was tested in a series of fourteen patients who responded to parenteral iron therapy with a rise in the haemoglobin and haematocrit values. The results are shown in Figure 5, and it can be seen that in the majority of cases a roughly parallel response was obtained. One patient, (No. 238, Appendix 6) developed a megaloblastic bone marrow between the first and second estimations (represented by the broken line in Figure 5); and as a result of folic acid therapy, she later showed a substantial rise in haemoglobin and haematocrit values. It can be seen from this case that the haematocrit value did not show any decrease during the phase of developing megaloblastosis, whereas the plasma volume and the red cell mass showed a fall. The inference from this case is, that some contraction of the plasma volume may occur during this type of episode, which counteracts the fall in red cell mass; resulting in maintenance of the peripheral haematocrit levels.

It was concluded from these experiments that, although the variations in plasma volume may have some effect on the peripheral haemoglobin and haematocrit values, individual errors introduced by assessment of the red cell mass, in terms of the body weight, surface area and ideal weight, would invalidate assessment of the patient's haematological status, in terms of the red cell mass.

Until such time as some simple and accurate method is evolved for the measurement of the lean body mass during pregnancy; I am of the opinion that the peripheral blood values, although not ideal, will still remain as the best criteria for the diagnosis and assessment of anaemia in pregnancy.

FIGURE 5.



To show the relative increases of haematocrit, and the red cell mass calculated in terms of surface area, in patients responding to parenteral iron therapy.

SUMMARY OF SECTION 2.

IRON DEFICIENCY ANAEMIAS IN PREGNANCY.

The typical changes of microcytosis and hypochromia usually associated with iron deficiency are only found in pregnancy after a state of severe iron deficiency has been in existence for a comparatively long period. The development of the relatively acute iron deficiency states in pregnancy may not show any evidence of abnormality in the red cell indices.

The Mean Corpuscular Volume (M.C.V.) is a more accurate parameter for the assessment of iron deficiency in pregnancy, than the Mean Corpuscular Haemoglobin Concentrate (M.C.H.C.).

Evidence of iron deficiency has been found in patients with haemoglobin values between 11.6 g. and 12.5 g. per 100 ml. of blood.

Good correlation has been demonstrated between the serum iron and percentage saturation of the Total Iron Binding Capacity (T.I.B.C.) and the response to parenteral iron therapy, but a considerable overlap of the values for the serum iron in patients responding to iron therapy with the normal range has also been found. The possible causes for this have been discussed, and it is suggested that the fall in the serum iron may be a secondary event in the development of the iron deficiency state.

It is difficult to establish the diagnosis of mild degrees of iron deficiency using the routine laboratory tests described. The severe iron deficiency states associated with microcytosis and low serum iron almost invariably show a brisk response to parenteral iron therapy.

SECTION 2.

IRON DEFICIENCY ANAEMIAS IN PREGNANCY.

At the Queen Victoria Maternity Hospital, it is routine practice to treat all antenatal patients with oral ferrous gluconate; but many patients fail to take the oral iron for various reasons. One of the commonest of these being that the patient feels well and can't believe the need for medication in her particular case. Some patients develop nausea, vomiting or diarrhoea whilst on the tablets. These symptoms are, of course, particularly common during the first five months of pregnancy, which is the time when the oral iron therapy is usually commenced, after the patient's first visit to the antenatal clinic. Unfortunately, these patients are then averse to taking further oral iron when the phase of morning sickness has passed.

Some patients seen during these investigations responded to the administration of ferrous gluconate. Cases seen during the first seven months of pregnancy predominate in this group, as it was usually the practice, if possible, to try this form of therapy first. A few patients manifested a severe degree of anaemia during this period; and it was considered that the risks to the foetus, entailed in a slow response to oral therapy, were not justified, and the patients were given parenteral iron therapy. This was occasionally carried out in intensive therapy on in-patients.

Scott and Govan (1949) believe that the absorbtion of iron may sometimes be defective during pregnancy. This was supported by my personal observation of many patients who had no apparent response in their anaemia, although they assured me they had taken their oral iron. So the giving of parenteral iron to these patients was usually commenced when the pregnancy had advanced beyond the seventh month.

Statistical analysis showed that no correlation was present between the response to oral iron therapy and the minimum haemoglobin, in twenty-eight patients who were assessed; and the lack of correlation in this assessment is almost certainly due to the multiplicity of factors influencing their rate of absorbtion of oral iron.

I therefore propose, in this present evaluation of the diagnosis of iron deficiency in pregnancy, to deal solely with those patients who showed a response to the parenteral iron therapy. I consider that, in this way, the maximum possible uniformity can be maintained in the findings. An additional feature in this method of presentation is that the patients were usually treated at the same stage of their pregnancies, (i.e. from the twenty-eighth to thirty-ninth weeks of gestation), and that the changes of blood volume during this period are at their minimum.

The dosage of iron dextran was calculated using the following formula taken from Holly's recommendation in 1958.

$$\frac{13.5 - y}{100} \times 4{,}000 \times 3.4 + 500 = \text{Total dosage of iron-dextran}$$

where y = the patients haemoglobin value in g. per 100 ml.

The average total dose for each patient came to 1,000 mg. of iron-dextran. It was found in practice that this amount was adequate, and at the same time it was not considered to be excessive as it was of the same order as the normal body stores, which had been depleted in the pregnancy.

The incidence of iron deficiency anaemia in pregnancy is known to be high in the United Kingdom and comparatively low in Australia (Morgan 1961). It is not proposed to dwell upon the incidence of this condition, but rather to evaluate the various laboratory criteria used for the diagnosis of iron deficiency in pregnancy, and to make available the findings in iron deficiency states for comparison with those in

thalassaemia minor.

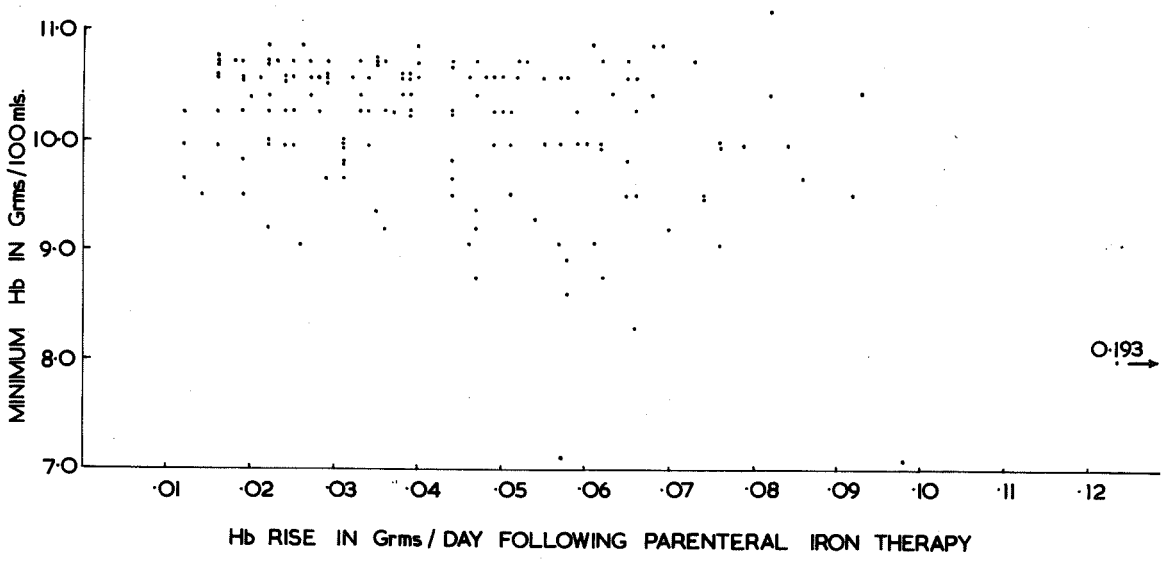
In evaluating the response to therapy during this survey, it was necessary to adopt some common criterion for the comparative measurement of response. Owing to the variations in time available during pregnancy for the response to therapy to take place, and also to the variable period between repeat haemoglobin estimations, it was decided to assess the response to therapy in terms of the daily rise in haemoglobin: (g. per 100 ml. of blood per day). Additional provisos were made, that no evidence of a response to therapy was accepted unless the total rise was in excess of 0.75 of a gramme of haemoglobin, and the rate of response was at least 0.012 g. haemoglobin per day.

Most of the patients accepted showed responses greatly in excess of one or other of these criteria. Moreover, no values for the peripheral haemoglobin were accepted as criteria for a response if they were taken during the last week of pregnancy, owing to the possibility of the haemoconcentration which, it has been reported, may occur during this period, (Lund 1951), 30 patients have been excluded from the survey results for this reason alone, although most of them showed evidence of a response to iron therapy.

Pre-treatment Haemoglobin Levels and the Response to Iron Therapy.

The total of one hundred and fifty-eight patients (Appendix 3), who showed a response to parenteral iron therapy had pre-treatment haemoglobin values ranging from 8.0 g. of haemoglobin per 100 ml. of blood to 11.2 g. of haemoglobin per 100 ml. There appeared to be some correlation of the rate of response to therapy with the severity of the anaemia, ($r = -0.22$, $p =$ less than 5%); but as can be seen from the scatter of results (Figure 6), the fact that an upper limit of 11.0 g. has been applied for the patients so treated, has obviously restricted the scatter

FIGURE 6.



Showing the correlation between the pre-treatment haemoglobin value, and the rate of haemoglobin rise due to parenteral iron therapy. Each point represents one patient.

of points. The statistical analysis of these figures from this point of view must, in consequence, be invalid. It would be expected that the severity of the anaemia would have some effect upon the rate of response to therapy, but to what extent this applies cannot be elicited from these figures.

The obvious restriction, and the scatter of results which has been illustrated, suggest that it is probable that many patients with haemoglobins in excess of 11.0 g. per 100 ml. would also respond to parenteral iron therapy.

Mean Corpuscular Volume and Mean Corpuscular Haemoglobin Concentration in Iron Deficiency Anaemia of Pregnancy.

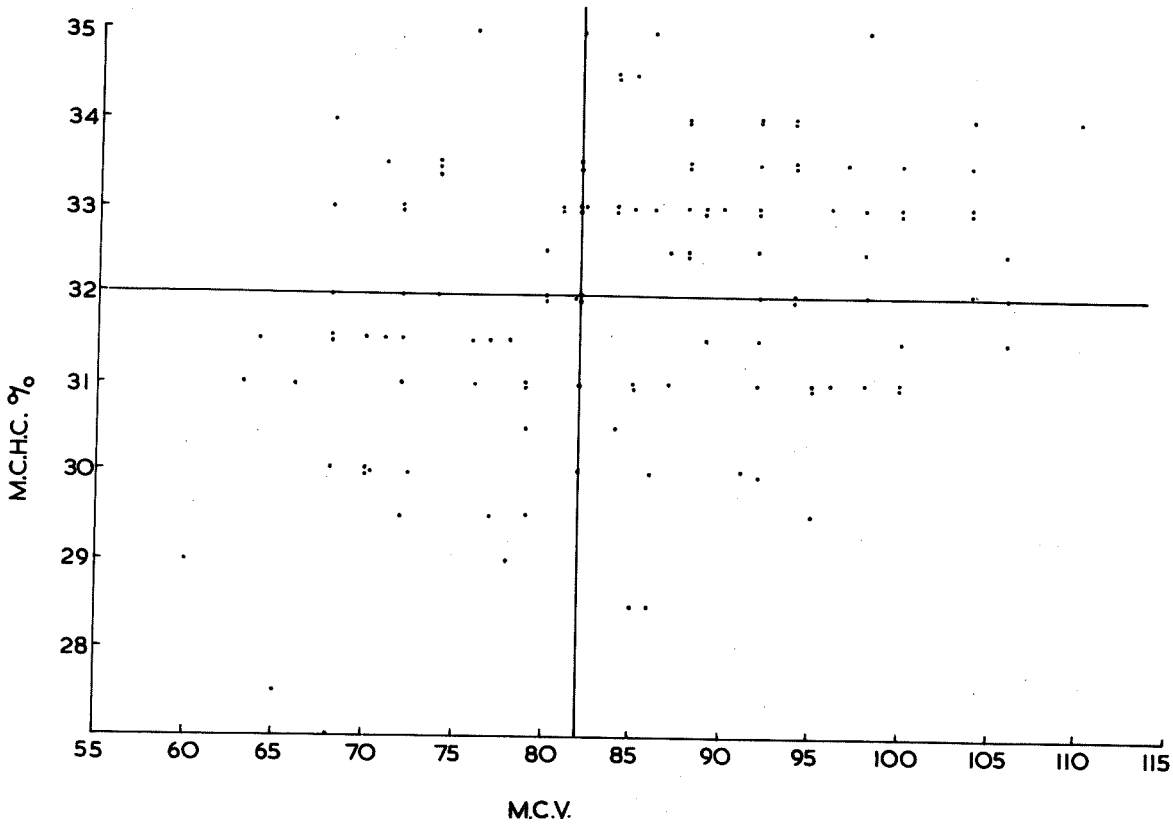
The relationship between the M.C.V. and the M.C.H.C. is shown in Figure 7. The variations in these results are considerable, but it does appear that there is a rough correlation between the M.C.V. and the M.C.H.C. in the hypochromic microcytic patients. This, of course is the expected relationship; and a further anticipated result is that many of the patients do not show any evidence of either hypochromia or microcytosis. This is due to the fact that the development of iron deficiency during pregnancy has a comparatively rapid onset, and the estimates of the red cell indices are largely influenced by the older populations of cells formed before the deficiency developed.

In an attempt to ascertain the relative accuracy of the M.C.V. and the M.C.H.C. as indices of the iron deficiency state of the patient, correlation of these two parameters with the response to iron therapy has been separately assessed.

Mean Corpuscular Haemoglobin Concentration and the Response to Parenteral Iron Therapy.

Only fifty-two out of one hundred and thirty-six (or 38%) of

FIGURE 7.



Showing the correlation between the Mean Corpuscular Volume, and Mean Corpuscular Haemoglobin Concentration in patients responding to parenteral iron therapy. There is considerable overlap of the lower limits for the normal ranges (indicated by lines at M.C.V. of 82 c.μ and M.C.H.C. of 32%.

the cases in whom pre-treatment data is available show evidence of a reduction in the M.C.H.C. It was found, moreover, that in many of the patients with values for the M.C.H.C. within the normal range of 32% to 36% showed a vigorous response to the parenteral iron therapy. (Figure 8). From this scatter of results, it can be seen that the correlation between the M.C.H.C. and the rate of response is very poor; (the coefficient of correlation (r) = 0.043). The probability that these findings are due to chance is greater than 10%.

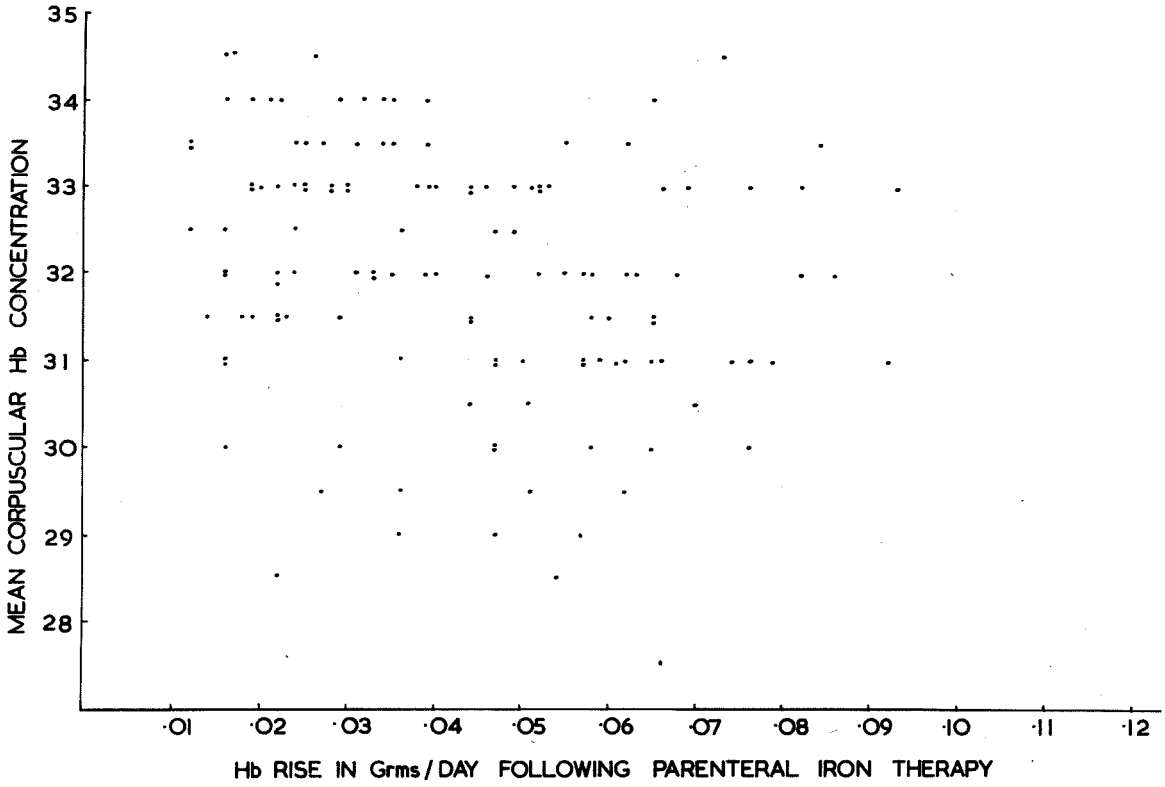
The Mean Corpuscular Volume, and Response to Parenteral Iron Therapy.

The difference between the values for the correlation coefficients, obtained with the M.C.V. and the M.C.H.C., have been tested by Fisher's z transformation test, and have been found to be significant. (p = less than 1%, greater than 0.5% for one tail).

When the M.C.V. is plotted against the response to therapy (Figure 9), it can be seen that the scatter of results is not as wide as was obtained with the M.C.H.C. and the response to therapy (Figure 8). This is demonstrated by the higher value obtained for the correlation coefficient r = 0.332, and there is a less than 5% probability that finding a correlation coefficient of this relatively high value is due to chance. It would appear, therefore, that the M.C.V. is a better guide to the rate of response to parenteral iron than the M.C.H.C. By inference, it would follow that the M.C.V. is a better guide to the degree of iron deficiency in pregnancy.

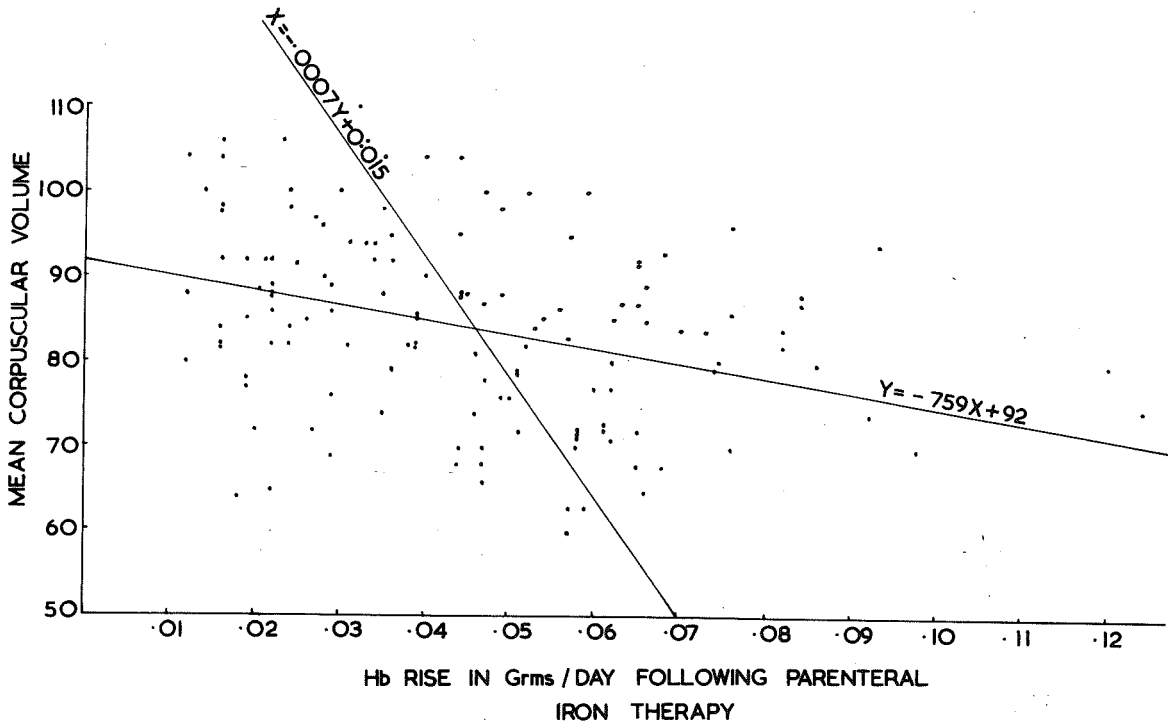
Owing to the fact that the calculation of the M.C.V. depends upon the red cell count, which is usually believed to be a grossly inaccurate measurement, it is somewhat surprising to find that the order of correlation obtained is high. Great attention has been paid, throughout these investigations, to ensuring that the highest possible accuracy

FIGURE 8.



Illustrating the lack of correlation between the pre-treatment value for the percentage Mean Corpuscular Haemoglobin Concentration, and the rate of response to parenteral iron therapy.

FIGURE 9.



Showing the correlation between the pre-treatment value for the Mean Corpuscular Volume, and the rate of response to parenteral iron therapy.

was maintained in the red cell counting techniques; and it seems that these efforts have been largely successful.

These results are assessed as indicating that, in general, a fair degree of confidence can be placed in the results for the values for mean corpuscular volume; which have been relied upon to a considerable extent in the diagnosis of the thalassaemia minor cases.

From the results obtained in the cases responding to parenteral iron therapy, it can also be seen (Figure 9) that responses were obtained, not only in the forty-seven cases who were microcytic, but also in thirty-three cases who were macrocytic. I considered the possibility that this finding was due to the macrocytosis sometimes associated with rapid red cell generation, (Dacie 1956); but found, in analysing the reticulocyte counts of those cases with M.C.V.s in excess of 87 cu. microns, that no evidence of correlation could be found: ($r = 0.15$, p is greater than 10%). Inspection of the scatter of points obtained in the correlation of the M.C.V. with the response to therapy shows that this area of macrocytosis appears to be an extension of the general regression of the points. The presence of macrocytosis in these cases cannot be explained by the present findings, but an obvious corrollary to these results would be that the presence of a mild degree of macrocytosis in pregnancy does not necessarily exclude iron deficiency, and indicate the presence of a deficiency of folic acid or B12 deficiency. The possibility of a mixed deficiency can't be dogmatically excluded, but it seems unlikely, as the majority of these patients had a substantial rise in their haemoglobin values.

Response to Parenteral Iron Therapy and the Reticulocyte Count.

In order that the normal range for the reticulocyte count in pregnancy could be determined, a series of reticulocyte counts was estimated on normal pregnant females at between thirty-one and thirty-six

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TABLE 1.

STATISTICAL EVALUATION OF RETICULOCYTE COUNTS IN NORMAL PREGNANT CONTROLS, AND IRON DEFICIENCY ANAEMIAS.

Patients	Number	Mean	S.D.
Normal controls	58	2.78	1.27
Iron deficiency anaemias	132	2.98	1.52

Groups Compared	F test on S.D.	t test on means
Normal controls Iron deficiency anaemias	<0.1% ***	<40% >30%

weeks of gestation. In the initial assessment of these results, in order to determine whether the haemoglobin level had any influence on the percentage reticulocytes, the cases were divided into two groups, in which patients with haemoglobin values of 12 to 13 g. per 100 ml. were compared with patients with haemoglobin values in excess of 13.0 g. per 100 ml. No variation attributable to the haemoglobin values was found, and the values were pooled.

The mean value in the fifty-eight normal patients for the reticulocyte count was 2.78%. When this value is compared with the mean value in the one hundred and thirty-two iron deficiency cases, (Table 1), it can be seen that the mean value for the iron deficiency cases is slightly increased, but that the difference is not statistically significant, it is a fairly general finding that in the non pregnant iron deficiency states, not associated with acute blood loss, the reticulocyte count is usually within the low normal range.

The above findings indicate that in pregnancy iron deficiency is not usually accompanied by a depressed level in the reticulocyte counts. This finding is probably attributable to the effects of the increasing blood volume on the marrow, which would be somewhat analogous to the effects of acute blood loss in the non pregnant patient.

There is always the possibility of blood loss from haemorrhoids; a condition to which the pregnant patient is particularly prone. A history of blood loss due to this cause could only be elicited in the very occasional case, however, and it is unlikely that this factor can have had any significant effect on these results.

Serum Iron Values in the Normal Pregnant Patient.

Serum irons, throughout these investigations, have been estimated by Kaldor's (1953) modification of the orthophenanthroline method of Kitze.

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TABLE 2.

COMPARATIVE NORMAL VALUES OF SERUM IRON AND T.I.B.C. IN MALES, NON-PREGNANT FEMALES AND PREGNANT FEMALES.

Authors		Control Patients No.	Control Patients Type.	Mean Serum Iron	Standard Deviation	T.I.B.C.	Standard Deviation.
Sturgeon	1959	31	Males	138	38	361	52
Hagberg	1953	26	Males	137	32	321	39
Roman and Wellby*	1957	22	Males	134	32	316	34
Gerritson and Walker	1954	48	Females	119	32	323	45
Sturgeon	1959	20	Females	122	38	360	50
Hagberg	1953	28	Females	123	44	338	31
Roman and Wellby*	1957	20	Females	129	37	317	38
Gerritson and Walker	1954	49	Pregnant females	120	46	403	58
Rath et al.	1950	18	Pregnant females	102	34	336	45
Sturgeon	1959	51	Pregnant females	118		514	
Hagberg	1953	21	Pregnant females	98		470	
Ibbotson	1961	15	Pregnant females	131	39.7	484	76

* Carried out in the Institute of Medical and Veterinary Science.

A standard iron solution was used as a routine check on the accuracy of the method. The T.I.B.Cs. were measured by the method of Rath and Finch (1949). All patients tested were fasting, and precaution was taken to ensure that they had taken no oral iron for at least forty-eight hours beforehand. All the specimens were collected between 9.0 a.m. and 10.0a.m. to eliminate the effects of diurnal variation: (Vahlquist 1941).

We tried to confine the estimations to patients at the thirty-second week of pregnancy; and the results quoted apply to patients between the twenty-sixth and thirty-sixth weeks of gestation, as mistakes in the estimated dates of delivery were found to influence the final assessment of the time of testing.

A considerable number of published results have quoted the normal ranges for the serum iron and T.I.B.C. in both the normal non pregnant and pregnant patient, and some of these are shown in Table 2. Normal ranges for the male and the non pregnant female have also been assessed in these laboratories, (Roman and Wellby, 1957); and during the course of this present investigation, a survey of the normal ranges for the serum iron and T.I.B.C. in pregnancy has also been undertaken.

It is generally considered that haemoglobin levels of the order of 12.0 g. per 100ml. are within the normal limits for pregnancy (Holly 1955). In the early stages the determinations of the normal serum iron ranges, patients known to have haemoglobin levels of about 12.0 g. per 100 ml. were tested. For reasons which will become apparent in the discussion of these results, a further series of more stringently selected normals had to be obtained from patients with haemoglobins of the order of 13.0 g. per 100 ml.

In the assessment of these results the normal pregnant patients have been divided into two groups - according to their haemoglobin level

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TABLE 3.

COMPARISON OF SERUM IRON, T.I.B.C. AND PERCENTAGE SATURATION OF T.I.B.C. VALUES IN TWO GROUPS OF NORMAL CONTROL PATIENTS WITH HAEMOGLOBIN VALUES OF 11.6-12.5 g.; AND IN EXCESS OF 12.6 g. PER 100 ml. BLOOD AT 28-34 WEEKS OF PREGNANCY.

Group	Estimation	Number	Mean	S.D.
Hb. $>$ 12.5 g.	Serum iron	15	131	39.7
Hb. $\leqslant$ 12.5 g.	" "	15	83.5	28.2
Hb. $>$ 12.5 g.	T.I.B.C.	15	484	75.9
Hb. $\leqslant$ 12.5 g.	"	13	519	71.1
Hb. $>$ 12.5 g.	% Sat.T.I.B.C.	15	27.2	7.2
Hb. $\leqslant$ 12.5 g.	" "	13	17.2	6.2

Group Compared	Estimation	F test on S.D.	t test on Means.
Hb. $>$ 12.5 g. Hb. $\leqslant$ 12.5 g.	Serum iron	Not significant	$<$ 0.1% ***
Hb. $>$ 12.5 g. Hb. $\leqslant$ 12.5 g.	T.I.B.C.	Not significant	$<$ 30% $>$ 20%
Hb. $>$ 12.5 g. Hb. $\leqslant$ 12.5 g.	% Sat. T.I.B.C.	Not significant	$<$ 0.1% ***

at the time of collection of the serum for the investigation. These groups comprised a total of thirty patients, fifteen of whom had haemoglobin levels between 11.6 g. and 12.5 g. per 100 ml. of blood (inclusive), with a mean level of 12.2 g. (S.D. 0.25). The other group had haemoglobin levels in excess of 12.5 g. per 100 ml., with a mean level of 13.2 g. (S.D. 0.16).

Analysis of the figures (Table 3) showed that there was a highly significant difference in the serum iron levels between these two normal control groups; and the mean level of 131 micro micrograms in the higher haemoglobin group was 47 micro micrograms higher than in the lower haemoglobin group. These figures are interpreted as indicating some degree of iron deficiency in those patients with haemoglobin levels of less than 12.5 g. per 100 ml.

Total Iron Binding Capacity in Normal Pregnant Patients.

In the two groups which have already been outlined, the T.I.B.C. is slightly lower in the higher haemoglobin group of control patients, but the degree of separation between the two groups is not significant: (Table 3).

Percentage Saturation of the T.I.B.C. by the Serum Iron in Normal Pregnant Patients.

The percentage saturation of the T.I.B.C. is exaggerated by the cumulative separation of the serum iron and the T.I.B.C.; and in view of the above findings, therefore, it is not surprising to discover that the degree of separation between the means of the two groups is highly significant: (Table 3).

Histograms for the normal values for the serum iron, T.I.B.C. and % Saturation are included in Section 3; Figures 23, 24, and 25.

Normal Ranges for the Serum Iron and T.I.B.C. Levels.

From the above data, and from some of the results yet to be presented in this section, it is fairly evident that the normal non pregnant range, for the serum iron, of 70-170 micro micrograms could include many patients who are mildly iron deficient. It is generally acknowledged that there is a rise in the level of the T.I.B.C. during pregnancy which is not associated with iron deficiency: (Rath et al. 1950; Bothwell et al. 1956); and it is possible that there is some rise in the normal serum iron level associated with this rise in the T.I.B.C. It cannot therefore be automatically assumed that the normal non pregnant range can be applied to pregnancy.

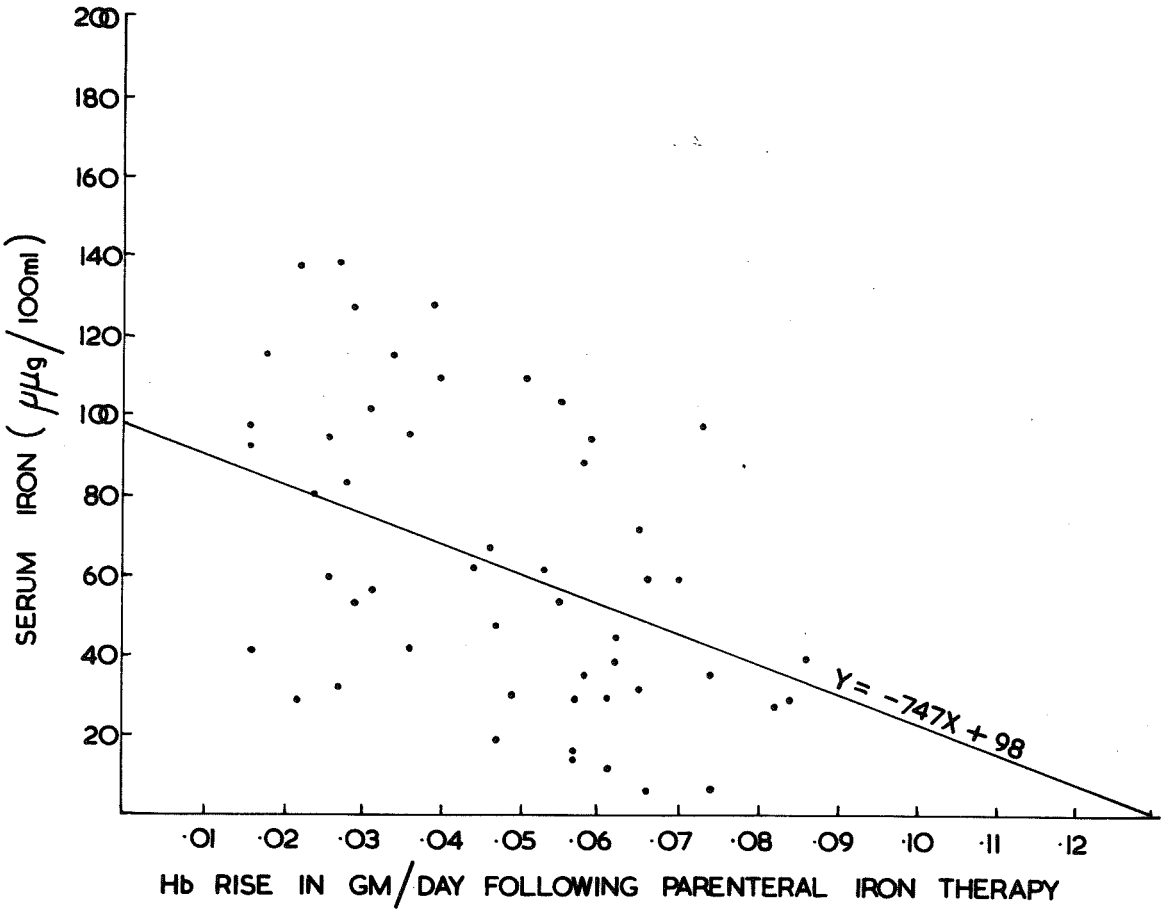
We are left then with the question: what precisely is the normal range ? It is noteworthy that there is one patient in the high haemoglobin normal control group who had a haemoglobin of 12.7 g. per 100 ml., and in whom the serum iron level was 59 micro micrograms and the percentage saturation of the T.I.B.C. only 12%; so it seems likely that even this group is somewhat biased by the inclusion of borderline iron deficiency patients.

Although it is appreciated that there may be some bias, I propose to accept the values found in the high haemoglobin group as the normal pregnant values for the serum iron, T.I.B.C. and percentage saturation, for comparison with the iron deficient and thalassaemic groups of pregnancy anaemia.

Serum Iron Levels and the Response to Parenteral Iron Therapy.

As can be seen in Figure 10, there was a highly significant degree of correlation between the serum iron levels and the rate of response to parenteral iron therapy: ($r = 0.387$, p = less than 1%, greater than 0.1%). One of the features which caused much difficulty in the

FIGURE 10.



Showing the correlation between the pre-treatment value for the serum iron and the rate of response to parenteral iron therapy.

initial stages of the investigations was the fact that many of these patients showed a definite response to the iron therapy when the serum iron level was within the normal range of 70 to 170 micro micrograms. Of the fifty-four patients assessed in this way, seventeen had a rise in their haemoglobin value when the pre-treatment serum iron level was in excess of 90 micro micrograms. Irrespective of at what point the lower limit of the normal range is set below the mean normal level of 131 micro micrograms, it would appear that there will be some patients who show a response to the iron therapy when their serum iron level is within the normal range.

The possibility was considered that this response might be due to the rise, sometimes found during the later stages of pregnancy in thalassaemia; a subject which will be discussed in a later section. Of the seventeen patients mentioned above, ten were of Greek or Italian origin. This proportion is representative of the overall figure of twenty-eight Greek and Italian patients out of the total fifty-four patients assessed in this manner. Moreover, the four patients showing a response, with serum iron levels in excess of 120 micro micrograms, were not Greek or Italian. So this possible explanation for the anomaly is fairly conclusively excluded.

T.I.B.C. Levels and the Response to Parenteral Iron Therapy.

The question of whether there is any additional rise in the T.I.B.C. level in iron deficiency in pregnancy above the usual 'non-specific' pregnancy rise had not been very clear until recently. Although Morgan (1961) did not establish any very definite relationship between the haemoglobin values and the serum iron levels, he was able to show fairly decisively that there was a rise in the T.I.B.C. levels in those patients who had lower haemoglobin levels, and there was also a fall in the T.I.B.C. levels in patients given oral iron.

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TABLE 4.

COMPARATIVE VALUES FOR THE T.I.B.C. IN NORMAL AND IN IRON DEFICIENT PATIENTS.

Group	Number	Mean	S.D.
Normal controls (Hb. > 12.5 g.)	15	484	75.9
Iron deficiency (All cases)	30	487	106
Iron deficiency (Response >.05 g. daily)	15	524	109
Iron deficiency (Response ≤.05 g. daily)	15	449	82.1

Group Compared	F test on S.D.	t test on Means.
Iron deficiency (Response >.05 g. daily) Normal controls (Hb. > 12.5 g.)	> 10%	< 15%, >10%
Iron deficiency response >.05 g. daily Iron deficiency response ≤.05 g. daily	> 10%	< 0.1% ***

The present results obtained with the patients under review are not so clear cut, but it can be seen (Table 3) that there was a slight rise in the T.I.B.C. levels in the normal control patients who had haemoglobin levels below 12.6 g. per 100 ml. which although not statistically significant is suggestive of a rise due to a mild degree of iron deficiency in these cases.

For the purposes of comparison with the normal controls the 30 patients showing a response to parenteral iron, in whom there is data available on the T.I.B.C. levels, have been divided into two groups according to the rate of response. It can be seen (Table 4) that there is a difference between the T.I.B.C. levels of these two groups, but it is also evident that as the mean value for the normal control group lies between these two iron responsive groups that any variations in the T.I.B.C. levels in these groups cannot be regarded as being of great significance.

It is probable that these findings can be attributed to at least two factors. Firstly, the method of Rath and Finch (1949) which was used for these estimations is probably inferior to the later methods which are at present being adopted. Secondly, in view of the fact that the T.I.B.C. is a protein and as such is liable to a very wide variation in the normal range during pregnancy as shown by the large standard deviations obtained in the normals; any rise in the individual patient which may be attributable to iron deficiency is largely masked by the wide scatter in the normal range. It is almost certain however both from the work of Morgan and from the high degree of correlation which will be shown to exist between the % saturation of the T.I.B.C. and the rate of response to parenteral iron therapy, that in the individual patient there must be some rise in the T.I.B.C. attributable to iron deficiency during pregnancy.

Percentage Saturation of the T.I.B.C. and the Response to Parenteral Iron Therapy.

The distribution of the points in Figure 11 shows that a high degree of correlation exists between the percentage saturation and the response: (r = 0.6, p = less than 0.1%). This value for r, obtained with the percentage saturation, is higher than the value for r in the correlation between the serum iron and the response to iron therapy. The difference between these two values for r was tested by Fisher's z transformation test, and it was found that t had a value of 1.196 on 76 degrees of freedom: this is equivalent to a value for p of between 10% and 15% for one tail. The difference is barely significant therefore; but the findings, nevertheless, strongly suggest that the percentage saturation of the T.I.B.C. is the better indicator of iron deficiency during pregnancy than the serum iron alone.

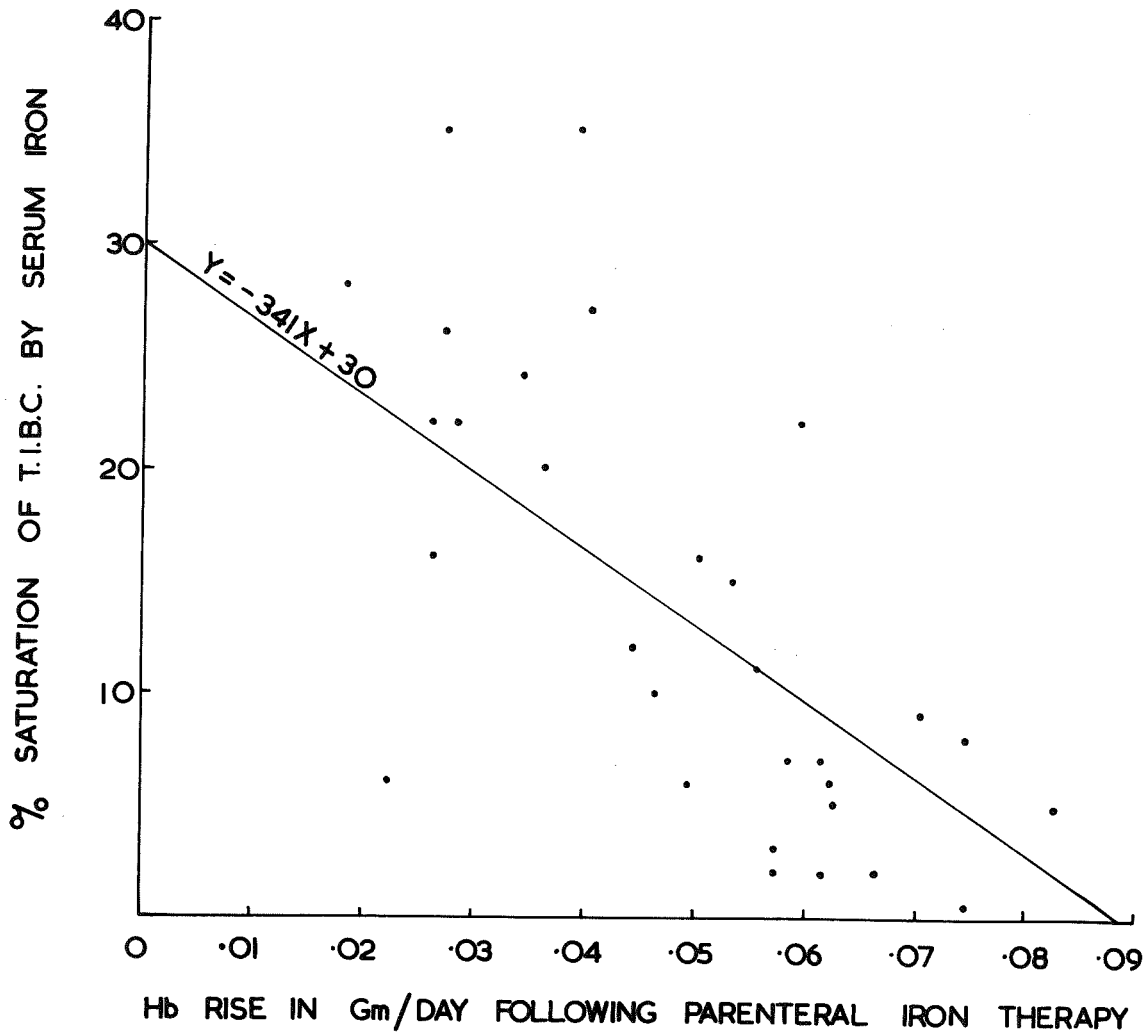
It can be seen in Figure 11, that one case lies outside the normal pattern of results, and shows a comparatively low response, with a percentage saturation of only 6%. This patient had an A_2 value of 2.7%, with a normochromic, normocytic blood picture, and there was no evidence suggestive of a thalassaemia minor. The total rise in her haemoglobin was 1.2 g. per 100 ml. between the twenty-eighth and thirty-eighth weeks of pregnancy; attaining a level of 12.0 g. at the thirty-eighth week, and although this appears to be a definite response, it is possible that there may have been some retardation due to a slight folic acid deficiency.

The percentage saturation of the T.I.B.C. in the high haemoglobin group of controls has a mean value of 27.2%, and it can be seen that many patients, with saturation levels which must be within the normal range, show a response to parenteral iron therapy.

The Diagnosis of Iron Deficiency During Pregnancy.

Sturgeon (1959) investigated the effects of parenteral iron

FIGURE 11.



Showing the correlation between the pre-treatment value for the percentage saturation of the T.I.B.C. and the rate of response to parenteral iron therapy.

therapy in pregnancy, and concluded that a response thereto indicated a pre-existing iron deficiency. This seems to be a reasonable assumption, and it has been applied throughout these investigations.

Under the described conditions, it has been demonstrated that the cell size seems to be a more accurate parameter for the assessment of iron deficiency in pregnancy than the cell chromicity: the reticulocyte levels are of singularly little assistance.

The percentage saturation of the T.I.B.C. by the serum iron has been found to be quite an accurate guide to response; but unfortunately, there is a gross overlap of the values of patients who have shown a response to iron therapy, with those of the normal range.

It is difficult to account for the fact that patients with a normal serum iron level show a response to parenteral iron therapy. It might be suggested that, even in the high haemoglobin group of control, there is, in fact, a bias introduced by the inclusion of iron deficient subjects, sufficient to account for these findings. But this seems hardly feasible when viewed against the agreement found with the values of Squires (1952) and Geritson and Walker (1954). They were working among the Bantu who are known to have a very high iron intake owing to the low phosphate content of their maize diet and to cooking in iron vessels.

A concept more difficult to confirm or refute has been put forward by Mandel (1959), who suggested that owing to the multiplicity of factors involved - many of which are at present undetermined - a wide normal range is not surprising, and that 60 micro micrograms may be a normal level in one patient, but abnormally low in another, who has a normal concentration of 120 micro micrograms per 100 ml. But if this hypothesis is correct, one would expect less correlation between the level of the serum iron and the response to therapy than is apparent with the present data.

In 1958, Beutler, Robson and Buttenweiser correlated tests for iron deficiency in the non pregnant state, and formed the opinion that the fall in the plasma iron was late in the sequence of events. Reid (1958) observed the onset of an iron deficiency anaemia in a non pregnant case, in whom repeated estimations on the serum iron, during two months, were normal.

I consider that the present findings confirm the opinion of the two latter authors, and suggest that the development of the hypo-ferraemia follows the occurrence of the slowing of haematopoiesis in pregnancy, due to the iron deficiency. There is obviously a common causal factor between the slowing of haematopoiesis and the hypo-ferraemia, although the linkage is only indirect.

These observations certainly detract from the value of the serum iron as an investigational aid in the diagnosis of mild degrees of iron deficiency; but in view of the findings to be described on the microcytic iron refractory anaemias, it is important to note that the simultaneous occurrence of a microcytic blood picture, in association with a normal serum iron and a failure to respond to parenteral iron, has not been observed in any of the patients at the Queen Victoria Maternity Hospital who were not of Mediterranean ancestry.

SUMMARY OF SECTION 3.

IRON REFRACTORY MICROCYTIC ANAEMIAS (THALASSAEMIA MINOR).

At an early stage of the survey of the anaemias of pregnancy it became evident that a high proportion of the cases of anaemia in the Greek, Italian and Cypriot populations were probably due to the presence of thalassaemia minor. These cases usually presented as microcytic hypochromic anaemias which were refractory to iron therapy. Over the first year of this survey, nineteen cases of this type were seen; and it was found that only 42% of these had raised foetal haemoglobin values.

It was not easy to diagnose these cases, without giving parenteral iron in an attempt to establish the presence of a response to iron therapy, and it was considered that the administration of parenteral iron to these patients was contra-indicated, because many of them have adequate iron stores.

The high incidence, of 1.3% in the Italian patients and 4.7% in the Greek patients attending the hospital made the problems of differential diagnosis, and method of treatment of these patients a matter of some urgency.

It is believed that in association with iron deficient type of blood picture, the presence of raised values for the minor component of haemoglobin, known as haemoglobin A_2, is virtually diagnostic. In consequence of this a considerable amount of work was carried out in an attempt to establish a simple and accurate quantitative method for the estimation of haemoglobin A_2.

This haemoglobin was originally isolated and estimated using starch block electrophoresis, but as this method had numerous technical difficulties, and moreover the number of specimens which could be estimated was somewhat restricted, it was considered that attempts to find

an alternative method would be of considerable value.

Attempts were made to quantitate these estimations using paper electrophoresis, and starch gel electrophoresis, with a barbiturate buffer, but it was found that these methods were not satisfactory.

A method using a tris buffer system with paper electrophoresis was finally used, and this was quantitated with brom-phenol blue dye. The factors influencing the accuracy of this method have been investigated, and it has been shown to give adequate separation and reproducible quantitation of the haemoglobin A_2.

The normal ranges in pregnancy were established, and the upper limit of the normal range for haemoglobin A_2 has been found to be 4.5% of the total haemoglobin.

The pathognomonic significance of raised haemoglobin A_2 values has been investigated by testing a series of anaemias, other than thalassaemia minor; and it has been shown that the only other forms of anaemia encountered with consistently raised haemoglobin A_2 values are the megaloblastic anaemias.

Sharp separation between the values in iron deficiency anaemias and thalassaemia minor have been established. It is considered that when associated with microcytosis and hypochromia, raised haemoglobin A2 values are almost diagnostic of thalassaemia minor, and that 'non-specific' raised values are only encountered in conditions which are not liable to be confused with thalassaemia

The haemoglobin A_2 levels in six cases of thalassaemia major in children are discussed, with particular reference to the diagnosis of thalassaemia minor in the parents. The significance of normal haemoglobin A_2 values, in cases considered to be thalassaemia minor on clinical or genetical grounds, are also discussed. The relevant literature on this

aspect is reviewed, and it is concluded that there are several varieties of thalassaemia minor. Raised haemoglobin A_2 values are associated with a form of thalassaemia known as beta thalassaemia, which is particularly prevalent amongst the Greek and Italian population. This type of thalassaemia is believed to be associated with an amino acid defect in the beta polypeptide chains of the globin molecule. It is believed that the other basic variety of thalassaemia (alpha), associated with a defect in the alpha polypeptide chains, is relatively uncommon in the Greek and Italian population.

In an attempt to establish the incidence of beta thalassaemia in the Greek and Italian populations, a survey of the haemoglobin A_2 values has been carried out in these people at the Queen Victoria Maternity Hospital. It was found that 2% of the Italian patients, and 6.8% of the Greek patients, had raised haemoglobin A_2 values. These were presumed to be cases of thalassaemia minor; and the haemoglobin levels, red cell indices, serum iron values and foetal haemoglobin levels of these patients were estimated during their pregnancies.

A total of thirty-two patients, including twenty-one Greeks, nine Italians and two Cypriots, were investigated as a result of this haemoglobin electrophoresis screening technique; and it has been shown that 91% of these patients had haemoglobin values of less than 11.0 g. per 100 ml. at twenty-four to twenty-eight weeks gestation, and that 72% had haemoglobins of less than this level at thirty-two to thirty-six weeks gestation. It is believed that these patients may have had a slight rise in their haemoglobin levels towards the end of the third trimester, when the rate of increase of the blood volume is slowing, and partial compensation for the anaemia is then possible.

Almost all the patients investigated under the above scheme

showed evidence of an abnormality in the red cell indices. These findings are compared to the observations in the iron deficiency cases.

For the purposes of the analysis of the findings, with regard to the reticulocyte counts and serum iron levels, the data from the thirty-two cases described has been compared with some of the data from the forty-two thalassaemia minor cases found during the general survey of the anaemias of pregnancy.

It was found that the mean reticulocyte counts are raised in thalassaemia minor during pregnancy; but that the rise is not great enough to make this investigation of much practical use in the differential diagnosis of the individual case. The possible reason for the raised value is discussed, and it is concluded that a major factor is the presence of increased red cell haemolysis in some of these patients.

The morphological appearances of the red cells in thalassaemia minor are discussed, and it is pointed out that the degree of poikilocytosis is disproportionate with the degree of anisocytosis, as compared to the findings in iron deficiency anaemia. Many of these cases also show punctate basophilia of the erythrocytes.

The mean serum iron level in the anaemia selected thalassaemic series is slightly lower than the mean value of the cases selected as a result of the electrophoretic survey; and the possibility that, in some of these anaemic cases, there may be a mild concurrent iron deficiency is considered. It is shown that overall, the serum iron levels in the thalassaemic cases are similar to the values obtained in the normal control series of patients with haemoglobins in excess of 12.5 g. per 100 ml.

The mean value for the T.I.B.C. in the Thalassaemia minor patients was found to be below the mean of the normal control values. The reason for this is discussed, and it is suggested that this may be due to the adequate iron stores in the majority of the thalassaemic patients.

Assessment of the normal values for the foetal haemoglobin levels during pregnancy has been carried out, and it was shown that there appeared to be a slight rise in this value associated with pregnancy, and the upper limit of normal was, in consequence, raised to 2% of the total haemoglobin. A survey of two hundred and forty-three Greek and Italian patients showed that occasional examples of raised haemoglobin F values were encountered, which did not appear to be associated with thalassaemia. It was found that only 21% of the A_2 survey series of thalassaemic patients had raised foetal haemoglobin values.

It was concluded that unless additional confirmatory evidence from other sources can be obtained, the foetal haemoglobin estimation is only of limited value for the diagnosis of thalassaemia minor.

A modified one tube technique in 0.3% buffered saline, for the assessment of the resistance of the erythrocytes to osmotic fragility, is described. This technique showed, when compared to a normal control series that twenty-six out of twenty-nine thalassaemic cases from the haemoglobin A_2 survey series had increased resistance of the erythrocytes to osmotic fragility. These results are compared to a small series of microcytic iron deficiency cases, and it is considered that the changes in this index are more marked in the thalassaemic corpuscles than in the comparable iron deficiency erythrocytes.

The effects of thalassaemia in pregnancy are discussed, with reference to the effects of this genetic abnormality in the production of anaemia during pregnancy. The relevant literature with respect to this effect, and to the incidence of the thalassaemia genes, is reviewed.

It is concluded that this condition, which has been largely overlooked previously, constitutes a major factor in the production of anaemia during pregnancy in the population under study; and that more attention should be devoted to it in the differential diagnosis of the

anaemias of pregnancy.

Three cases of other types of abnormal haemoglobins, namely: haemoglobin S trait, haemoglobin C trait, and haemoglobin Lepore, have been seen during these investigations, and the other haematological findings in these cases are briefly described.

SECTION 3.

IRON REFRACTORY MICROCYTIC ANAEMIAS. (THALASSAEMIA MINOR)

The first case seen, after the commencement of the survey, was a multigravida of Greek birth who first attended the antenatal clinic when she was twenty-eight weeks pregnant. Her haemoglobin at this time was 8.3 g. per 100 ml.; and the peripheral blood showed a microcytic hypochromic type of anaemia, with a mean corpuscular volume of 77 cu. microns, and a mean corpuscular haemoglobin concentration of 29%. The direct Coombs test was negative, but there was a serum bilirubin of 1.2 mg. per 100 ml., and with excess urobilinogen in the urine. The serum iron was 150 micro micrograms per 100 ml. of serum, with a total iron binding capacity of 380 micro micrograms per 100 ml. of serum. The combination of a normal serum iron with the microcytic hypochromic anaemia, and the presence in the blood film of numerous poikilocytes and some target cells, was suggestive of a congenital form of anaemia of the thalassaemic type. Accordingly, studies of the fragility of the red cells were carried out, and they showed an increased resistance to osmotic fragility, which became more marked after twenty-four hours incubation, as is a feature of thalassaemia minor; (Dacie, 1960). The auto-haemolysis studies showed no variation from the normal, being 0.5% after twenty-four hours incubation, and 2.2% after forty-eight hours incubation. It seemed reasonable to assume that this was a case of thalassaemia minor, and the patient was transfused with four pints of blood. A further transfusion was required before delivery, as the haemoglobin again fell to 10.0 g. per 100 ml. During the immediate post-natal period the haemoglobin again fell to 8.4 g., but spontaneous recovery took place, and when the patient was seen, at the six weeks post-natal examination, the haemoglobin level had risen to 12.1 g. per 100 ml.

A second patient was seen shortly afterwards, who presented a

similar type of blood picture and a haemoglobin level of 10.0 g. per 100 ml., but in whom there was no evidence of splenomegaly, raised serum bilirubin, or excess urobilinogen in the urine. This patient failed to show any response to 800 mg. of iron dextran given parenterally, and the foetal haemoglobin was 4.2%, using the method of Singer et al (1951) in which the upper limit of normal was accepted at that time as 1.7% of the total haemoglobin. A course of folic acid (5 mg. three times daily) and vitamin B_{12} failed to produce any significant haematological response. It was therefore decided that this patient must be another case of thalassaemia minor. In this case the clinical course was not so severe, and it was more typical of the many cases seen subsequently: the diagnosis of this type of case was more difficult to confirm.

During the first twelve months of the survey a total of nineteen cases was seen, which were similar in most respects to the second of the two cases already outlined. (A resume of these cases is included in Appendix 4). Of these, six cases were of Italian birth; which number, when compared to the total four hundred and fifty-seven Italian patients booked for antenatal treatment at the hospital over this period, gave an incidence of 1.3% of the total. Similarly, the thirteen cases of Greek or Cypriot birth, when compared to the total number of two hundred and seventy-three patients of these nationalities, gave an incidence of 4.7% of the total number of Greek and Cypriot patients attending the hospital. This apparently higher incidence in the Greek patients was tested, using the chi-square test, and found to be highly significant.

Of the nineteen cases, twelve were given parenteral iron; and of those so treated, there was a slight rise in the haemoglobin level in three cases. But in none of those three was there a sustained rise exceeding a total of 0.7 g. haemoglobin per 100 ml., and all three had a

raised foetal haemoglobin level.

Seven of the aforementioned nineteen patients were not given parenteral iron, on the grounds: (a) that they had failed to respond to such a course in a previous pregnancy, in which they had also had a microcytic hypochromic anaemia; or (b) that they had a raised foetal haemoglobin.

Of the total nineteen patients, nine, or 42% had raised haemoglobin F values. In eleven of the nineteen cases, serum iron determinations were carried out; and values within the normal non pregnant range of 70-170 micro micrograms per 100 ml. were found in nine of them. One value of 50 micro micrograms was found in case No. 176, but this patient showed no response to parenteral iron therapy. The other case, No. 174 had a miscarriage at an early stage of pregnancy, and further investigation was not possible; but it was found, that in a previous pregnancy, there had been no response of a microcytic hypochromic anaemia to large doses of iron dextran given parenterally.

Studies on the red cell osmotic fragility were carried out on cases No. 159 and No. 169 and in both of these resistance to osmotic fragility was found. It was felt, however, that this could not be used as a routine test, owing to the demands it would place on the laboratory facilities, and to the fact that the hypochromic microcytic anaemias associated with iron deficiency also show some increase in resistance to fragility. (Daland and Worthley, 1935; Dacie, 1960).

It was obvious, therefore, from this preliminary evaluation that many, if not all, of these cases were thalassaemia minor. All the cases of microcytic iron refractory anaemia encountered at the Queen Victoria Maternity Hospital have been of Mediterranean ancestry; but we considered that in the individual case it was difficult to exclude the

possibility of other causes, such as pyridoxine deficiency.

There was moreover the example of one case in whom the foetal haemoglobin level was 1.8%, and the haemoglobin A_2 level was normal, (Dr. C. Curtain). In spite of a severe microcytic hypochromic type of anaemia, this patient only had a rise of 0.7 g. per 100 ml. in the haemoglobin level after 42 days of oral iron therapy. In view of the uncertainty as to the diagnosis this case has been excluded from the present results. This type of case gave rise to some difficulty in diagnosis. It has subsequently been appreciated that when compared with the reactions of the Northern European patients with microcytic anaemia, this is an unsatisfactory response; and as will be outlined later, it has been appreciated that many cases of thalassaemia minor do show some rise in the haemoglobin level during the third trimester.

Gerald and Diamond, (1958) have suggested that a diagnosis of thalassaemia, occurring without genetic proof in the shape of children with the homozygous major form of the condition, can only be substantiated in the presence of a microcytic type of blood picture, combined with an elevation of the A_2 haemoglobin value on electrophoresis of the haemoglobin.

This component was isolated by Kunkel and Wallenius, (1955); and raised values were shown to be associated with thalassaemia minor by Kunkel and co-workers in 1957. They and other workers in this field, (Gerald and Diamond, 1958; Josephson, 1959), have used a method of starch block electrophoresis for the estimations of this component; but unfortunately the technique is costly of both time and material, requiring many runs using the same batch of starch grains before adequate separation can be obtained; (V. Minnich, 1960). I therefore decided to try to find an alternative method. Whilst these trials were being carried out, Dr. C. C. Curtain of the Baker Institute, Melbourne, investigated a number

of haemoglobins for me.

Electrophoresis of Haemoglobin, Using the Moving Boundary Method of Tiselius.

Haemoglobins from some of the nineteen cases outlined above were examined by Dr. Curtain, who initially screened them, in a semiqualitative manner, with starch gel electrophoresis; and subsequently examined those cases thought to have high A_2 values, with the moving boundary technique.

He found, with these methods, that four out of the seven submitted had definitely raised values for the haemoglobin A_2 component. Of the other three, one case (No. 164, Appendix 4) has since had a later pregnancy, (No. 210, Appendix 5), and was then found to have a raised haemoglobin A_2 value by the quantitative tris buffer method. In one of the other cases, also, there was a further pregnancy with associated raised A_2 values; (No. 171, Appendix 4, and No. 198, Appendix 4). In the third case, the diagnosis was based on a combination of a raised F value, and the failure of a microcytic hypochromic anaemia to respond to oral iron. It is now thought that this diagnosis is not certain, and this case has been excluded from the results as it is not regarded as a proven thalassaemia minor.

Paper Electrophoresis of Haemoglobin with a Barbiturate Buffer.

The flat paper type of electrophoresis tank, as described by Zuelzer, Neel and Robinson (1956), was constructed, and a barbiturate buffer at pH 8.6, (Dacie, 1956) was used. It was found that in runs of sixteen hours, with a constant voltage power supply delivering 255 volts, adequate separation between the haemoglobin A_2 and the main haemoglobin A component was not obtained. Visual inspection of these papers showed that an increased density of the trail was often obtained in those patients considered to be probable thalassaemia minor. It was felt, however, that

this method did not give sufficiently constant results for the basis of a firm diagnosis.

This method was not used, therefore, for the purpose of separating haemoglobin A_2, but it has subsequently been used for the identification of other abnormal haemoglobins: (Figure 28).

Haemoglobin Electrophoresis Using Starch Gel and a Barbiturate Buffer.

This method was based on that of Curtain's (1958), using 0.25 molar barbiturate buffer at pH 8.6

The formula used in making up this buffer was:-

Sodium Barbitone 10.0 g.

Sodium Acetate (Anhydrous) 4.01 g.

0.1 N Sulphuric Acid 68.4 ml.

Water to 2 litres.

For making the starch gel this buffer was diluted with an equal quantity of water, the resultant strength being 0.25 molar. The full strength (0.5 molar) buffer was used in the electrode tanks.

The hydrolysed starch was obtained from the Connaught Laboratories in Canada. 15 g. of this starch was added to 150 ml. of buffer; after geling with heat the trapped air was removed by negative pressure, and the gel poured into perspex moulds of 25 cms. length x 2 cms. width x 0.5 cms. depth; haemoglobin solution was applied to strips of filter paper inserted in a slit made in the starch gel. The electrophoresis was run for sixteen hours at 4.0 degrees centigrade, with power from a 255 volt (constant voltage) supply. After completion of the run the strips were split down the centre and stained by the ortho dianisidine method of Owen et al. (1958).

I found that good separation of haemoglobin A_2 could be achieved; but no satisfactory method of quantitation, other than visual inspection,

proved satisfactory. I did find, however, that some form of visual quantitation could be obtained, if the runs were made under standard conditions and 0.02 ml. of haemolysate, of the strength of 10 g. haemoglobin per 100 ml., was applied on each occasion. On completion of a large number of runs, the strips were placed in order of increasing amounts of haemoglobin A_2, by an unbiased observer, and those patients above the rather arbitrary point at which the normals were thought to have been eliminated were considered to have raised values for haemoglobin A_2. These values are shown in the resume of patients in Appendix 4.

By the use of this technique, a further twelve patients were considered to have raised haemoglobin A_2 values.

It was fairly obvious that these semi-quantitative methods were unsatisfactory for the unbiased selection of patients, particularly in those cases who appeared to have borderline results. It was therefore decided to attempt to quantitate the electrophoretic method which Craddock-Watson, Fenton and Lehmann, (1959), had described for separation of haemoglobin A_2 on paper, using a tris buffer.

Paper Electrophoresis of Haemoglobin Using a Tris Buffer at pH 8.6

Wilkinson and Wilkinson (1960), have investigated the factors influencing the accuracy of the brom-phenol blue dye for the quantitation of protein, separated by paper electrophoresis in barbiturate buffer. They showed the importance of carefully standardised conditions in the staining and washing of papers; and in particular the necessity for attaining a stable green colour of the dye during the process of washing the papers.

All specimens were collected using di-potassium sequestrene as the anti-coagulant. The haemolysates were prepared, as for the foetal haemoglobin estimation, by the Toluene method of Singer, Chernoff and

Singer (1951). The haemolysates were converted to carbon-monoxy haemoglobin using coal gas after the estimation of the haemoglobin F when required. The alkali denaturation method does not give reproducible results in the presence of carbon-monoxy haemoglobin.

Electrophoresis was carried out in two vertical tanks, of the type described by Flynn and Mayo (1951), in each of which nine paper strips (Whatman No. 3 M.M.) could be placed. The tanks were modified so that variable lengths of paper could be used; and under our conditions it was found that a shorter paper, - length of 25.5 x 4.0 - with a power supply of 255 volts, gave a higher voltage gradient over the paper and better separation of the haemoglobin components than the longer strips (36 cm.) used by Craddock-Watson et al. We then found that the haemoglobin A2 tended to move towards the cathode, and we thought that this was probably due to an electro-osmotic effect.

The tris buffer (described by Craddock-Watson et al.) was used, and this was adjusted to pH 8.6 using boric acid. On each paper, .02 ml. of haemolysate was applied with a micropipette, and the paper immediately moistened with buffer on both sides, to within a quarter of an inch of the point of application of the haemolysate. The buffer then ran up to both sides of the haemolysate, and this method was found to give the sharpest definition of the bands. The electrophoresis was allowed to run for sixteen hours at room temperature.

After completion of the run the papers were dried at 90-100 degrees centigrade, and fixed for ten minutes in an ethanol solution containing 10% mercuric chloride and 10% glacial acetic acid. It was found to be essential to discard the fixative after each use, as prolonged standing with the buffer salts gave rise to some precipitation, which resulted in artificially high values for the small component. Accordingly,

only small quantities, sufficient to moisten the papers, were used on each occasion. This fault gave rise to considerable difficulties during the first two months of these trials, and it is thought that any values obtained during this period may be erroneous, so they have been eliminated from the results.

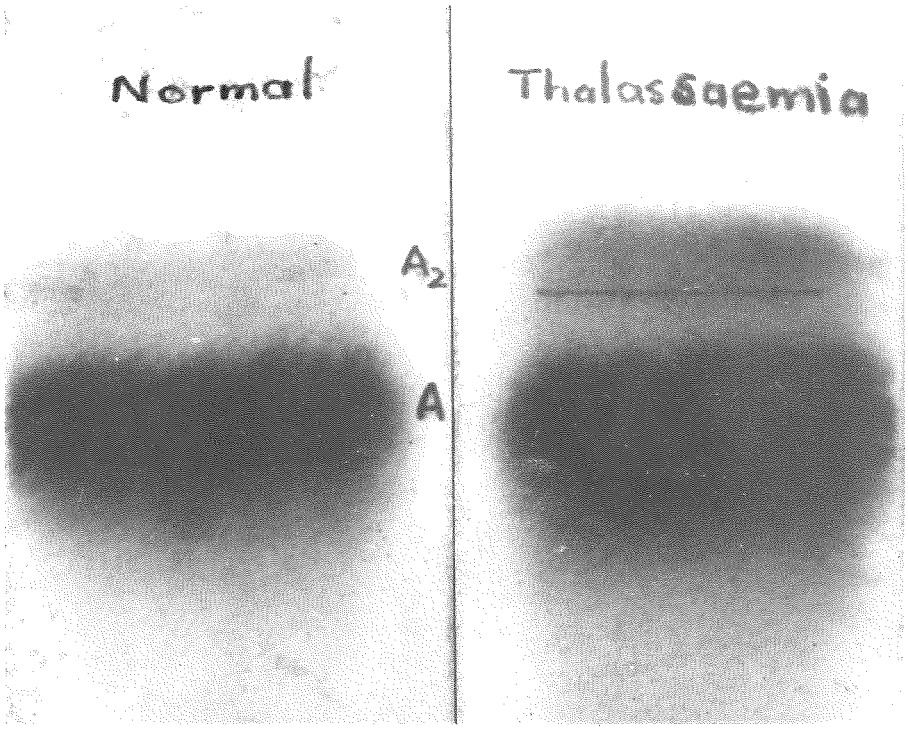
Upon completion of the fixation, the papers were dried, washed in two changes of distilled water for ten minutes, and thoroughly dried again. Staining was then carried out for ten minutes, with an ethanol solution containing 1% brom-phenol blue and 1% glacial acetic acid. A solution of 0.5% acetic acid in 25% ethanol was used for washing, as the addition of ethanol was found to give more rapid elation of the excess dye. Three washes of ten minutes duration and two of fifteen minutes were found to give a stable green colour. After drying, the papers were marked and divided at the point of least colour between the two bands (Figure 12). The dye from the A_2 band was eluted with 10 ml. of 1.5% (w/v) sodium carbonate in 50% (v/v) methanol, and that from the main A band with 30 ml. of the same solution. This latter solution was subsequently diluted eleven times to bring the optical density within the most senitive region of the instrument. The optical densities of the two eluates were measured at 595 mμ. in a Unicam S.P. 400 absorptiometer. Using this technique, satisfactory separation and staining were obtained.

It was necessary to demonstrate that this method gave reproducible results, and a number of experiments were undertaken for this purpose.

Variations in pH.

Craddock-Watson et al. had demonstrated that with their technique an extra band of protein, which did not stain with specific haemoglobin stains, could be found running at a slower rate than A2. We also encountered this

FIGURE 12.



Typical electrophoretic patterns obtained from the blood of normal and thalassaemia minor patients.

band, but as the quantity appeared to increase when the pH was increased to 9.2, we considered that this could be a denaturation product. For this reason, care was taken to ensure that the buffer was not allowed to rise above pH 8.6, at which the band was usually minimal in quantity.

Linearity of Dye Uptake.

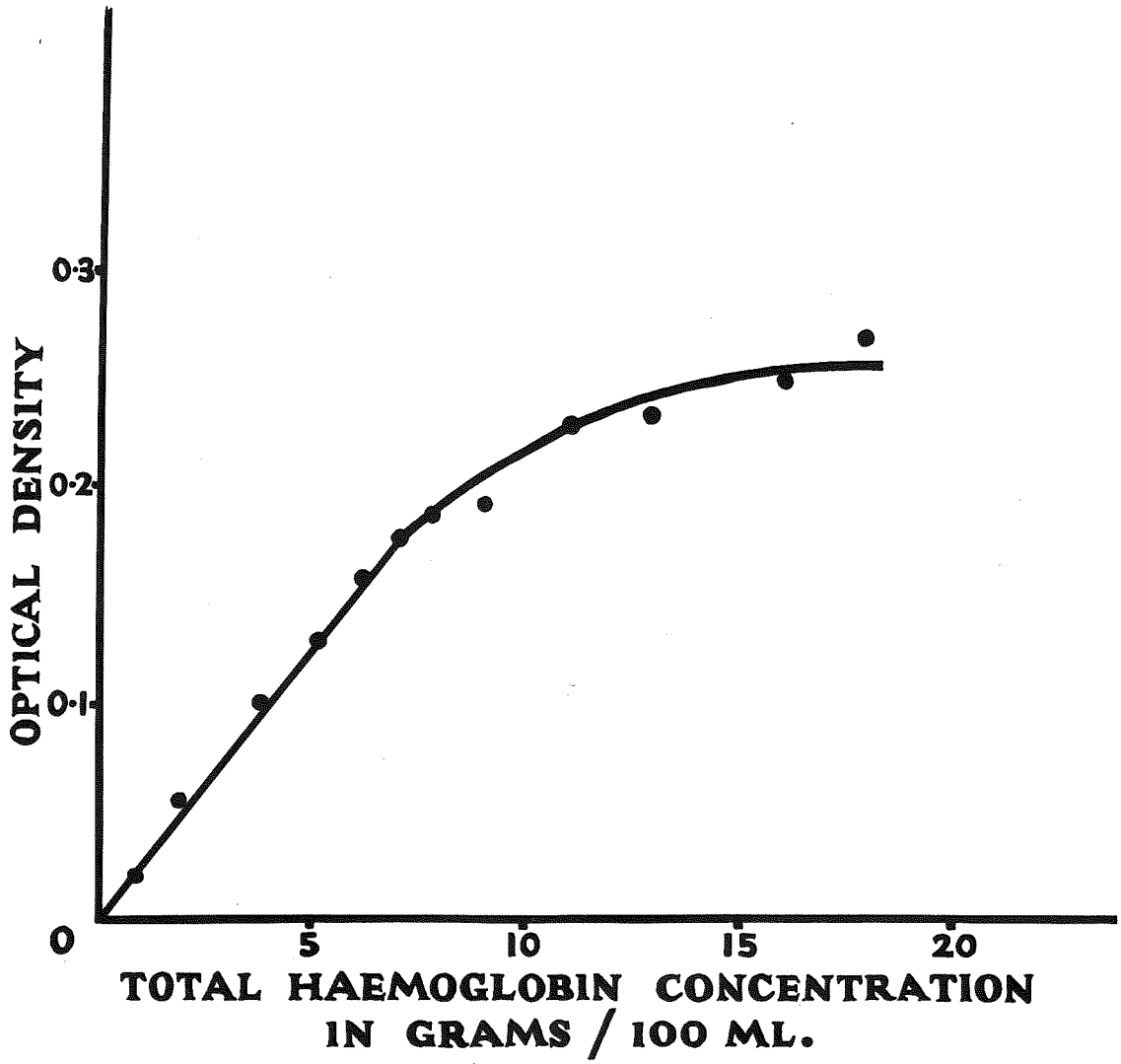
By serial dilutions of the dye it was demonstrated that Beer's Law was obeyed over the range of densities in use, and that the density readings were well within the maximal sensitivity range of the instrument. Wilkinson and Wilkinson (1960) have demonstrated that this linearity may not always hold, particularly at higher density values.

Standard curves for haemoglobins A and A_2 were constructed by subjecting .02 ml. samples of varying concentrations of haemoglobin solutions to electrophoresis. The concentration of the solutions under test ranged from 1 to 19 grams of haemoglobin per 100 ml. of haemolysate. Blanks were also tested, and the values deducted from the readings of the other components. The results obtained are shown in Figures 13 and 14, and it can be seen that departure from linearity of dye uptake by the haemoglobin A occurs at concentrations above 7 g. of haemoglobin per 100 ml. of haemolysate. As the A_2 band usually comprises less than 10% of the haemoglobin A band, linearity would be anticipated throughout the range of solutions applied; and above 7 g. of haemoglobin per 100 ml. of haemolysate a relative increase in the percentage of haemoglobin A_2 would occur. This experiment was repeated several times, and on each occasion similar results were obtained. From this time onwards, all haemolysates have been diluted to the range of 4.5 g. to 7.0 g. of haemoglobin per 100 ml., before application to the paper.

Accuracy of Haemoglobin A_2 Estimations.

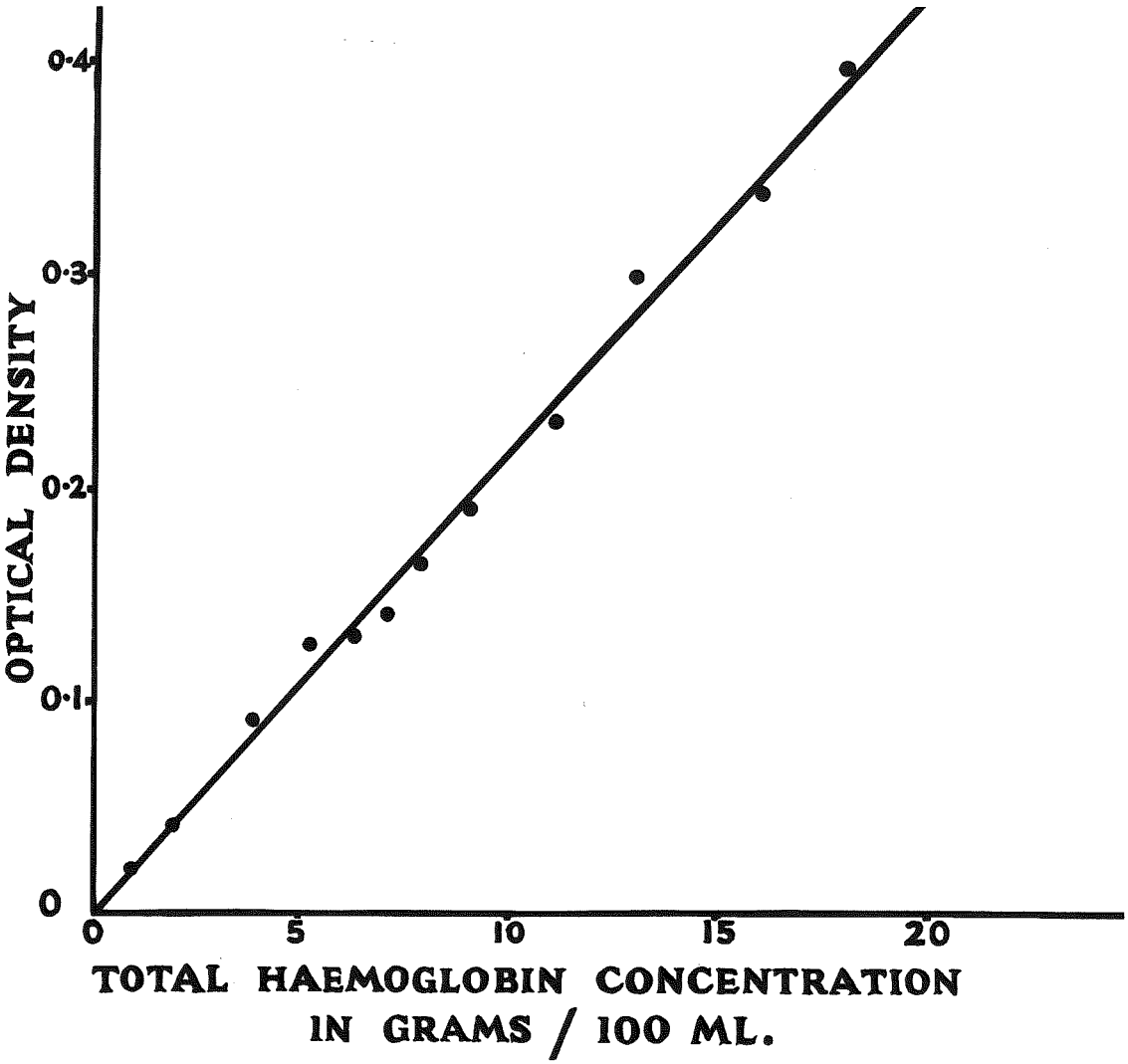
It was necessary to get some idea of the accuracy and reproducibility of this method; and electrophoretic estimations of the

FIGURE 13.



The optical densities of the diluted dye eluates obtained from the A haemoglobin band, plotted against the original haemoglobin content of the haemolysates which were subjected to electrophoresis. Linearity can be seen to fall off at concentrations in excess of 7 g. of haemoglobin per 100ml.

FIGURE 14.



The optical densities of the diluted dye eluates obtained from the A_2 haemoglobin band, plotted against the original haemoglobin content of the haemolysates which were subjected to electrophoresis. Linearity holds throughout the range of concentrations used.

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TABLE 5.

VALUES FOR A_2 AS PERCENTAGE OF TOTAL HAEMOGLOBIN CONTENT FROM TWO NORMAL AND TWO THALASSAEMIC SUBJECTS WITH MULTIPLE ESTIMATIONS ON FIVE SUCCESSIVE DAYS.

Type of Subject	Total No. of Esti-mations	Mean Value for % A_2	Same Day S.D.	Same Day % Error	Different Days S.D.	Different Days % Error
Normal	69	3.3	0.28	8	0.39	12
Thalassaemic	30	6.4		4		6

haemoglobin A_2 value, on at least three samples from each of two normal controls and from two cases of thalassaemia, were made, for this purpose, on five successive days. In these estimations the technique used was exactly the same as the routine subsequently employed.

The results of the percentage A_2 in these groups were submitted to an analysis of variance, with the results shown in Table 5. From these results it can be seen that the percentage error did not differ greatly between the normal and the thalassaemic patients, being slightly less in the thalassaemic cases than in the normal. There was a significantly greater variation between runs on different days than on the same day; and as there was no evidence of a systematic day effect common to all patients, this variation was attributed to slight differences in the degree of separation between the A and A_2 components, and possibly to some variation in the cutting between bands.

In assessing these results it should be borne in mind that the absolute quantity of A_2 component on each paper is very small:- something in the order of 50 micrograms of protein. It was considered that as two thirds of all the results should lie within one standard deviation (i.e. $\pm$ 0.4%) of the true value, this degree of error was not excessive, particularly if values within $\pm$ 0.5% of the upper limit of normal were subjected to repeat estimations in any doubtful cases.

Normal Range of Haemoglobin A_2 Values.

Evaluation of the normal range for the haemoglobin A_2 values, expressed as a percentage of the total haemoglobin was carried out on eighty-six normal pregnant patients of Northern European origin and on one hundred and fifteen normal non-anaemic patients of Mediterranean origin. The figures obtained are shown in Table 6.

The normal range obtained with this method is slightly higher

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TABLE 6.

NORMAL VALUES FOR PERCENTAGE A_2 HAEMOGLOBIN.

Type of Case	Total No.	Quantity of A_2 as Percentage of Total Haemoglobin		
		Mean	Range	S.D.
Normal pregnant patients of northern European origin	86	3.2	1.4-4.3	0.61
Normal pregnant patients of Mediterranean origin	115	3.2	1.4-5.3	0.64

than the values obtained with the starch block method. Kunkel et al. (1957) reported a mean value in normals of 2.5% on different starch blocks, with a standard error of 0.11, and a coefficient of variation of 12.4%. This finding is reiterated by Masri and Josephson (1958) who found a mean value of 2.55%, with a range of 1.2% to 3.5%. Marinone, Bernasconi, Morsiani and Lucci (1956), (quoted by Silvestroni and Bianco, 1959), found a mean normal value for haemoglobin A_2 of 2.16%, using the starch block method. Other methods often give higher results for the haemoglobin A_2 fraction: Yakulis et al. (1960), using a method employing tris with agar gel on glass slides, found a normal range of 4.1% to 9.7%; and their values in thalassaemia minor ranged from 10.2% to 19.1%.

From the figures in Table 6, evaluation of the normal range from the standard deviation would indicate that 96% of all normal results should be included within the range of 2.2% to 4.4% of haemoglobin A_2: this being the expectation from a double tailed normal distribution. It would be anticipated that 2% of all normal results would be higher than this figure; and in practice, we accepted 4.5% as the upper limit for the normal range. This figure would include 98.4% of all the normal values.

Cases with haemoglobin A_2 values falling within the range of 4.5% to 5% were subjected to repeat estimations in an attempt to minimise the effects of experimental error on the classification of these particular individuals

Evidence to be produced at a later stage will indicate that there was some lowering of the normal range during the course of these investigations. The number of normals falling outside the normal range would therefore be lower than is indicated by the above figures. This would have the effect of making the separation between the normals and abnormals more critical, but it was considered that the upper limit of

TABLE 7.

HAEMOGLOBIN A_2 VALUES IN NON-PREGNANT NORMAL CONTROLS, AND NON-THALASSAEMIC ANAEMIAS.

Group	Number	Mean	S.D.
Non-Pregnant Normal Controls	50	2.85	0.45
Iron Deficient	21	2.88	0.68
Megaloblastic	21	3.91	0.72

STATISTICAL COMPARISON OF THE HAEMOGLOBIN A_2 VALUES IN NON-THALASSAEMIC TYPES OF ANAEMIA.

Groups Compared	Probability Value for F test.	Probability Value for t test.
Iron Deficiency & Megaloblastic	Not Significant	< 0.05% ***
Normal & Megaloblastic	< 1.0%, > 0.1%	< 0.05% (a) *
Normal & Iron Deficient	< 5.0%, > 1.0%	< 45%, > 40% (a)

The t test probability value quoted is for 1 tail of distribution.

(a) With reservations owing to significant value for the F test on the standard deviations.

4.5% for the haemoglobin A2 should continue to be adhered to as this was the standard originally adopted.

Values for Haemoglobin A_2 in Various Forms of Anaemia, other than Thalassaemia Minor.

In order to be able to assess the significance of raised values for the haemoglobin A_2 component, it was necessary to determine whether any examples of raised values could be found in other forms of anaemia.

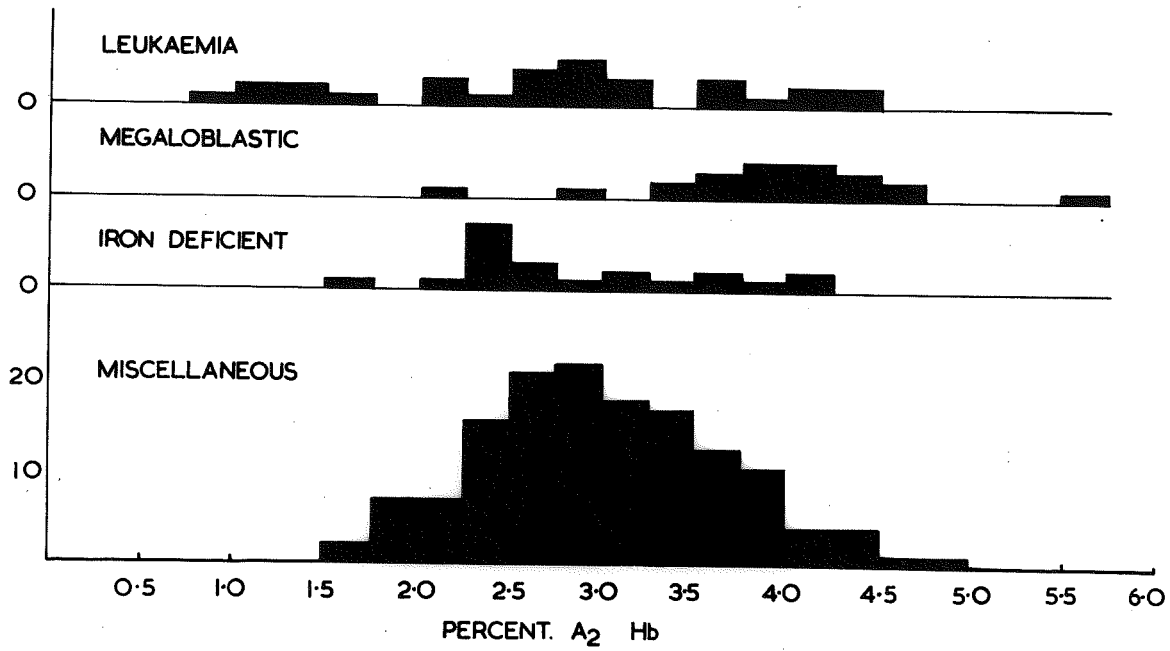
Blood specimens from one hundred and ninety-six cases of various types of anaemia, obtained from the routine haematology laboratory at the Institute of Medical and Veterinary Science, were submitted to haemoglobin electrophoresis.

A series of fifty normal, non pregnant controls were also tested for comparison with these results. It was found (Table 7) that they had a slight reduction in the mean value obtained for the percentage haemoglobin A_2 compared with the previously tested pregnant controls. This variation could be due to minor improvements in technique, and not necessarily to any real difference between the pregnant and non pregnant values. This belief is supported by the retrospective observation of a slight reduction (Figure 16) in the mean values of the Greek and Italian populations in the A_2 survey which will be described later. In these latter cases the mean normal value for the haemoglobin A_2 in the pregnant Greek population is 2.88% (S.D. 0.64), and for the pregnant Italian population 2.81% (S.D. 0.67).

The values for the haemoglobin A_2 results in the anaemia cases have been divided into four main groups (Figure 15):- megaloblastic; iron deficiency; leukaemias of all types, and a miscellaneous group which included a wide variety of anaemias other than those specified above.

It can be seen that raised haemoglobin A_2 values were encountered

FIGURE 15.



The distribution of Haemoglobin A_2 values found in various forms of Non-thalassaemic Anaemias (Non-pregnant).

in three out of the twenty-one megaloblastic cases tested, and in two from the miscellaneous group. The latter had diagnosis of Sjögren's syndrome and uraemia associated with renal failure, and their respective A_2 values were 4.9% and 4.6%.

Inspection of the histogram shows that a wide scatter of results is present in the leukaemic cases, but that none of them had raised A_2 values.

The histogram also suggests that among the megaloblastic anaemias the cases with raised values were part of an upward shift to the whole group. This is borne out by the statistical analysis of the figures (Table 7). The mean value for the megaloblastic cases is 3.9% compared with the normal mean volume of 2.85%.

The F test on the standard deviations of these two groups was significant, and shows that the scatter of the megaloblastic results is considerably greater than the normal.

The t test on the difference between the two means is mathamatically significant, but this can only be accepted with reservation owing to the high value obtained in the F test on the standard deviations.

Although the mean value for the iron deficiency cases is not significantly different from the normal, their scatter of results approximates that of the megaloblastics, and in this case it is valid to carry out the t test between the means of the two of them. It can be seen that there is a highly significant separation between the means for the iron deficiency cases and the megaloblastics.

It can be presumed from the foregoing that the A_2 values in megaloblastosis tend to be higher than normal, and although there is a suspicion that the values in iron deficiency may be slightly lower, it cannot be proved by the present data.

For the purpose of this thesis, in which attention is paid to the differential diagnosis of thalassaemia minor and iron deficiency, it is interesting to note that raised values are not encountered in iron deficiency. In the other anaemic conditions the high A_2 values do not exceed 5% of the total haemoglobin, with the exception of the megaloblastic anaemias in which occasional high values exceeding 5% may be encountered. It would appear therefore, in so far as any laboratory tests can be said to be completely specific, that the pathognomonic significance of raised haemoglobin A_2 values in the diagnosis of thalassaemia minor is reasonably reliable.

It may be irrelevant to the main theme of this thesis; but with regard to the significance of raised A_2 levels, it is of interest to note that during this survey we have encountered three cases (one pregnant, two non pregnant), of Anglo-Saxon or Germanic origin, with raised A_2 values associated with an iron deficiency type of anaemia. These cases showed all the other features of thalassaemia minor in the form of normal serum iron and increased resistance of the red cells to osmotic fragility; and studies have revealed similar types of pictures with raised A_2 values in other members of their families.

The variation between the A_2 values in the different forms of anaemia is also of interest, and these findings are in partial agreement with those of Josephson et al. (1958) who found evidence of low values in thirty cases of iron deficiency anaemia, and raised values in five out of nine megaloblastic cases. They also found some evidence of raised values in hereditary spherocytosis. Kunkel et al. (1957) also observed lower values in iron deficiency.

No theoretical explanation of these variations in A_2 values in the various forms of anaemia has yet been brought forward, but as these

findings are a fairly constant feature of the few reports which have so far appeared, it seems likely that they have some bearing on the synthesis of the haemoglobin molecule. It is possible that this might support Zuelzer's (1961) contention that haemoglobin A_2 is a primitive form of haemoglobin, and that raised values are encountered when there is an enzymatic blockage at some point in the synthesis of the normal Beta polypeptide chains of the globin molecule.

Although it is interesting to speculate on this point, its significance must remain as pure conjecture until more data on the structure and synthesis of haemoglobin A_2 is available.

Values for Haemoglobin A_2 in Thalassaemia Major and the Significance of Normal Haemoglobin A_2 Values and the Diagnosis of Thalassaemia.

Gerald and Diamond (1958), suggested that the minimum diagnostic criterion for the diagnosis of the thalassaemia trait is a reduction in the value for the mean corpuscular volume, with an increased A_2 value. They investigated the parents of twelve children with thalassaemia major, and in all twenty-four of these cases raised A_2 values were found. Kunkel et al. (1957), in their original assessment of haemoglobin A_2 values, reported two cases in which the mothers of children with thalassaemia major had normal haemoglobin A_2 values; and as a result, they estimated the probability of correctness of diagnosis of thalassaemia minor as 94%, when using the haemoglobin A_2 value as the criterion for diagnosis. Josephson (1959), stated that he had not encountered any examples with normal haemoglobin A_2 values in over one hundred and fifty cases of classical thalassaemia minor.

During the course of this present investigation, haemoglobin electrophoretic studies have been carried out on six thalassaemia major children, together with their parents and siblings. In all twelve parents raised haemoglobin A_2 values were found; but in two of the thalassaemia

major children normal values for the haemoglobin A_2 with raised haemoglobin F values were found. This finding is in agreement with Kunkel and Wallenius (1955), and Phaedon Fessus (1959), who found normal haemoglobin A_2 values in six out of twenty-one cases of thalassaemia major. An interesting observation by Gerald and Diamond (1958), suggests that the haemoglobin A2 values were very similar within the individual pedigrees of six families whom they investigated, and they believe that there may be some familial genetic determination of the haemoglobin A_2 values.

Three of the mothers of our thalassaemia major cases were delivered at the Queen Victoria Maternity Hospital during the survey. One of these mothers, who subsequently had a thalassaemia major child, was missed during the first year of the survey as her haemoglobin level did not fall below 11.0 g. per 100 ml., and she was therefore not investigated during her pregnancy. It was this case, in particular, which gave emphasis to the subsequent conclusion that the screening technique previously adopted was almost certainly missing some of the cases of the thalassaemia trait. The other two cases were numbers 173 and 191 in Appendix 4.

We have encountered one family in whom there was an hereditary, microcytic, hypochromic, iron refractory anaemia which resembled thalassaemia minor. All members of this family had normal haemoglobin A_2 values, and raised foetal haemoglobin values. We have also encountered one other case in which there was a syndrome resembling thalassaemia minor, with a normal haemoglobin A2, but in this case we have not been able to investigate any other members of the family to establish an hereditary pattern for this anaemia. When these two cases are reviewed against the total of fifty-five individual cases, (this figure excludes all cases investigated as members of thalassaemic families, siblings of thalassaemia major cases and the pregnant cases reviewed at the Queen Victoria Maternity

Hospital; but includes the twelve parents of the thalassaemia major cases), it would appear that the occurrence of the thalassaemic syndrome, with normal haemoglobin A_2 values, is low in the Greek, Italian and Cypriot populations.

This finding is borne out by the fact that during the period in which quantitative haemoglobin A_2 investigations have been carried out, we encountered no cases, at the Queen Victoria Maternity Hospital, of iron refractory microcytic hypochromic anaemia in which normal haemoglobin A_2 values were found. This observation excludes the case (Table 11) in which the Lepore haemoglobin was present.

It would appear that cases of thalassaemia minor with normal haemoglobin A_2 values, though infrequent, do occasionally occur. Ingram and Stretton (1959), attempted to explain this apparent anomaly by postulating that there are two main groups of thalassaemia minor, which they have called alpha and beta thalassaemia. They suggest that in beta thalassaemia there is a substitution of an amino acid in the beta polypeptide chain of the globin, which has a charge similar to that of the normal amino acid which has been substituted. By the present electrophoretic methods, this results in the abnormal haemoglobin having the same electrophoretic mobility as the normal haemoglobin A molecule: Ingram and Stretton (1959), Stretton (1960) has demonstrated, by the finger printing technique, that the alpha chains of haemoglobin A_2 appear to be identical with the alpha chains of haemoglobin A; but that in place of the normal beta chains, haemoglobin A_2 has a pair of polypeptide chains which are chemically distinct from the normal beta chains; he labelled the latter "delta chains".

Ingram and Stretton (1959), have postulated that in the form of thalassaemia which they call Beta, there is a "hidden" amino acid substitu-

tion in the beta chains of the haemoglobin, resulting in a blockage in the synthesis of the beta chains, and a relative increase in the synthesis of the alpha chains. In consequence, there is some increase in the synthesis of the haemoglobin A_2 (alpha and delta chains), and the foetal haemoglobin which is composed of alpha and gamma polypeptide chains; Hunt (1959).

Ingram and Stretton (1959), also postulated that there was a further class of thalassaemia, in which a hidden amino acid substitution took place in the alpha chains with, in consequence, no elevation of the haemoglobin A_2 component in these cases. They believe that each of these two classes may contain a number of varying types of thalassaemia with differing "hidden" amino acid substitutions. If they are correct, a wide diversity of haematological effects would not be surprising in the thalassaemic syndrome. Zuelzer (1961), has objected to this theory on the grounds that the alpha type of thalassaemia should have a depressed level of foetal haemoglobin, since this haemoglobin contains alpha chains. It would be anticipated, therefore, that if this theory of Ingram's and Stretton's is correct, cases of beta thalassaemia minor, with normal or low haemoglobin A_2 levels, would also have normal haemoglobin F levels; which is contrary to the usual findings in these cases. Zuelzer accepts the hidden amino acid theory, but suggests that haemoglobins A_2 and F are primitive forms of haemoglobin, and are probably incomplete Beta polypeptide chains. His objection may not be valid, however, because as Hunt and Lehmann (1959) have pointed out, the alpha chains of haemoglobin F and haemoglobin A are probably controlled by different genes. They demonstrated that haemoglobin "Barts" is composed solely of gamma chains; and it would appear that, in infants with this abnormal haemoglobin, there is a complete suppression of alpha chain production. The interesting

feature in these cases, is the fact that in later life with the transition to adult haemoglobin production, the synthesis of the alpha chains is then normal; and it would appear probable that the alpha chains of normal haemoglobin A, and those of haemoglobins A_2 and F, are probably controlled by different mechanisms.

Nevertheless, the terminology of Ingram and Stretton appears to be accepted for the present, and the forms of thalassaemia associated with raised haemoglobin A_2 levels are being called Beta Thalassaemia.

Survey of Haemoglobin A_2 Values in the Greek and Italian Antenatal Patients.

As I have already outlined; using the criterion of a microcytic hypochromic anaemia resistant to iron therapy, a large number of patients, presumed to be thalassaemia minor, were found in the first twelve months of the anaemia survey. Some of these patients had haemoglobin levels only just below the arbitrary level of 11.0 g. haemoglobin per 100 ml. which had been adopted as the limit for investigation in the survey. It was considered probable, however, that some cases had been missed.

It was therefore decided to vary the procedure in an attempt to find the true incidence of the thalassaemia trait, and to determine its clinical effects.

Beginning in April, 1960, all the specimens of blood taken from Italian and Greek (non-private) patients, on their first visit to the antenatal clinics at the Queen Victoria Maternity Hospital, were submitted for electrophoresis of the haemoglobin. Those patients with values for the percentage haemoglobin A_2 in excess of 4.5% were followed through pregnancy, wherever possible, with at least three determinations of the red cell indices; with serum iron values at the thirty-second week of pregnancy, and serial estimations of the A_2 and foetal haemoglobins. This survey was terminated after it had run for fourteen months.

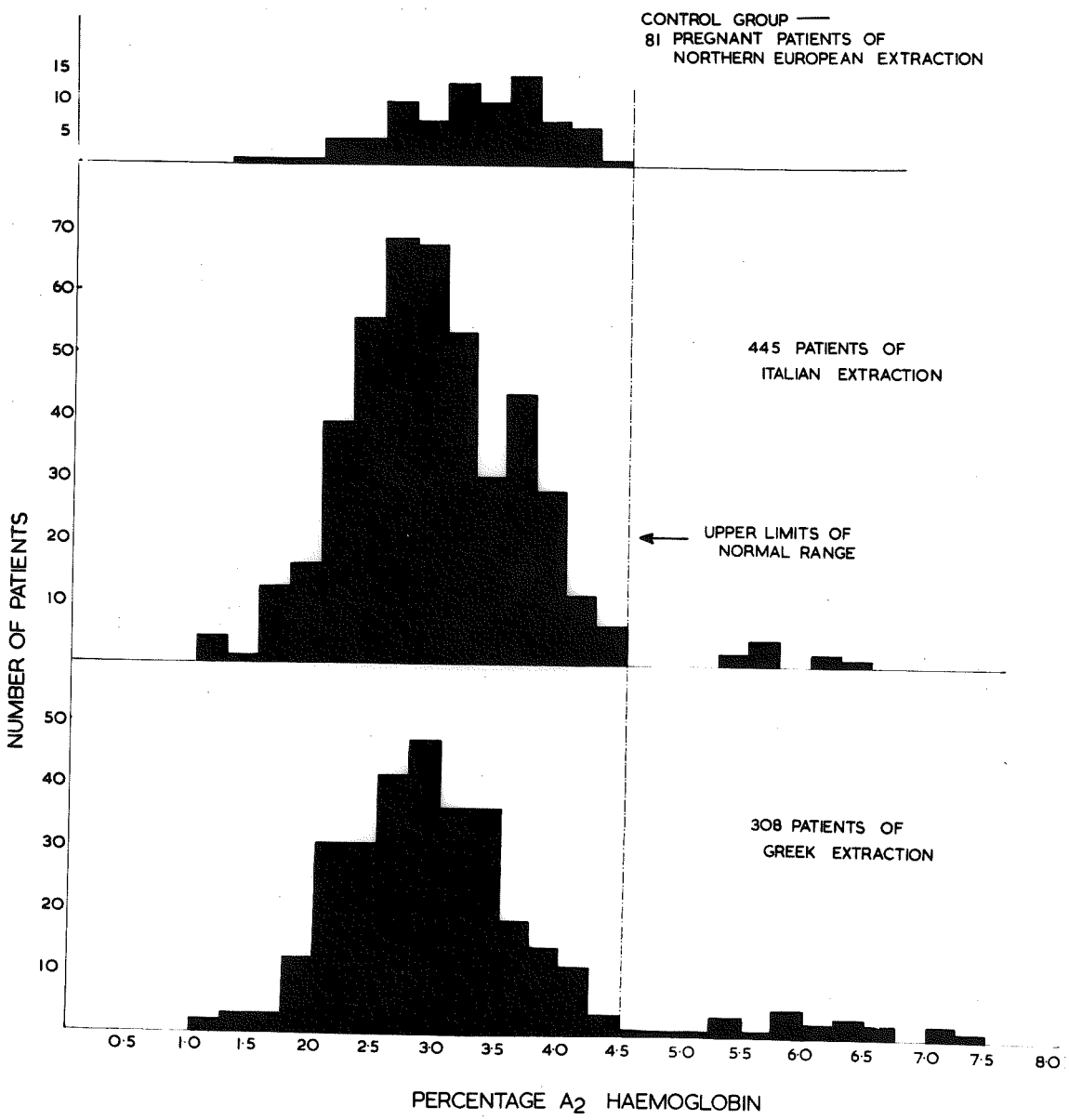
In addition to the difficulties occasioned by the language barriers, there was often some reluctance on the part of these patients to attend the routine antenatal clinics, and it was necessary to design the experiment in such a way as to cause the minimum inconvenience to them. We were therefore unable to introduce investigations of too tedious or painful a nature; and it was for this reason that bone marrow biopsies were not carried out. This was regrettable, because the opportunity to examine these specimens for haemosiderin and P.A.S. positive granules would have been welcome. In addition, the finding of Jandl et al. (1959), of a repeated megaloblastic change in a case of thalassaemia minor, and the observation of Chanarin, Dacie and Mollin (1959), that some degree of folic acid deficiency may occur in association with haemolytic anaemias, emphasised the possibility of a megaloblastic change in some of these cases.

It was assumed, for the purpose of this investigation, that a raised haemoglobin A_2 value was synonymous with the presence of the gene or genes for thalassaemia minor.

With the accumulation of a large number of patients, it could be seen that a bimodal form of distribution of results was formed, in which the majority assumed a normal distribution type of curve within the normal range, with a second population forming a prolonged tail in the area of raised values for the haemoglobin A_2 values; (Figure 16). This was, of course, the result that had been anticipated, and the point of interest centered upon those patients who came within the group which fell outside the normal range.

Detailed analysis of this latter group showed that of four hundred and forty-five Italian patients examined, nine, or 2% of the total, had raised haemoglobin A_2 values; and of the three hundred and

FIGURE 16.



Haemoglobin A2 values found in the electrophoretic survey of Greek and Italian antenatal patients, compared with a normal control group.

eight Greeks, twenty-one, or 6.8% of the total, had raised A_2 values.

Similar investigation of twenty-three patients of Cypriot birth revealed two cases with raised values for this component. These two, together with the nine patients of Italian origin and the twenty-one of Greek origin, gave a grand total of thirty-two patients with raised A_2 values: found as a result of the electrophoretic survey of the haemoglobins.

Recent examination of the hospital records has revealed that approximately 10% of the Greek and Italian patients attending the ante-natal clinics have been missed from the survey for various reasons. Of this 10%, two patients with raised haemoglobin A_2 values; one of whom was anaemic, were found at a later stage in their pregnancy. The results from these two patients have been excluded from this section so that the incidence should remain accurate. Their results have been included in the overall resume (Appendix 4), which includes all thalassaemic patients encountered throughout the three years investigations, other than those included in the haemoglobin A_2 survey. The same procedure has been followed in two patients, also found to be thalassaemic, who were born in Australia; one of whom had Greek parents (Case No. 199), while the other had been an adopted child who believed her father to have been Italian, (Case No. 200).

The apparent finding of a higher incidence among the Greek population than in the Italian was examined using the chi square test with Yates correction. It was found that the value for chi was 0.85 on 1 degree of freedom, which gave a corresponding value for p of less than 0.5%, greater than 0.1%. It seems fairly certain, therefore, that in the Greek population under examination there was a higher incidence for the thalassaemia trait than in the corresponding Italian section.

The discussion of the findings in this investigation will be

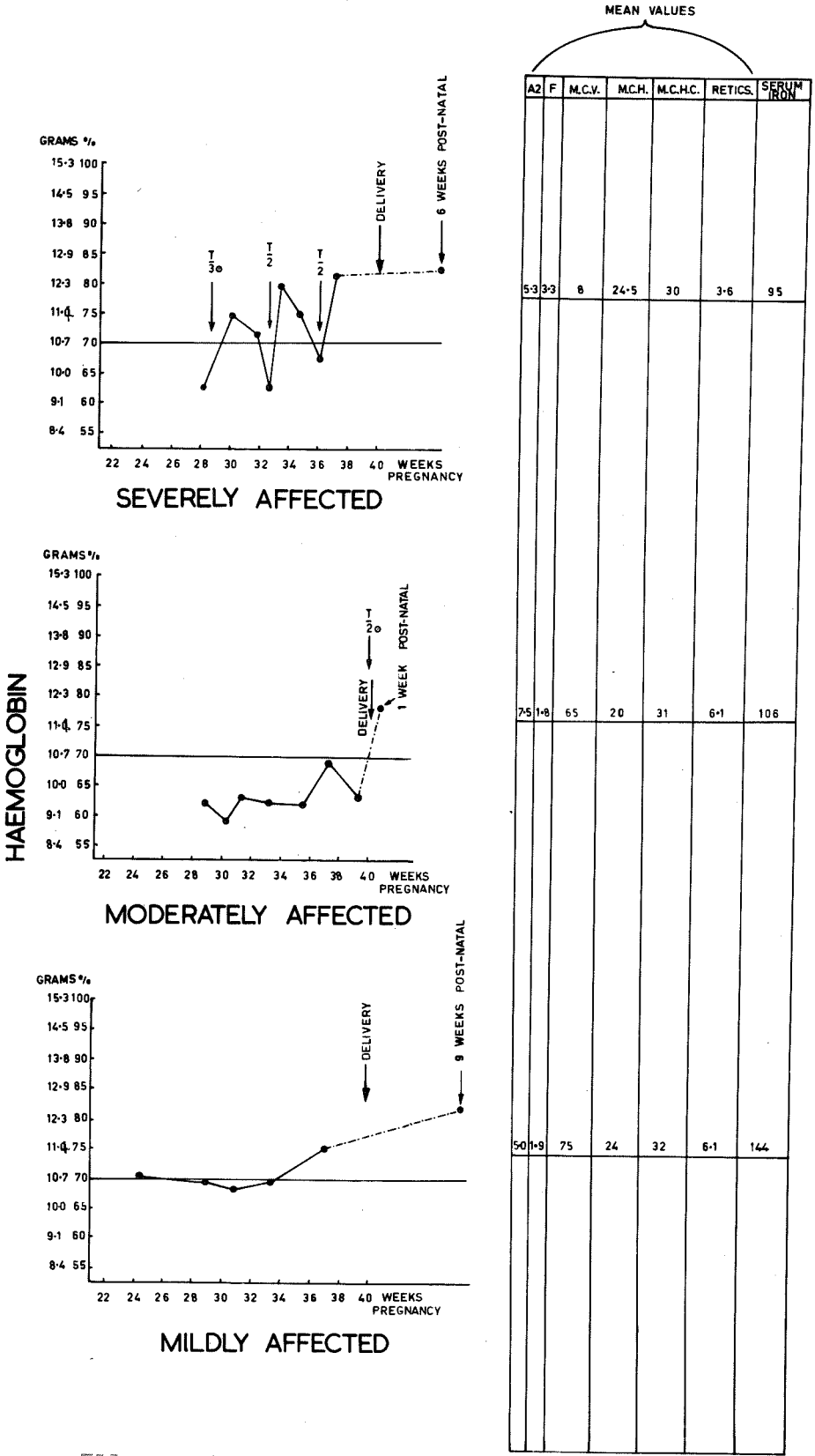
confined, wherever possible, to the thirty-two patients found in the haemoglobin A_2 survey. In some sections it is necessary to introduce some findings from the larger group of thalassaemic patients who were discovered during the general survey of anaemias of pregnancy; this is for the purpose of comparison, or in order to increase the numbers for more accurate statistical analysis. To avoid confusion, I propose to call this latter group Anaemia Survey Thalassaemic Cases, and the former group the A_2 Survey Thalassaemic Cases.

Haemoglobin Values during Pregnancy in Patients with Raised Haemoglobin A_2 Levels.

The typical course of the condition in three patients with raised haemoglobin A_2 values is illustrated in Figure 17. It can be seen that in the more mildly affected case there is a tendency for the haemoglobin to rise during the last trimester. Some of the patients with raised haemoglobin A_2 levels showed an even more dramatic rise than is demonstrated in this illustration; and it appears probable that these patients become anaemic during the period of rapid expansion of the blood volume, but when the rate of expansion slows, during the third trimester, the erythropoietic capacity overtakes the rate of plasma volume expansion, with a corresponding rise in haemoglobin. Of the twenty-three out of thirty-two cases seen at the twenty-fourth to twenty-eighth week of gestation, twenty-one, or 91%, had haemoglobin values of less than 11.0 g. per 100 ml.; whereas at the thirty-second to thirty-sixth week of gestation, twenty-one out of the thirty-two cases, or 72%, had haemoglobins of less than 11.0 g. per 100 ml. This overall rise in haemoglobin levels can also be seen by visual inspection of the histogram: Figure 18.

The mean level for the haemoglobin in these patients, at twenty-four to twenty-eight weeks, is 10.3 g. per 100 ml. of blood; and at thirty-

FIGURE 17.



Illustrating the haematological course during pregnancy in three typical cases with raised Haemoglobin A_2 values. (T. indicates transfusion).

two to thirty-six weeks it is 10.8 g. per 100 ml. of blood. The respective standard deviations for the two groups are 0.67 and 0.59. Statistical analysis of these results, by the F test, showed that there was no significant difference between the standard deviation, and it was legitimate to apply the t test to the means. It was demonstrated by this test that the rise in the mean haemoglobin levels between the twenty-fourth to twenty-eighth weeks and the thirty-second to thirty-sixth weeks of gestation was highly significant. The Null Hypothesis that observed differences are ascribable to chance, was refuted: P = Less than 1%.

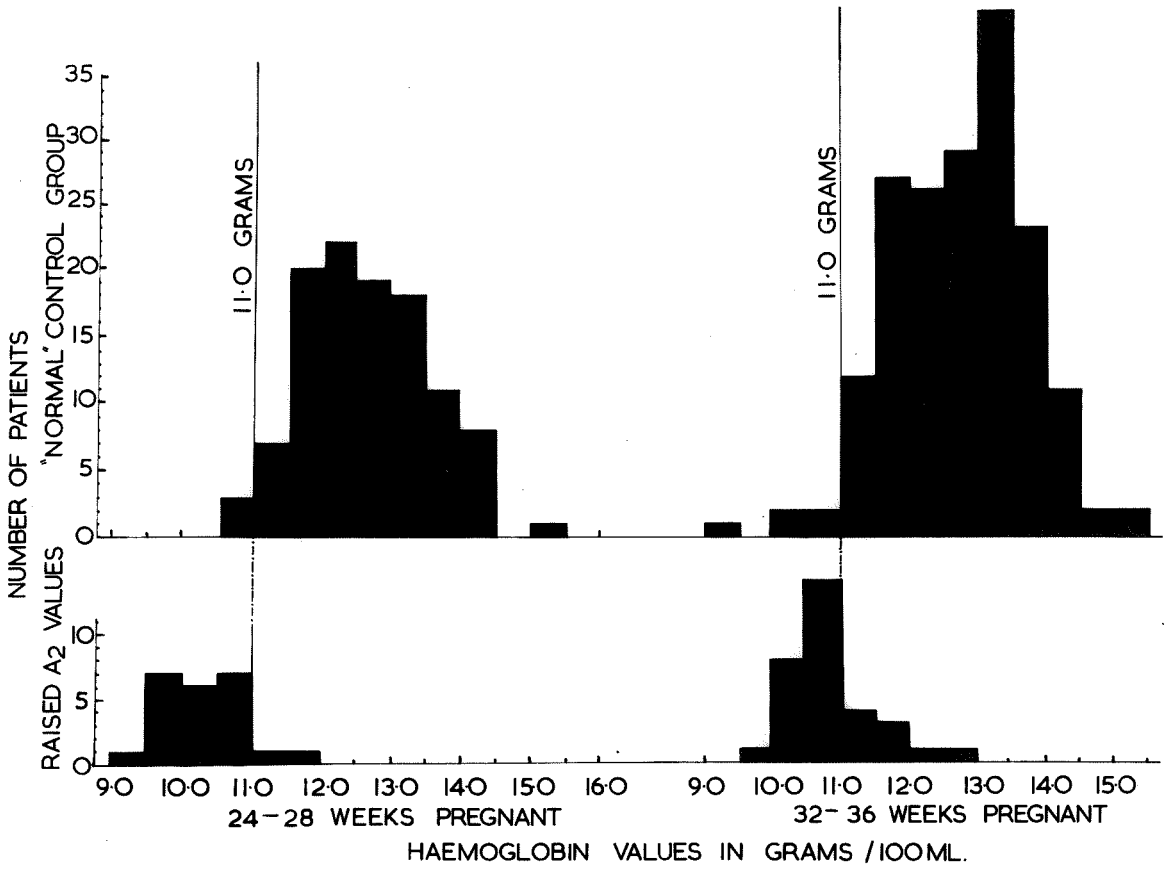
The possibility that there might be some correlation between the degree of anaemia i.e. the minimum haemoglobin level and the haemoglobin A_2 value in individual patients was also examined, but it was shown that no correlation existed: ($r = -.03$; p is greater than 10%).

From the haemoglobin levels, it was apparent that under the previous criteria employed, at least four out of the thirty-two cases would have been missed, because in none of these cases did the haemoglobin fall below 11.0 g. per 100 ml. A number of the other cases might also have been missed as the fall below this figure of 11.0 g. was only of short duration, and it might have been considered, by the clinician in charge of the case, that they were showing a slight response to oral iron: they would not, therefore, have been referred for investigation. The fact that, in these cases mentioned, the haemoglobin might have been lower at some previous stage in their pregnancy cannot be entirely eliminated as some did not attend for antenatal treatment until late in the third trimester.

The Red Cell Indices in Patients with Raised Haemoglobin A_2 Values.

The mean values for the mean corpuscular volume and the mean corpuscular haemoglobin of thirty out of the thirty-two cases is shown in Figure 19. From this it can be seen that, with one exception (Case

FIGURE 18.



Haemoglobin values in the patients with raised Haemoglobin A_2 values at two stages of pregnancy, compared with a randomly selected group of 'normal' patients.

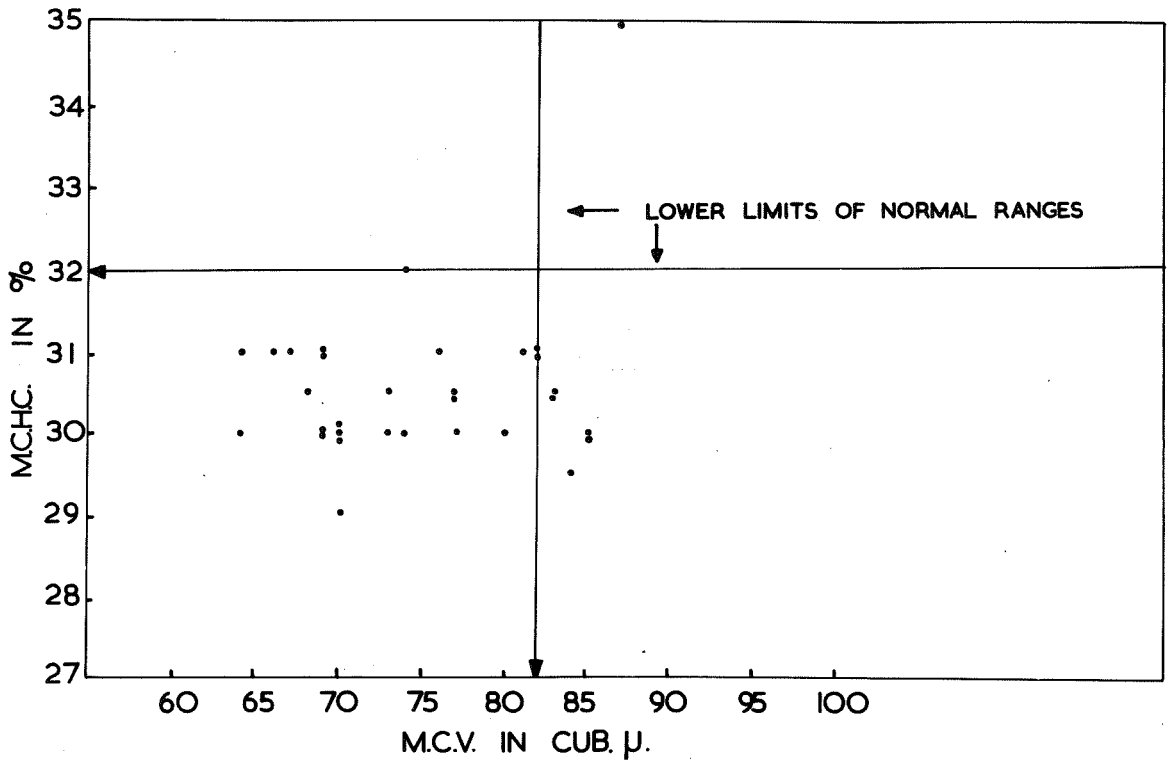
No. 207), all had low values for one or other of these indices. In this one exception shown, there was only one estimation of the red cell indices which appeared normal, but the haemoglobin was down to 10.0 g. per 100 ml.

One of the chief problems in an investigation of this type is the difficulty of obtaining accuracy, even with a high degree of technical skill, in the red cell indices: particularly in those dependent upon the red cell count. It is believed that the values in the case under discussion were probably erroneous; but unfortunately, we were unable to get a further estimation of the red cell indices in this patient, owing to her irregular attendance at the antenatal clinic.

The estimations of the red cell indices in two more of the thirty-two patients were missed through minor lapses in the organisation.

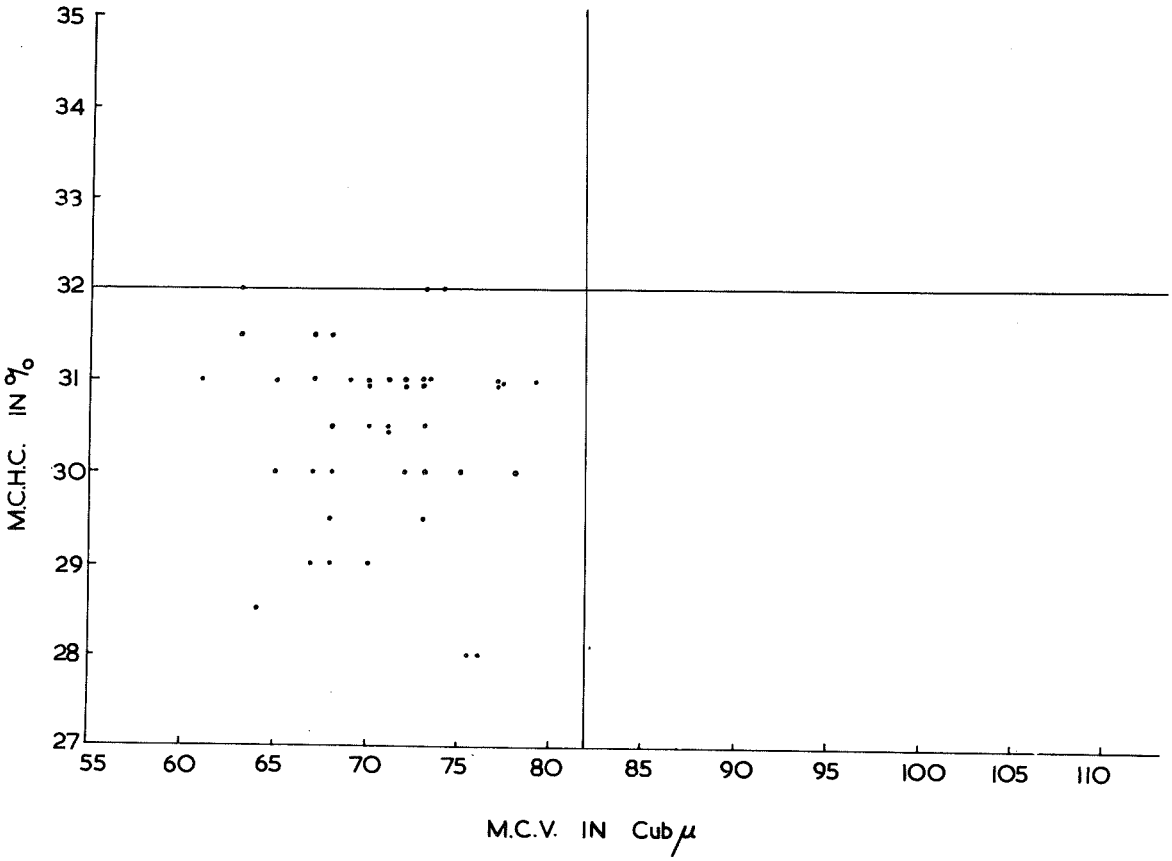
These findings indicate that most of the patients with raised A_2 values do show some other haematological evidence of the thalassaemia trait during pregnancy. It is of interest to note that, although some of these patients show a marked reduction in mean corpuscular volume; during the total three years observation, in none of the cases has the mean corpuscular haemoglobin concentration been reduced below 28%. My personal impression is, that in severe iron deficiency with a reduction in the M.C.V., the reduction in the M.C.H.C. is often more marked than that seen in thalassaemia cases with a comparable M.C.V. The results from the M.C.V. and M.C.H.C. in the anaemia selected thalassaemic group and the results from the iron deficiency series are shown in Figures 20 and 7. It can be seen that the overlap in these groups is too great for proof of this suggestion. Wallerstein and Aggeler (1956), and Mooney (1952), also believed that the reduction in red cell chromicity was less marked than the reduction in red cell size in thalassaemia minor.

FIGURE 19.



Correlation of the mean values for the M.C.V. and M.C.H.C. in the patients with raised haemoglobin A_2 values found during the electrophoretic survey.

FIGURE 20.



Correlation of the mean values for the M.C.V. and M.C.H.C. in thalassaemic patients found in the general survey of anaemias of pregnancy.

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TABLE 8.

STATISTICAL EVALUATION OF RETICULOCYTE COUNTS IN THALASSAEMIA MINOR.

Patients	Number	Mean	S.D.
Normal Controls	58	2.78	1.27
Iron Deficiency	132	2.98	1.52
Anaemia Survey Thalassaemic	40	3.61	1.47
Hb. A_2 Survey Thalassaemic	26	3.90	1.53
Pooled Thalassaemic	66	3.86	1.49

Groups Compared	Probability for F test on S.D.	Probability for t test on Means.
Anaemia Survey Thalassaemic Hb. A_2 Survey Thalassaemic	Not Significant	$<50\%, >40\%$
Pooled Thalassaemic Normal Control	$<10\% >5\%$	$<0.1\%$ ***
Pooled Thalassaemic Iron Deficiency	$>10\%$	$<0.1\%$ ***

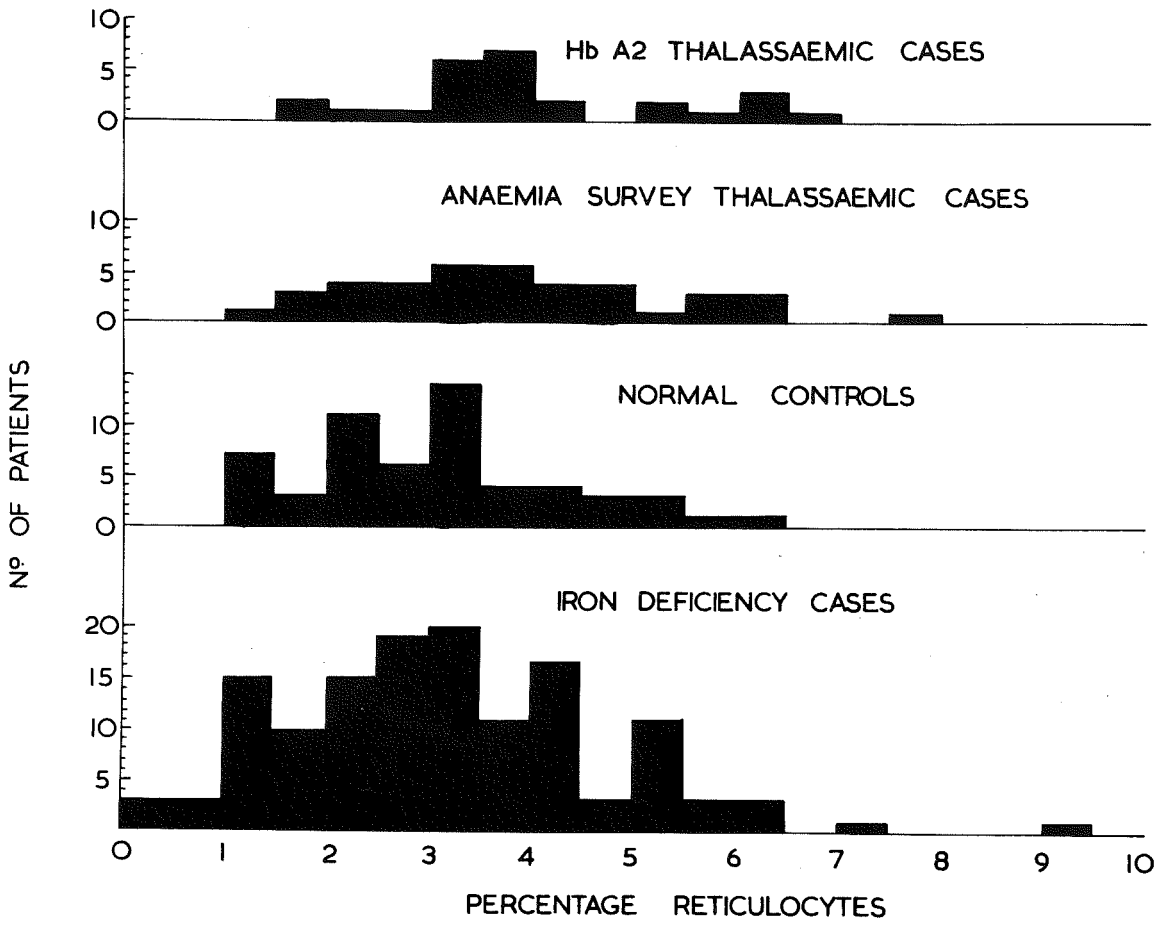
Therefore the Mean reticulocyte count in the Thalassaemic cases are higher than the Normal and the Iron Deficiency cases.

The reticulocyte counts in the cases with raised haemoglobin A_2 values had a mean value of 3.86% with a standard deviation of 1.49%. This mean value is higher than the mean value of 2.78% encountered in the normal controls, and the Student's **t** test applied to this variation demonstrated that it was highly significant. (p = less than 0.1%, greater than 0.01%) (Table 8).

For this particular test, the variances of the reticulocyte counts of the thirty-two cases found by the haemoglobin survey technique, and the variances of the forty cases found during the general survey of anaemias, were pooled; after it had been established by the F test, on the standard deviations, and the **t** test on the means, that there was no significant difference between the two groups. This procedure was carried out in order that the t test on the differences between the means of the reticulocyte counts of the control and the thalassaemia cases should be more critical (see Table 8). It was also found that the values for the reticulocyte counts were significantly higher in the thalassaemia cases

Although the reticulocyte count is not of great value in the differential diagnosis of thalassaemia minor in pregnancy, owing to the wide overlap of the results with the normal values and iron deficiency values (Figure 21), this finding of a difference in the mean values is of some interest, because the anaemia in these cases could be due either to defect in erythropoiesis, or to a combination of this with some increase in haemolysis. It appears probable from this result, and from the occasional finding of a raised serum bilirubin level and excess urobilinogen in the urine, that haemolysis does have a significant effect in the production of the anaemia. Further evidence in favour of a haemolytic element in some cases of thalassaemia minor was produced by Kaplan and Zuelzer (1950), who transfused Cr, 51 labelled erythrocytes from a series of thalassaemia minor

FIGURE 21.



Comparison of the reticulocyte count in thalassaemic and iron deficient patients, compared with a normal control group.

cases into a series of normal subjects. From the variations in the rates of elimination, they formed the opinion that the erythrocytes showing marked morphological defects might be more rapidly eliminated.

Although the above evidence indicates that haemolysis is a major factor responsible for the rise in the reticulocyte counts, there may be additional factors playing minor parts. It is possible for example, that some retardation in the maturation of the reticulocyte is present. Some evidence in support of this might be deduced from the finding by Bannerman, Grinstein and Moore (1959), of some delay of C_{14} and Fe_{59} uptake by the erythrocyte precursors in thalassaemia. They suggested that the synthesis of protoporphyrin, and the combination of iron with protoporphyrin may be slowed in thalassaemia.

Foetal Haemoglobins.

Throughout these investigations, the foetal haemoglobins have been estimated using the Singer 1 minute alkaline denaturation test, (Singer et al. 1951). In the first two years the upper limit of normal had been accepted as 1.7%. For reasons which will be outlined below this upper limit of normal was later increased to 2% of the total haemoglobin.

After commencing the survey of haemoglobin A_2 levels, it was decided to examine the foetal haemoglobin levels of a series of patients who had normal haemoglobin A_2 levels, in order to ascertain whether any cases of a thalassaemia like syndrome, associated with raised foetal haemoglobin levels and normal haemoglobin A_2 levels, were present. Simultaneously with these estimations, a series of non pregnant normals was also examined.

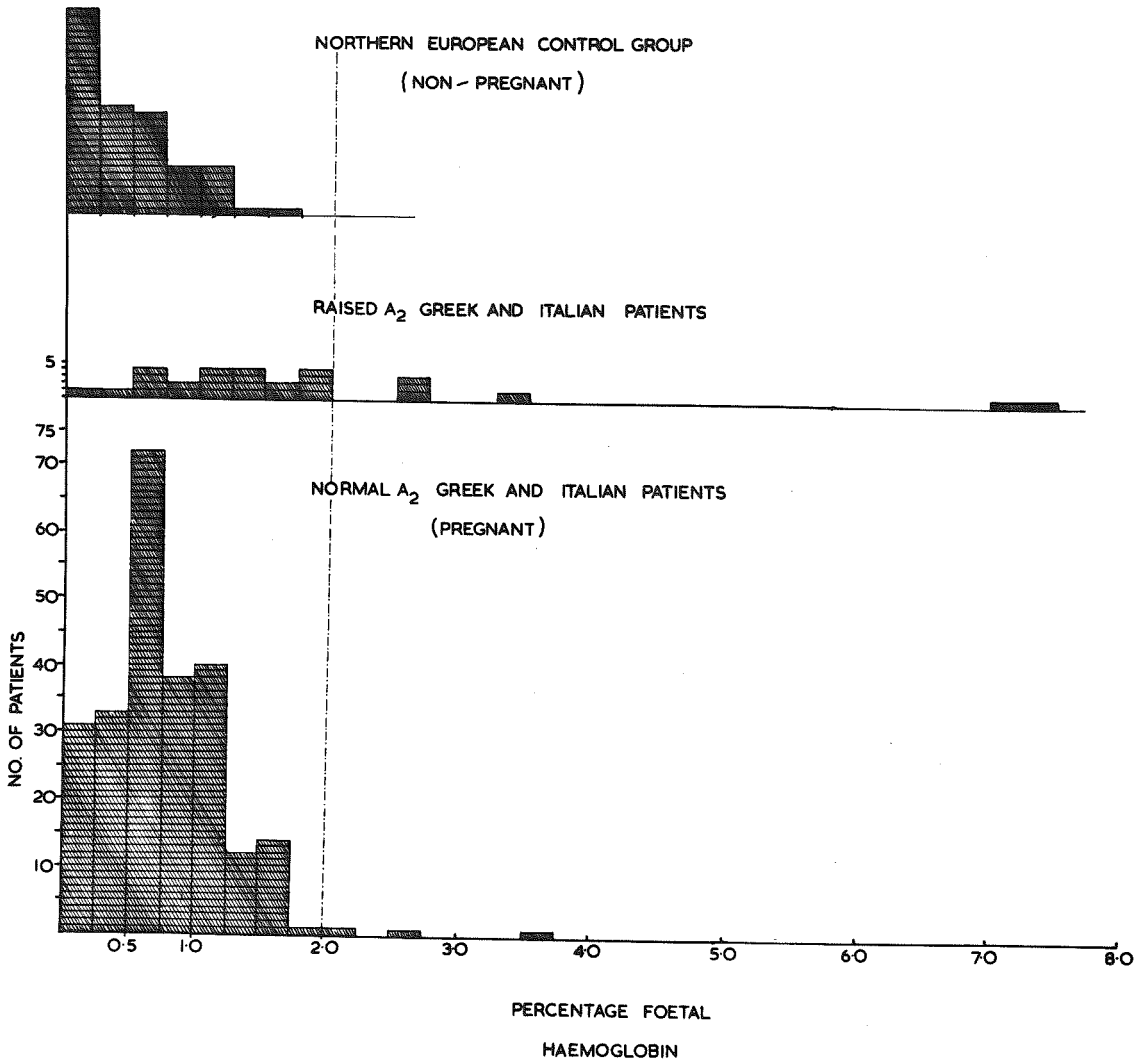
It is not possible to apply the normal measures of statistical analysis to these results, as the normal non pregnant controls obviously do not follow the pattern of a normal distribution. Inspection of the

histogram (Figure 22) shows that there does appear to be an increased quantity of foetal haemoglobin in the pregnant Greek and Italian patients who had normal haemoglobin A_2 levels. It was decided, in view of this, to adopt an upper limit of normal of 2% for the foetal haemoglobin level in future.

Of the two hundred and forty-three Greek, Italian and Cypriot patients, with normal haemoglobin A_2 levels, who were thus examined, it was found that three had raised foetal haemoglobin levels. One of these was only just in excess of the 2% adopted as the upper limit of normal. This patient had a minimum haemoglobin level of 11.2 g. per 100 ml., but no other data on her is available.

In the other two patients there was no evidence of anaemia, the minimum haemoglobin levels being 12.8 g. and 12.0 g. The examination of the red cell indices showed that both patients were normochromic and normocytic, and the infants delivered were both normal. In the two cases, which I have encountered, of foetal maternal bleeding of sufficient severity to raise the foetal haemoglobin level of the maternal blood, the infants exhibited severe anaemia; and it is considered improbable that foetal maternal bleeding, of a degree consistent with foetal haemoglobin levels in the mother of 3.7% and 2.7%, would be consistent with the delivery of normal infants. In both of these cases there was a fall in the serially estimated values during the course of the pregnancy: in one of them the fall was from 3.7%, at the twenty-fifth week of pregnancy, to 3.4% at the thirty-second week. In the other case the fall was from 2.7%, at the twenty-sixth week of pregnancy, to 1.6% at the thirty-ninth week: this last value being within the normal range. The possibility of these variations being associated with variations in the haemoglobin levels was also considered, but there was no evidence of any rise in the haemoglobin level

FIGURE 22.



Foetal haemoglobin levels in Greek and Italian pregnant patients with raised haemoglobin A_2 values, and in Greek and Italian patients with normal haemoglobin A_2 values compared with non-pregnant normal controls.

which could account for them, on an absolute quantitative basis.

These two cases comprise approximately 1% of the total normal Greek and Italian patients examined. The explanation for the raised values is obscure, but it may possibly be that, in times of stress in the erythropoietic system, there is a tendency to revert to the foetal haemoglobin synthesis. Some support for this is apparent in the occasional finding of high F values in other forms of anaemia. We have encountered a value of 3.1% in a megaloblastic anaemia, and one of 10.8% in an erythroleukaemia. The upward shift of the foetal haemoglobin levels in the normal pregnant Greek and Italian patients could also be attributed to this factor.

The advisability of calculating both the foetal haemoglobin and the A_2 haemoglobin in terms of absolute amounts was considered, but it was rejected for the following reasons:-

1. If this had been carried out, the variation in the normal values would have been increased, because there is no evidence of correlation with haemoglobin values.
2. The percentage of the various haemoglobin components appears to be similar in all the cells. This has been discussed by Ingram and Stretton (1959) with regard to the haemoglobin S; and we have personally demonstrated, by the foetal haemoglobin staining technique of Zipursky et al. (1959), that the amount of foetal haemoglobin appears to be evenly distributed amongst the red cells in thalassaemia major.
3. It is normal practice, among all workers in this field, to calculate the quantities of the haemoglobin components as a percentage of the total. To depart from this convention would render comparisons difficult, and such departure would

not appear to be justified under the circumstances.

Of the thirty-two patients found in the haemoglobin A_2 survey, foetal haemoglobin estimation results are available in twenty-eight. As can be seen in Figure 22, six of these patients, or 21%, had values in excess of 2% foetal haemoglobin. The comparative figures for the anaemia survey thalassaemic cases are fifteen with raised values (i.e. in excess of 2% F) out of a total of forty-one cases; or, 36% of the total.

This last figure is on a biased selection of cases and is probably higher than the true value.

From the above results, it is evident that estimation of foetal haemoglobin, though a useful adjunct to the other methods of investigation, is not a reliable criterion for the exclusion of the diagnosis of thalassaemia; and in addition, could occasionally produce false positive results if used as the sole criterion for the diagnosis of thalassaemia minor.

These findings are in agreement with those of Fessas, (1959) who found that in most cases the haemoglobin F values are entirely normal or borderline (2% to 3%), and that only six out of fifty cases had values in excess of 3%.

The possibility of any negative or positive correlation of haemoglobin F with the minimum haemoglobin level during the pregnancy was investigated and no evidence of any relationship could be found. In addition serial determinations on the A_2 survey thalassaemic cases failed to show any evidence of a constant variation in foetal haemoglobin levels associated with the stage of pregnancy.

Peripheral Blood Smears and the Diagnosis of Thalassaemia Minor.

The red cell morphological appearances are well known to include anisocytosis, poikilocytosis, target cell formation, microcytosis

and hypochromia. These features are also found in iron deficiency anaemia, and interest naturally centres on the differential diagnosis between these two conditions.

The morphological appearances cannot be quantitatively evaluated, and assessment must depend upon the opinion and experience of the observer. No single feature can be diagnostic and I merely aim at giving my personal impressions on the preponderance of changes which may suggest the diagnosis.

The poikilocytosis present in thalassaemia minor usually appears to be more marked than would be expected in an iron deficiency case showing a similar severity of anaemia and anisocytosis. Obvious poikilocytes can frequently be seen in cases in whom the haemoglobin level is within the normal range, and as the severity of the anaemia increases the poikilocytosis usually becomes gross.

The hypochromic appearance of the erythrocytes is usually more obvious from the film appearances than one would have expected from the degree of lowering of the M.C.H.C. found. This may be due to the thalassaemic corpuscle being reduced in thickness relative to its diameter, the hypochromic appearance being thereby exaggerated. Target cell formation is due to the same cause, but in my experience this feature is usually present in thalassaemia, but in so variable a degree and with such dependence upon small variations in technique in making the smears, that it is of no great value in differential diagnosis.

Punctate basophilia of the erythrocytes is sometimes present, and this feature was studied in detail in the first nineteen cases seen. The films of these cases were viewed under a phase contrast microscope, and with this technique it was found that ten of them showed punctate basophilia. Owing to the fact that this finding is not specific for

thalassaemia minor its importance in differential diagnosis is considerably reduced, and its only real importance is the suggestion of the possibility of thalassaemia minor in cases in which punctate basophilia is present, and the avoidance of the assumption that the appearances must be due to toxic phenomena, such as lead poisoning.

In summary, therefore, it is evident that the peripheral smears from cases of thalassaemia minor may be suggestive of this condition, but certainty of the diagnosis can only be established by other means.

Serum Iron Levels During Pregnancy in Patients with the Thalassaemia Trait.

The variation in the serum iron levels in normal "non-anaemic" controls has already been dealt with in the section on iron deficiency and anaemia of pregnancy. For the purpose of comparison with the thalassaemic patients, therefore, I propose, in this section, to use only the values from the control series of patients with haemoglobin levels in excess of 12.5 g. per 100 ml.

Serum iron levels were obtained in seventeen of the patients from the A_2 survey thalassaemic series, and in thirty of the patients from the anaemia survey thalassaemic cases. In the haemoglobin A_2 survey, only five thalassaemic cases had estimations of the T.I.B.C. of the serum. This number is insufficient for adequate statistical analysis, and discussion of the results with regard to this protein will therefore concentrate upon estimations of the T.I.B.C., and the percentage saturation of the protein by the serum iron, in twenty-two cases from the anaemia survey thalassaemic series.

With the exception of two cases, all the serum iron estimations were carried out between the twenty-eighth and thirty-fourth weeks of gestation. One of these exceptions has been excluded, as the patient was having a miscarriage at the time the blood was taken.

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TABLE 9.

STATISTICAL EVALUATION OF THE SERUM IRON, T.I.B.C. AND % SATURATION OF THE T.I.B.C. IN NORMAL CONTROLS AND THE THALASSAEMIC CASES.

Group of Patients	Number	Mean	S.D.
SERUM IRON			
Normal Controls	15	131	39.7
Anaemia Survey Thalassaemic	16	121	45.0
Hb. A_2 Survey Thalassaemic	30	154	67.4
T.I.B.C.			
Normal Controls	15	484	75.9
Anaemia Survey Thalassaemic	22	407	78.3
Hb. A_2 Survey Thalassaemic	5	432	149.7
% Sat. of T.I.B.C.			
Normal Controls	15	27.2	7.9
Anaemia Survey Thalassaemic	22	31.3	9.6
Hb. A_2 Survey Thalassaemic	5	57	27.9

Groups Compared	F test on S.D.	t test on Means.
SERUM IRON		
Controls / Anaemia Survey Thalassaemic	Not Significant	$< 50\%, > 40\%$
Anaemia Survey Thalassaemic / Hb. A_2 Survey Thalassaemic	$< 5\%, > 1\%$ *	$< 5\%, > 2.5\%$(a)
T.I.B.C.		
Controls / Anaemia Survey Thalassaemic	Not Significant	$< 1\%, > 0.1\%$ **
Anaemia Survey Thalassaemic / Hb. A_2 Survey Thalassaemic	$< 5\%, > 1\%$ *	$< 40\%, > 35\%$
% Sat. T.I.B.C.		
Controls / Anaemia Survey Thalassaemic	Not Significant	$< 10\%, > 5\%$
Anaemia Survey Thalassaemic / Hb. A_2 Survey Thalassaemic	$< 0.1\%$ ***	$< 5\%, > 2.5\%$(a)

(a) Not acceptable as significant.

The mean values and standard deviations for the serum iron values, together with the total iron binding capacities and percentage saturation, are shown in Table 9.

There were four chief points of interest to be elucidated in this part of the investigation.

Firstly: to determine whether there was any evidence of iron deficiency in the thalassaemic cases. As can be seen from Table 9, the mean value for serum irons in the A_2 series thalassaemic cases is slightly higher than in the anaemia survey thalassaemic cases. This variation, when tested by the t test, shows that the difference is just within the significant level: (p = less than 5% greater than 1%, for one tail of the distribution).

Many of the anaemia selected thalassaemic cases were treated with parenteral iron and although there did not appear to be any appreciable response to this therapy, it is conceivable that some cases may have been slightly iron deficient.

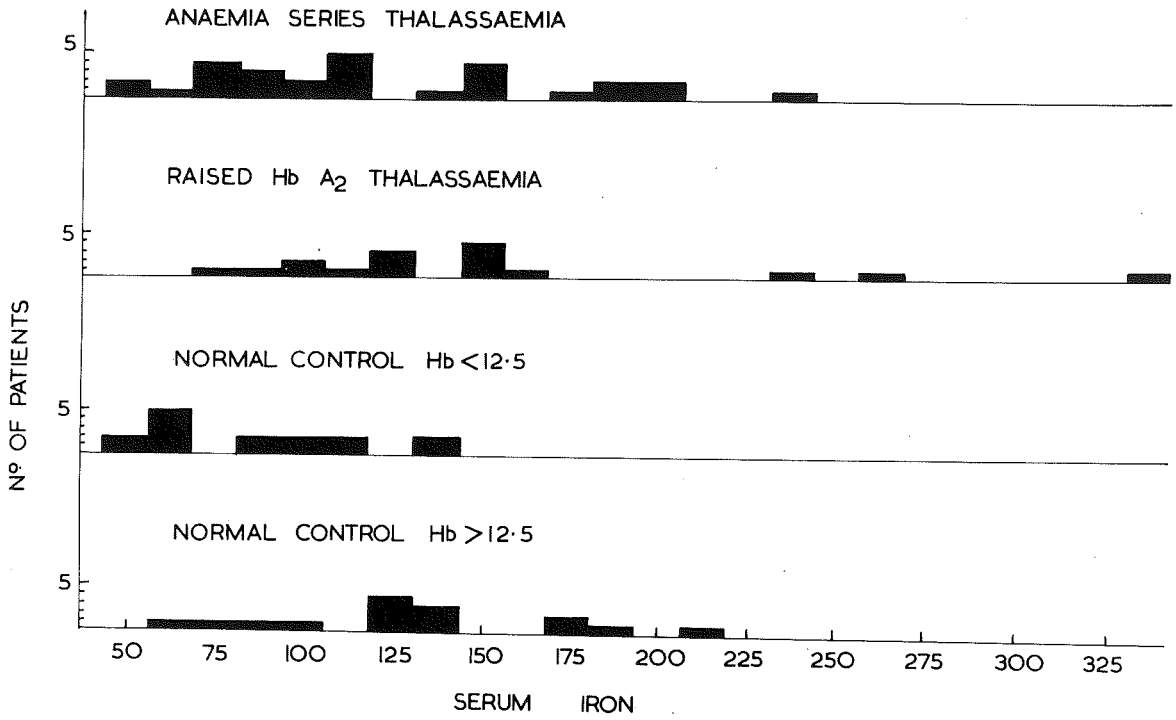
Secondly: whether the serum iron values obtained in the control series were at an optimal level.

The mean serum iron values for the control series fell between those of the two groups of thalassaemic patients, and it would appear likely that the levels obtained in the control series are near to their optimal value. (Figure 23).

Thirdly: whether the T.I.B.C. levels in the thalassaemic cases were similar to those obtained in the control patients.

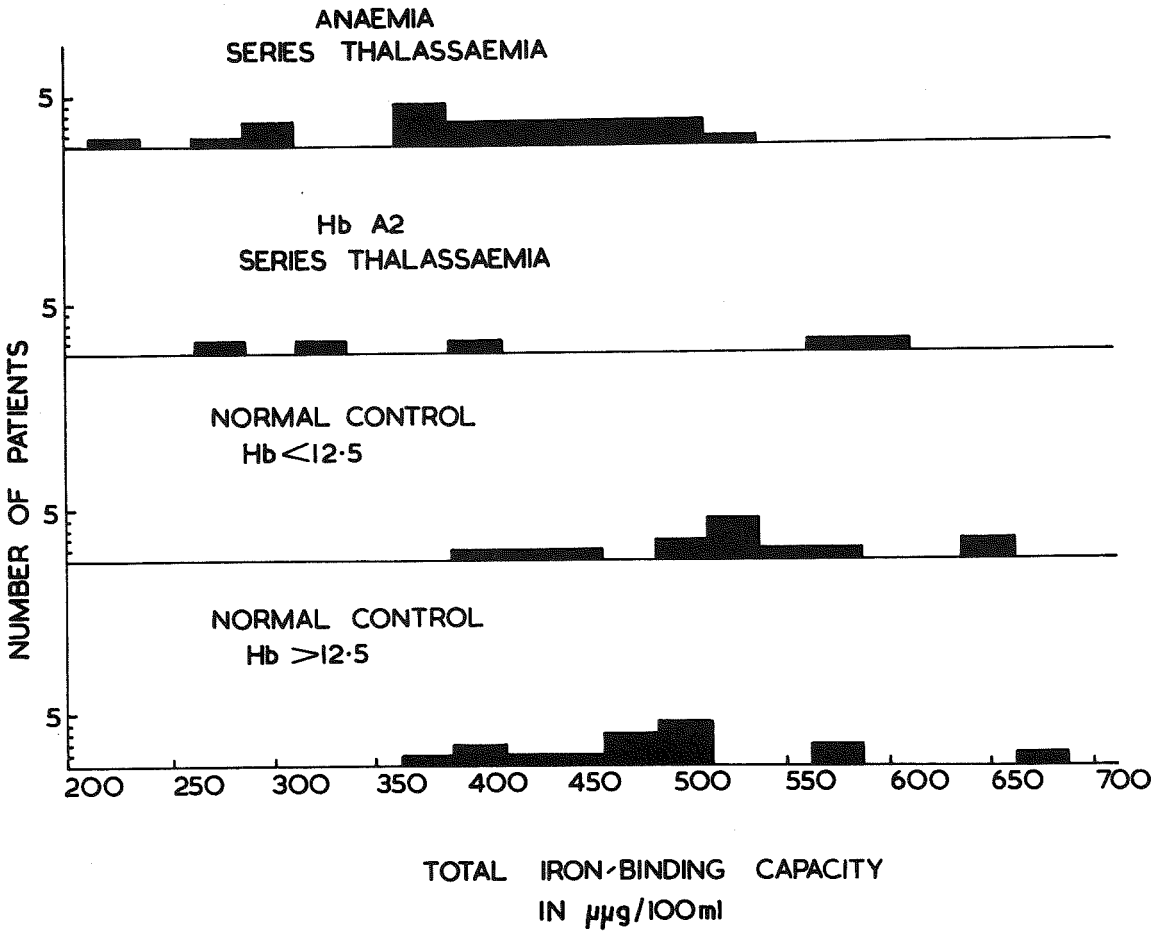
The mean level for both groups of thalassaemic cases is below the mean level for the control series; and in the case of the larger anaemia selected thalassaemic group this difference is significant: (p is less than 1.0%, greater than 0.1%). (Figure 25).

FIGURE 23.



Serum Iron values in the two groups of thalassaemia patients compared with the values found in the normal control patients with haemoglobin values of 11.6-12.5 g/100 ml., and a second group of normals with haemoglobin values above 12.5 g/100 ml.

FIGURE 24.



T.I.B.C. values in the two groups of thalassaemic patients compared with the two groups of normal control patients.

And fourthly, whether the values for the percentage saturation of the T.I.B.C. in the thalassaemic cases were similar to those in the control series.

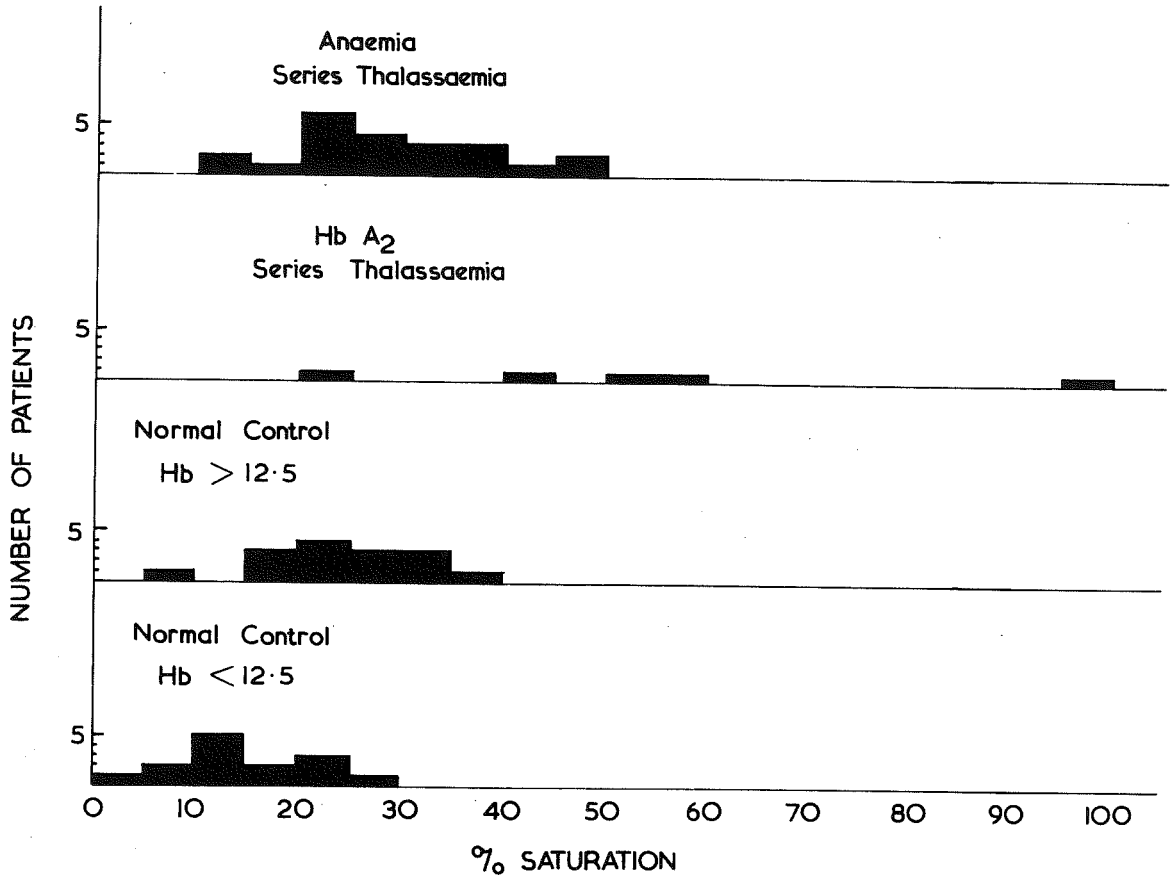
The slightly higher value obtained in the thalassaemic patients is not significantly different from the mean for the control patients: (P is less than 10%, greater than 5%). A higher value was obtained in five patients from the A_2 series, with a very wide scatter of results - as shown by the probability value of 0.1% obtained in the F test between these and the anaemia survey thalassaemic cases. This result is heavily biased by the finding of one patient with complete saturation of the T.I.B.C. among the five thalassaemic patients from the A_2 survey; (see Figure 25).

From these results, I have concluded that there is no good evidence indicative of iron deficiency in either the control series or the thalassaemic patients. The low T.I.B.C. in the thalassaemic cases is a point of interest, but in the absence of evidence of iron deficiency in the control patients, the obvious explanation: of a rise in these latter patients due to iron deficiency, is excluded. Speculation on this point might suggest that the thalassaemic patients have adequate iron stores in the bone marrow, and that the transportation of iron from the other body stores is at a minimum. But this can only remain as an unsolved detail until more knowledge of the controlling factors in the transportation and storage of iron is available.

Erythrocyte Fragility in Thalassaemia and Iron Deficiency Cases.

The increased resistance of thalassaemic red cells to fragility in hypotonic saline is well known, (Dacie, 1960). Mooney (1952), after a familial study of thalassaemia minor, found that increased resistance to

FIGURE 25.



% Saturation of the T.I.B.C. by the serum iron in the two groups of thalassaemic patients compared with the two groups of control patients.

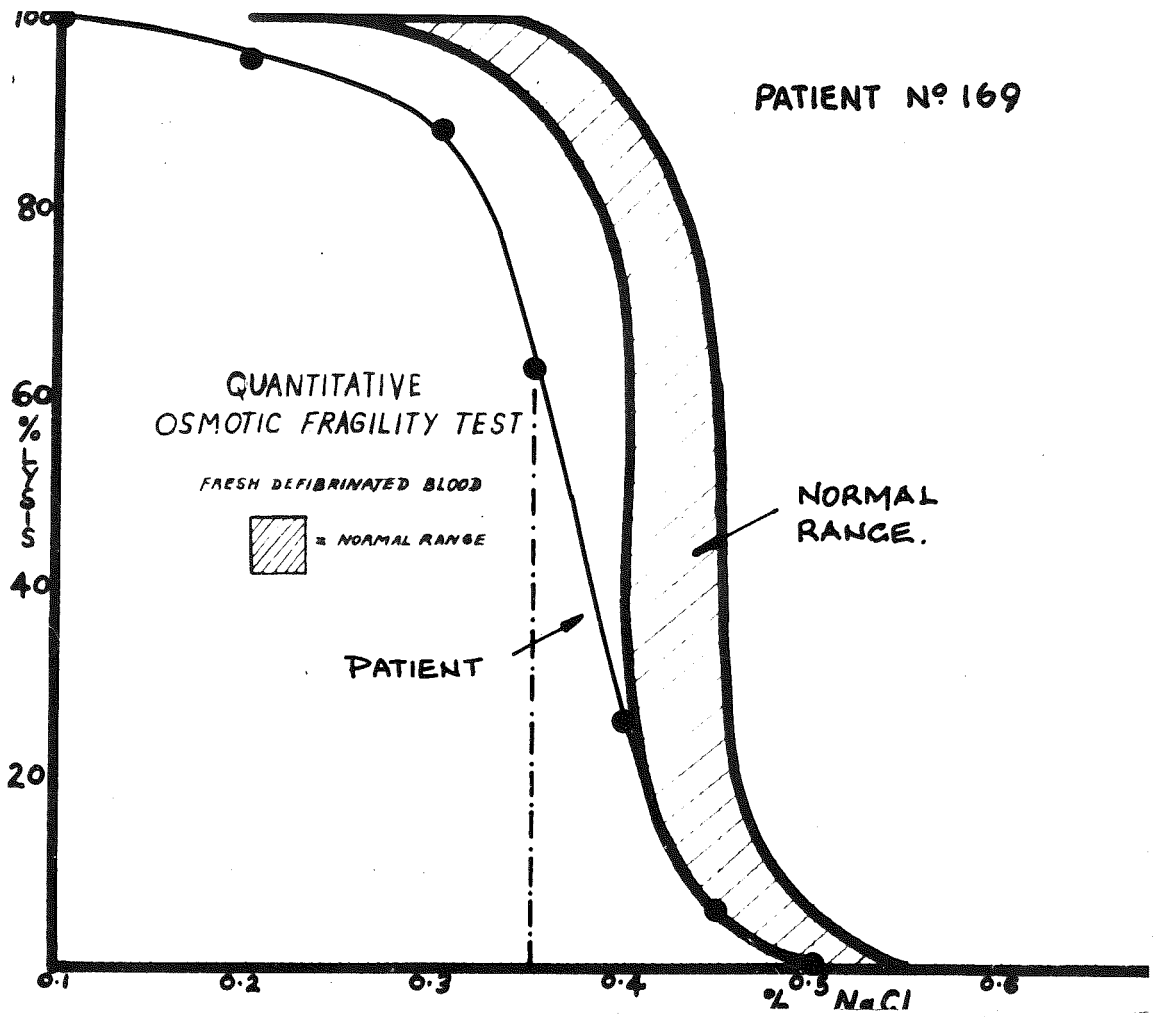
osmotic fragility by the red cells was the only abnormal finding in some cases. Silvestroni and Bianco (1959), have screened large numbers of the Italian population for the thalassaemic trait using a technique in which they measured the percentage of the red cells resistant to fragility, in 60%, 50%, 40% Tyrode's solution (Silvestroni and Bianco 1945). Increased resistance to osmotic fragility is also found in the hypochromic microcytic cells of iron deficiency states (Daland and Worthley, 1935); and in sickle cell anaemia (Dacie, 1956).

As I previously mentioned, complete quantitative fragility studies have been carried out in only two of the patients in this present study. In these cases, a maximal divergence from the normal was observed to take place at 0.35% saline, (Figure 26). A modified one tube test was evolved, in which the haemoglobin estimation was performed in the usual manner by adding 40 cu. mm. of blood, taken in di-potassium sequestrene, to 10 ml. of 0.04 (v/v) ammonia solution. A further 40 cu. mm. of the blood was then added to 10 ml. of 0.35% buffered saline. After it had been standing for thirty minutes at room temperature, this suspension was centrifuged; and the haemoglobin of the supernatant solution was estimated and compared with the haemoglobin value obtained by the oxy-haemoglobin method. This result was then expressed as a percentage haemolysis.

At a late stage in this investigation, it was found that repeated estimations in the same patient showed occasional high values. We attributed this to technical error - due to partial resuspension of the centrifuged deposit of the cells. This would have the effect of reducing the separation between the normal and the abnormal results and probably accounts for the occasional anomalous results in the thalassaemic patients.

Results from this test are illustrated in Figure 27; and when compared to the control group of normal pregnant patients, it can be seen

FIGURE 26.



Showing the increase in resistance to osmotic fragility by the erythrocytes of a thalassaemia minor patient and illustrating the maximum divergence from the normal range at 0.35% buffered saline.

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TABLE 10.

STATISTICAL EVALUATION OF THE POSSIBLE CORRELATION BETWEEN THE RESISTANCE TO OSMOTIC FRAGILITY AND THE RED CELL INDICES IN THALASSAEMIA.

Parameters Compared	Number	Correlation Coefficient (r)	P
M.C.V. & % Fragility	27	.078	> 10%
M.C.H. & % Fragility	28	.062	> 10%
M.C.H.C. & % Fragility	27	.113	> 10%

that twenty-six, out of the twenty-nine patients tested, had increased resistance of the red cells to osmotic fragility in the hypotonic saline.

A control group of iron deficiency anaemias, all of whom showed microcytosis and hypochromia, were also tested; and it can be seen that, although there was some increase in resistance to hypotonic saline, the changes were not usually so gross as those encountered in the thalassaemic group.

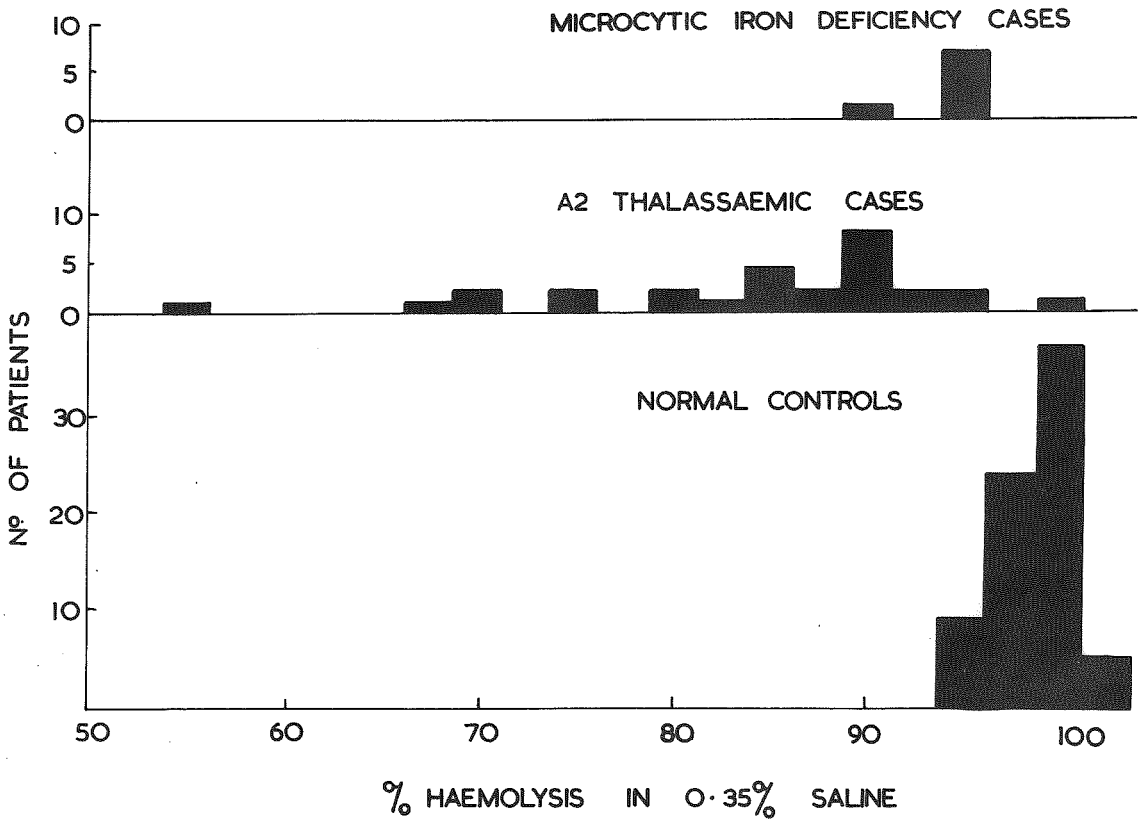
We also investigated the possibility that the degree of resistance to hypotonic saline in the thalassaemic red cells might be related to the mean corpuscular volume, or the mean corpuscular haemoglobin, or the mean corpuscular haemoglobin concentration.

The values for the correlation coefficient for these red cell indices, and the percentage haemolysis, are shown in Table 10; and it can be seen that there is no significant correlation for any of these indices.

The Interaction of the Thalassaemia Trait with Pregnancy.

The literature on this subject is confined to single case reports appearing at irregular intervals. Goldberg and Schwartz (1954) reported a case of Mediterranean anaemia in a negro who developed a megaloblastic anaemia in three successive pregnancies. In the second of these there was some doubt as to whether the response was to folic acid or vitamin B_{12}. On each occasion the development of the megaloblastic change was accompanied by a gross exacerbation of the anaemia, and some reduction in the hypochromia. Whilst discussing the association of generalised siderosis in Mediterranean anaemia in a series of thirteen cases, Ellis and Schulman (1954) mention that one of their cases had an anaemia of pregnancy. Hammond and Nazum (1946) and Torrance (1958) report single cases presenting as anaemia of pregnancy; in the latter case the patient had an exploratory laparotomy and splenectomy for a tumour in the left hypochondrium. This

FIGURE 27.



Showing the resistance to osmotic fragility (.035% saline) by the erythrocytes of patients with raised haemoglobin A_2 values, compared with a group of microcytic iron deficient pregnant patients and a normal control group of pregnant patients.

emphasises the necessity for the appreciation and recognition of this condition as a cause of splenomegaly. Grignani and Sulis (1960), working in Cagliari, reported an interesting case of a very severe anaemia in pregnancy. The patient had a foetal haemoglobin of 80%, but they could find evidence of haematological abnormality in only one of her parents, and they had difficulty in deciding whether to classify the case as thalassaemia major or minor.

There have not been any reports of a series of cases of thalassaemia in pregnancy, and only brief mentions have been made of this problem. Lund (1951), in discussing serum iron values in pregnancy, says that he has excluded sickle cell disease, mediterranean anaemia and Holly's refractory anaemia from his series. Lehmann (1960) in reviewing the techniques of diagnosis of the haemoglobinopathies, mentions that thalassaemia minor may present as an anaemia of pregnancy.

There has been a greater interest in the problem of Sicklaemia and pregnancy. Abrams and Schwartz (1959), whilst reporting a further six cases, reviewed the literature and were able to find a total of one hundred and forty cases reported up to that time. Sickle-cell thalassaemia (microdrepanocytic disease) in pregnancy has been reported by Brown and Ober (1958). The reason for the dominant interest in the effects of pregnancy on the sickling syndromes is due to the comparatively high mortality and morbidity due to the tissue infarctions which are associated with sickling of the red cells.

It seems clear from the small amount of information in the literature and from the data collected in the present survey, that the increase in the blood volume during pregnancy presents a challenge which the defective erythropoietic system of the thalassaemic patient is unable to meet, and these cases therefore frequently present as an anaemia of

pregnancy.

These patients may be fairly well compensated in the non pregnant state and in the eleven cases which have so far been followed up, after sufficient time had lapsed for full recovery from pregnancy to take place, five had haemoglobin values in excess of 11.9 g. per 100 ml.

The Incidence of Thalassaemia Minor.

As has been demonstrated, the incidence of this condition, in patients selected because they showed anaemia in pregnancy, was 1.3% in the Italians, and 4.7% in the Greeks. As has already been discussed this incidence is almost certainly below the true value, and it is probable that the incidence of β. thalassaemia, of 2.0% in the Italians and 6.8% in the Greeks, is more accurate. In three of the Greek cases from the haemoglobin A2 survey series, we failed to obtain any confirmatory evidence to support the diagnosis. If these three cases are eliminated the incidence in the Greek population under study would then be 5.9%. Our data suggests that the incidence of alpha thalassaemia is comparatively low in the Greeks and Italians, and in this we are at variance with Zuelzer et al (1961) who saw thirty-nine Beta thalassaemic cases to fourteen alpha thalassaemics in Greeks and Italians. It seems obvious that bias on one side or the other, due to case selection must account for our difference of opinion. It is possible that we have missed cases because the level of haemoglobin A_2 was our principal criterion for diagnosis, but on the other hand if Zuelzer's ratio of three Beta to one alpha is correct it is surprising that we have not seen several cases presenting as anaemia during our survey in which we have seen over thirty-two cases of Beta thalassaemia. Reports from workers amongst the negro populations suggest that the incidence of alpha types of thalassaemia is probably commoner amongst those people (Went and MacIver, 1961).

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TABLE 11.

TO SHOW THE HAEMATOLOGICAL RESULTS IN 3 CASES WITH ABNORMAL HAEMOGLOBINS.

Investigation	Case 1.	Case 2.	Case 3.
Abnormal Hb. Tris Barbiturate	S S	C C	Lepore Nil
% Abnormal Haemoglobin	25	39	10.1
% A_2 Haemoglobin	2.3	-	3.6
% Foetal Haemoglobin	0.9	0.5	4.6
Sickling	+++	Nil	Nil
Minimum Haemoglobin Value g/100ml.	12.2	12.5	11.5
Mean M.C.V.	93	90	71
Mean M.C.H.	31	29.5	22
Mean M.C.H.C.	32	32	31.5
Mean Reticulocytes %	5.0	11.2	4.6
Anisocytosis	+	+	++
Poikilocytosis	-	+	++
Target Cells	-	-	occas.
Serum Iron	97	-	57
T.I.B.C.	-	-	-
% Sat. T.I.B.C.	-	-	-

A considerable amount of work has been carried out on the incidence of the various forms of haemoglobinopathies (Figure 29), but the actual incidence of the thalassaemia genes has been comparatively neglected. This is largely due to the difficulties of making a definitive diagnosis of this condition. Silvestroni and Bianco (1959) have surveyed large numbers in the population of Italy using their red cell fragility screening method, and they have shown that the incidence varies considerably in different localities, and is maximal in the Po Valley where they found 10% of the population with red cells showing osmotic resistance. They give an average incidence for Italy of between one and two percent, which is in agreement with our figures. From a retrospective assessment based on the incidence of thalassaemia major, Neel and Valentine (1945) calculated a theoretical incidence for the thalassaemia gene of 4% amongst the Italian population in Boston.

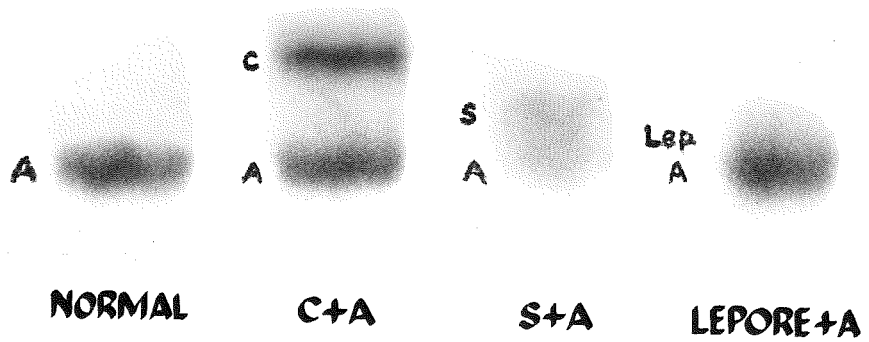
Haemoglobinopathies, other than Thalassaemia, encountered during the Haemoglobin A_2 Survey.

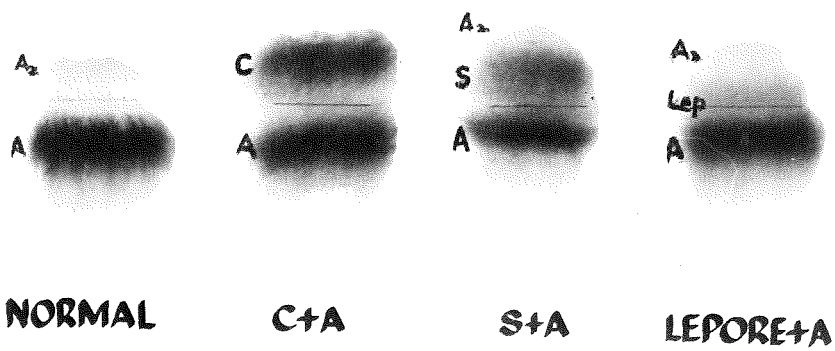
During the course of the haemoglobin electrophoretic survey, we encountered three cases showing abnormal haemoglobin components. (Figure 28).

As can be seen from Table 11, the only patient who showed any clinical effects was No. 3, whose blood contained the Lepore haemoglobin. This condition, which has many features in common with thalassaemia minor, was first described by Gerald and Diamond in 1958 (b). They separated a small component from between the A and the A_2 bands by using starch block electrophoresis on the blood of an infant. Lehmann (1960b) reports that this condition is not uncommon amongst the Greek population, and he has also shown that this haemoglobin can be separated on paper using a Tris buffer: (Lehmann and Sharih, 1961).

FIGURE 28.

ELECTROPHORESIS OF ABNORMAL HAEMOGLOBINS



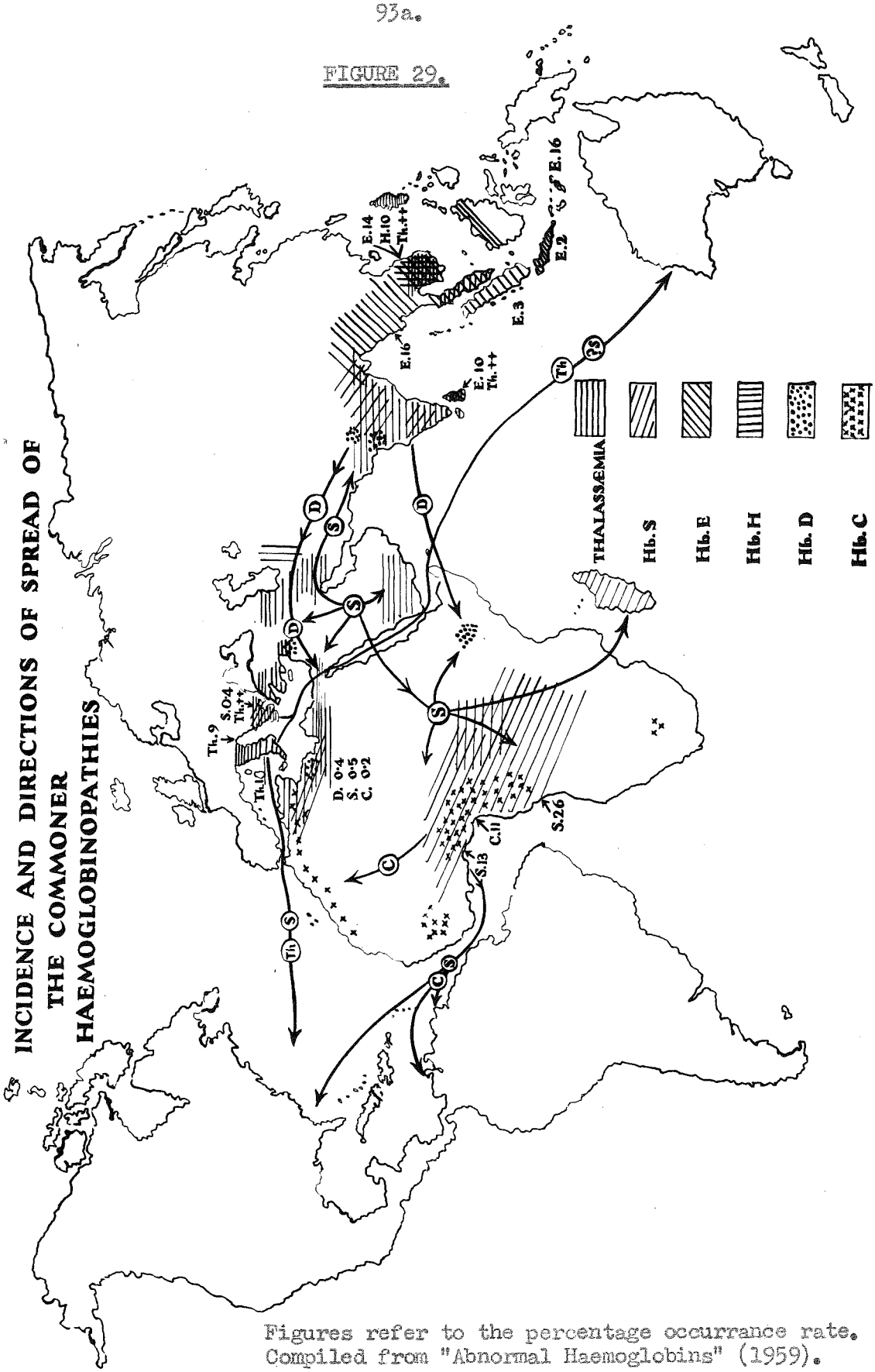


Comparative separation of the abnormal haemoglobins, with tris and barbiturate buffers.

The red cells of the patient with the sickle cell trait showed sickling properties when treated with sodium meta bisulphite. The erythrocytes of the patient with the haemoglobin C trait did not show the target cell formation which is frequently a striking feature of these cases.

Haemoglobin S is not uncommon amongst either the Greek or the Italian populations, and an incidence of 31% and 32% has been found in some of the villages in the Chaldiki Province of Northern Greece: (Deliyannis and Tavlarakis, 1955). Haemoglobin C is common in Ghana, (Figure 29), and also occurs sporadically in Algeria, (Lehmann, 1959). This is a rare abnormal haemoglobin among the Italians, but its occurence has been reported previously, (Erlandson, Smith and Schulman, 1956). An interesting sidelight on this haemoglobin is the suggestion by Mourant, (1954) and Lehmann, (1959) that is is a mutant form of haemoglobin S.

FIGURE 29.



Figures refer to the percentage occurrance rate.
Compiled from "Abnormal Haemoglobins" (1959).

SUMMARY OF SECTION 4.

IRON REFRACTORY NORMOCYTIC AND MACROCYTIC ANAEMIAS OF PREGNANCY.

The incidence of megaloblastic anaemia of pregnancy, in this survey, has been shown to be one case in one thousand, two-hundred and fifty patients. This figure is lower than those published from the industrial areas in the United Kingdom. The implications of this finding have been discussed, and it is suggested that dietetic factors play a major part in the pathogenesis of this condition.

A further nine cases were described, in which some response to combined folic acid and vitamin B_{12} was observed. It seems probable that mild degrees of deficiency of these substances can occur during pregnancy without producing a frank megaloblastic change in the bone marrow. This finding is discussed, with relation to megaloblastic change of the bone marrow, as a criterion for diagnosis.

IRON REFRACTORY NORMOCYTIC AND MACROCYTIC ANAEMIAS OF PREGNANCY.

In view of the reports of high incidence of megaloblastic anaemias of pregnancy which have appeared in the United Kingdom over the last decade, it was thought that this condition might account for a substantial proportion of the anaemias coming within the terms of this survey.

During the course of the thirty-eight months in which the anaemia survey has been conducted, seven thousand, five hundred and seventeen non-private patients have been booked for antenatal treatment at the Queen Victoria Maternity Hospital.

Of these patients, over four hundred have been referred for investigation of their anaemia; and only five of them have shown the classical features of a megaloblastic bone marrow, associated with a reticulocyte response and rise in the haemoglobin level as a result of therapy with folic acid.

A summary of the case histories of these patients is inserted below.

Case No. 233. A thirty-five year old aboriginal who had had seventeen previous pregnancies and who first attended for treatment at thirty-two weeks gestation. Her haemoglobin, at that time, was 10.0 g. per 100 ml., the M.C.V. was 103 cu. micra, the M.C.H.C. 33%, and the reticulocytes 3.2%. The patient was brought into hospital as an in-patient for treatment of bronchitis and pleural effusion; and at that time (34 weeks gestation) the haemoglobin had fallen to 8.2 g. per 100 ml., the M.C.V. was 93 cu. micra and the reticulocytes 1.8%. A shift to the right in the polymorph series was noted, and bone marrow examination showed a megaloblastic change in the erythroid series. The patient was transfused

on account of the anaemia, and folic acid therapy (15 mgm. daily) was commenced. The maximum reticulocyte response was 8.2% on the seventh day after commencement of therapy. The rate of rise in the haemoglobin level was difficult to assess owing to the transfusion, but a continuous rise from the post-transfusion level took place, to 12.1 g. per 100 ml. at one week before delivery. Delivery of a 7 lb. 7 oz. living male infant was uneventful.

Case No. 234. A gravida 4, aged thirty-six and born in South Australia, who complained of continuous vomiting and anorexia throughout pregnancy. This behaviour was said to be completely different from that of all her previous pregnancies. At the 10th week of pregnancy her haemoglobin level had been 14.7 g. per 100 ml., and at the thirty-fifth week it was 11.0 g. Three weeks later, when she was admitted to hospital in premature labour, the haemoglobin level had fallen to 8.5 g. per 100 ml. and a stillborn infant was delivered. The M.C.V., at that time, was 100 cu. micra and megaloblasts were found both in the bone marrow smears and in the buffy layers from the peripheral blood. Folic acid (30 mgm. daily) was given, and the maximum reticulocyte response was 24.4 on the tenth day after commencement of treatment. In spite of transfusion immediately after the bone marrow was taken, the haemoglobin had only risen to 9.0 g. on the twenty-first day of therapy, and vitamin B_{12} (100 micrograms twice weekly) was added to the folic acid therapy. Serum was taken for B_{12} assay prior to this treatment, and the combined serum B_{12} level was subsequently found to be 420 µµg. per 100 ml., with a free B_{12} level of 20 µµg. per 100 ml. A second reticulocytosis was observed reaching a maximum of 14% on the fifth day, and the haemoglobin level rose to 13.5 g. after four weeks of B_{12} therapy. An alcohol test meal showed a normal level of hydrochloric acid, with no evidence of achlorhydria. This patient has been followed up, since

the cessation of the B_{12} therapy, and there has not been any evidence of a fall in the haemoglobin level during these examinations.

Case No. 235. An aborigine, gravida 8, aged twenty-nine, who was first seen at the twenty-first week of gestation when her haemoglobin was 10.7 g. per 100 ml. This patient only attended the clinic at irregular intervals. At the thirty-first week 950 mg. of iron dextran had been given, but the haemoglobin fell to 9.5 g. at the thirty-third week of gestation, and she was admitted for investigation. The M.C.V., at that time, was 104 cu. microns; the M.C.H.C. was 34.5%; the reticulocytes 0.4%. A bone marrow showed a megaloblastic change, and oral folic acid (15 mgm. daily) was given. A maximum reticulocyte response of 16.4% was observed on the sixth day of therapy, and the haemoglobin had risen to 12.0 g. per 100 ml. after twenty-five days of therapy.

Case No. 236. A twenty-six year old patient, gravida 6, whose first two pregnancies were uneventful, but who had subsequently had three stillbirths due to erythroblastosis foetalis. This patient lived in the country, at Barmera, and first attended the antenatal clinic when thirty-six weeks pregnant. Examination of the light absorbtion pattern of the amniotic fluid, at that time, suggested a pre-hydropic infant. At this visit it was noted that there was a definite hypersegmentation of the polymorphs in the blood smear. The haemoglobin was 10.2 g. per 100 ml., and when the patient was followed up, it had fallen to 8.5 g. per 100 ml. within a fortnight. The M.C.V. was 85 cu. microns, the M.C.H.C. 37% and the reticulocytes 0.6%. A bone marrow showed a megaloblastic change, and iron stains on the marrow section revealed a normal haemosiderin content. The patient was given folic acid (10 mg. daily) and attained a maximum reticulocyte response of 10.4% on the eighth day of treatment. The picture was made even more confused by the finding of a raised bilirubin in the

serum, which attained a maximum level of 2.8 mgm. per 100 ml. (direct 2.6 mgm.; indirect 0.2 mgm.) on the eighth day of folic acid therapy; and there was an increased urinary excretion of urobilinogen (17 mg. in twelve hours) at the same time. A positive direct coombs test was also obtained when the bone marrow was performed, but this result could not be repeated later. The titre of antibodies in the maternal serum was 1/32, but in view of the history and findings on the amniotic fluid, surgical induction of labour was undertaken after the patient had been transfused. The infant was hydropic and failed to respond to exchange transfusion. Delivery and transfusion eliminated the possibility of assessing the haemoglobin response in the antenatal period, but the haemoglobin rose to 13.0 g. per 100 ml. during the second post-natal week.

This patient was also found to have an enlarged spleen during the post-natal period, and she stated that it had been enlarged since she was twelve years old, at which time the possible diagnosis of leukaemia had been suggested. In view of this finding, associated with the evidence of haemolysis during pregnancy, studies of the red cell fragility and auto-haemolysis were undertaken in both the patient and her mother, who was also alleged to be anaemic. All the haematological findings on these patients showed no abnormality, and the possibility of a conjenital form of haemolytic anaemia is there by excluded.

Although the picture is not clear cut in this case, most of the findings are consistent with a megaloblastic anaemia of pregnancy, with the additive complication of a rhesus factor incompatibility between the mother and foetus, and the possibility of a transient haemolytic anaemia in the mother.

Case No. 237. The patient, a gravida 3, aged thirty-one, was a known epileptic, controlled for thirty-three months on dilantin and pheno-

barbitone. The haemoglobin in this case gradually fell from 13.5 g. per 100ml., at seven weeks gestation, to 11.0 g. at twenty-six weeks. A more rapid fall then took place, to 10.2 g. at twenty-nine weeks. The serum iron, at that time, was 232 micro micrograms per 100 ml., and the iron binding capacity of the serum was fully saturated. A bone marrow showed a megaloblastic change, and a maximal reticulocyte response of 17% was observed on the eleventh day of folic acid therapy (15 mgm. daily).

Case No. 234, of this series of five proven megaloblastic anaemias, is of particular interest because she failed to show the expected rise in haemoglobin on folic acid therapy; and subsequently showed an apparent response to the B_{12} therapy in spite of the normal serum level. In some ways, this case shows a resemblance to the tropical cases, as reported by Patel and Kocher (1950) and Tasker et al. (1958), and the other isolated cases reported by Killander (1958) and Loewenstein et al. (1953), in whom a response to B_{12} therapy was obtained. The possibility of a classical Addisonian type of Pernicious Anaemia appears to be eliminated by the presence of free acid, and one possible explanation is that the patient's B_{12} reserves had been eliminated by the continuous vomiting and poor diet during pregnancy, combined with the demands made by the foetus, and increased erythropoietic activity. This is difficult to reconcile with the finding of a normal serum vitamin B_{12} level, unless there had been some mobilisation of the B_{12} due to the folic acid therapy. The pathogenesis in this patient remains obscure, but it is evident that, in some way, the B_{12} therapy had a potentiating effect upon the erythropoiesis. As I have already mentioned in the historical section, Killander (1958) has also reported three cases of megaloblastic anaemia of pregnancy, with normal serum B_{12} levels, who responded to B_{12} therapy.

It is also of interest to note that all five of these cases of

mine had unusual features. One was associated with intractable hyperemasis; one was associated with erythroblastosis foetalis; one was on dilantin therapy, and two occurred in aboriginals, in whom a high incidence of this condition has been noted both in Adelaide and Perth: (W.R. Pitney, 1959).

In addition to the five classical cases already noted here, another ten cases have been observed in whom the anaemia was less severe, (Appendix 6), but in whom there was some rise in haemoglobin following combined folic acid (15 mgm. daily) and B_{12} therapy (100 micrograms weekly). Bone marrows were carried out in seven of these cases, and assessed, both personally and by at least one other unbiased haematologist, before the response to therapy was known; and in all seven cases, the marrow was considered not to show the features of megaloblastic change.

In retrospect, Case No. 238 is considered to show some evidence of macropolycytes and an occasional transitional megaloblast; but in view of the previous unbiased assessment, the patient has been retained in the normoblastic group. It may be significant that this patient, in whom the marrow findings were borderline, showed the most vigorous response of all the ten patients in this normoblastic group; she had also had a megaloblastic anaemia in a previous pregnancy. Recurrences in later pregnancies are a recognised phenomenon; Forshaw et al. (1957) observed three relapses in a total of nineteen patients under observation.

This finding agrees with Lawson and Bolton in Australia (1951), who treated sixteen cases of macrocytic anaemia with folic acid, and found that some response to folic acid was indicated by a rise in the haemoglobin values. Not all of these cases would be acceptable under the criteria of diagnosis used in this survey, as the possibility of iron deficiency exists, particularly in two cases seen at very early stages of

pregnancy. These authors were of the opinion that sternal marrow biopsy did not always assist in the diagnosis. Their cases have a feature in common with the present descriptions, in that they were investigated in Australia and that the anaemia was not usually as severe as in those reported overseas.

The incidence of five proven cases of megaloblastic anaemia and one case showing transitional megaloblasts in a total hospital population of seven thousand, five hundred and seventeen patients, over the period of thirty-eight months; or one in one thousand, two-hundred and fifty, is considerably below the figure of one in one-hundred and ninety confinements quoted by Forshaw et al. (1957) from Lancashire, England, and that of Giles and Shuttleworth (1958). The latter carried out marrow biopsies on all patients with haemoglobin values of less than 9.5 g. per 100 ml., and found sixty-two cases with megaloblastic anaemia during their pregnancy, and twenty-eight with megaloblastic anaemia during the puerperium.

These cases were encountered during a fourteen month survey of three thousand, one hundred and ninety-nine patients, and their incidence of megaloblastic anaemia is one in thirty-nine pregnancies. Lillie, Gatenby and Moore, (working in Dublin in 1954), found an incidence of one case of megaloblastic anaemia in every two-hundred and eighty-eight pregnancies; and they thought that only one of their fourteen cases had an adequate diet. Balfour (1927) drew attention to the extremely high incidence in India, and described the findings in one-hundred and fifty cases; he also noted that the incidence appeared to be seasonal, in that the majority of the cases occurred in the second half of the year. Das Gupta, (1954) quoted an incidence of fifty-eight per one-hundred deliveries in Calcutta, India. Davidson (1952) thought that the incidence in Edinburgh

was one in two thousand confinements, and he remarked that the incidence during the war years was two to three times greater than in the post-war era; he attributed this variation to the low animal protein content of the diet during wartime. Thompson (1957) found that the incidence of this form of anaemia was increased during the Spring and Summer: a variation which he, also, believed to be due to dietetic factors. Thompson pointed out that the folic acid content of beef was very high, but believed most of this would be destroyed by cooking, and the main source of folic acid in the diet would be from green vegetables. Loewenstein et al., Pick and Philpott (1953) saw eighteen cases of megaloblastic anaemia in three years; but they did not see any more cases during further survey lasting eighteen months, in which time all their antenatal patients were given 4.5 micrograms of vitamin B_{12} and 3 mg. of folic acid daily.

Giles (1960) has also used routine administration of folic acid in the prophylactic treatment of his cases, and found that megaloblastic anaemia thereby was eliminated. He also found that the overall haemoglobin levels in these patients were significantly higher than in a control group; and he interprets these findings as indicating that a large number of women have some folic acid deficiency in pregnancy; but in only a few, with an inherited disposition, would this depletion result in florid megaloblastosis.

As previously discussed, the folic acid absorption test of Chanarin, Anderson and Mollin (1958) showed some deficiency of absorption of folic acid in all their cases of pregnancy; but they weren't able to demonstrate any additional impairment of absorption in their cases of megaloblastic anaemia.

The relatively low incidence of frank megaloblastic anaemia of

pregnancy observed in this present survey is in agreement with the personal observations of many obstetricians who have worked in Australia and the United Kingdom, and who nearly all believe that there is a relatively low incidence in Australia.

It seems almost certain that the dietetic intake of folic acid plays a major part in the pathogenesis of this condition. The present observations, that the cases in which frank megaloblastosis has occurred have additional complicating factors, or are known to have a poor diet, support this contention.

Thompson (1957) has pointed out that, although dietary intake of folic acid undoubtedly plays a major role in the pathogenesis of folic acid deficiency, it cannot be assumed that dietetic factors are solely responsible for all cases with megaloblastic anaemia of pregnancy.

It seems fairly evident that factors of varying rates of absorption, and variations in susceptibility of the patient, must also play some part.

Many authors have maintained that a certain diagnosis of megaloblastic anaemia of pregnancy can only be made if unequivocal evidence of megaloblastosis of the bone marrow is present: (Callender, 1944; Israels and Da Cunha, 1952; Loewenstein et al., 1953). Other authors have been willing to accept some reduction in this criterion and to take the presence of: giant metamyelocytes (Giles and Shuttleworth, 1958); transitional megaloblasts (Das Gupta, 1954) or macronormoblasts (Scott and Govan, 1952), as evidence in favour of a megaloblastic form of anaemia.

It is well known that in these borderline types of morphological assessments it is not easy to attain unanimity in the opinions of the observers, and it becomes extremely difficult to compare different sets of reported results. The advent of the more sensitive methods of serum folic

acid assay offers hope for the elucidation of many of these problems of variations in incidence, host susceptibility and absorption encountered in the study of folic acid deficiency states.

TABLE 12.

CASES OF ANAEMIA ENCOUNTERED COMPARED WITH THE TOTAL NUMBER OF HOSPITAL BOOKINGS.

Figures in parentheses refer to percentage of row A.

Birthplace.	Australia	Italy	Greece	Cyprus	U.K.	Other	Totals
(A) Total No. of Hospital Bookings.	3700	1467	875	64	473	929	7507
Iron responsive (Oral & Parenteral)	86 (2.3) (b)	44 (3.0)	30(3.4)	-	20(4.2)	28 (3.0)	208 (2.8)
Proven & Probable Thalassaemic	3 (a)	24 (1.6)	39 (4.5)	4	-	-	70 (0.9)
Possible Thalassaemic	-	-	2	-	-	-	2
Responding F.A. or B_{12}							
1 Megaloblastic	6	-	-	-	-	-	6 (0.08)
2 Normoblastic	8	-	1	-	-	-	9 (0.1)
Toxic	9	1	1	-	2	2	15 (0.2)
Holly's Hypoplastic	7	-	-	-	1	-	8 (0.1)
Acute blood loss	1	-	-	-	-	-	1
Insufficient data	37	18	6	2	10	10	83 (1.1)
Totals (Anaemia)	157	87	79	6	33	40	402

SUMMARY OF THE INCIDENCE OF THE ANAEMIAS OF PREGNANCY.

In this final summary some indication of the relative incidence of the various types of anaemia encountered will be covered; and the problems which are peculiar to the anaemias of pregnancy in South Australia will be outlined. In order to place in perspective some proportionate idea of the incidence of the various forms, it will be necessary to include several forms of anaemia which have not been dealt with in this thesis: for example, Holly's (1953) hypoplastic anaemia of pregnancy which is a normocytic or macrocytic anaemia of unknown aetiology, and is refractory to all known forms of therapy including iron, folic acid and vitamin B_{12}. Another group of anaemias included those of toxic origin secondary to infections such as cystitis and pyelitis, bronchiectasis, osteomyelitis and tuberculosis.

These other forms of anaemia have not been dealt with in this thesis, because it is considered that they are already well known. They are merely included for the purpose of completing the data on the comparative incidence of the various types of anaemia.

A further group of patients has been included in the analysis (Table 12) under the heading "insufficient data". The majority of these cases presented at too late a stage in pregnancy for completion of the necessary investigations and treatment. Many of them are considered to be suffering from iron deficiencies and some showed evidence of a response, but as previously explained, the intervention of the delivery of these patients rendered the assessment of the response unreliable and they have therefore been excluded. There is no evidence to suggest that this group contained any cases of megaloblastic anaemia.

When interpreting the data in Table 12 it must be appreciated that the patients therein are confined to the cases referred for

investigation, and although attempts were made to make the coverage as comprehensive as possible, some bias due to the obvious interest in the Greek and Italian patients might have arisen. It is of interest to note that the incidence of iron deficiency is shown to be higher in the Greek and Italian patients than in the comparative population of Australian birth. The incidence is even higher among patients born in the British Isles, and it is believed to be due to the failure of these patients to make good their pre-existing iron deficiency since emigration. In this respect, it has been noted during this work that the most severe cases of iron deficiency anaemia have usually been encountered in patients who have migrated from Europe within the last few years.

Although the problem of iron deficiency in pregnancy is not so great in Australia as in Europe, it still accounts for at least 50% of the anaemias of pregnancy met in the non-private patients at the Queen Victoria Maternity Hospital.

The incidence of gross folic acid deficiency is lower in Australia than in many of the more recently reported, comparative figures from the British Isles, and it is almost certain that this is also due to variation in the dietetic habits. It has been noted, however, that some cases may have mild deficiencies which may not show the classical features of a megaloblastic anaemia of pregnancy; this complicates the comparative interpretation of the various reported incidence figures for this condition.

It is obvious that the diagnostic problems imposed by the migrant populations from the Mediterranean area are now of major importance in the study of anaemias of pregnancy in South Australia.

As can be seen from the quoted figures, Thalassaemia is responsible for anaemia of pregnancy in 0.9% of the total number of

TABLE 13.

MIGRANT POPULATIONS RESIDENT IN SOUTH AUSTRALIA AND AUSTRALIA.

(Figures obtained from the Deputy Commonwealth Statistician, South Australia).

	ITALIANS				GREEKS			
	South Australia		Australia		South Australia		Australia	
	Male	Female	Male	Female	Male	Female	Male	Female.
Known to be resident 31.12. 1957	9,723	5,673	120,686	71,244	3,074	2,189	37,972	23,594
Net immigration 1958 (i.e. less emigration)	Not available		1,945	8,052	Not available		925	3,831
Known to be resident 31.12. 1958	9,723	5,673	122,631	79,296	3,074	2,189	38,897	27,425
Approx. No. of Thalassaemia Minor cases expected (a)	194	113	2,452	1,585	181	129	2,295	1,618

(a) Calculated from the percentage of proven cases in haemoglobin A_2 survey, i.e. 2.0% of Italians and 5.9% of Greeks.

It is assumed that there is no evidence of sex-linkage for Thalassaemia.

patients of all races attending the hospital over the period of this survey. There is a particularly high incidence among the Greeks.

It cannot be assumed that the relative proportion of the anaemias of pregnancy encountered in the population under study will necessarily apply to other countries, or even to other centres in Australia, as there must inevitably be some bias introduced by the areas from which the migrants come, the proportions of various nationalities in the population under examination and the dietetic habits of the patients.

Economic factors play a large part in determining the type of patients attending a particular hospital; and this has had a big influence in this investigation because the Queen Victoria Maternity Hospital attracts a relatively large proportion of the migrant population and the poorer sections of the community in general.

Figures for the migrant populations of Greek and Italian origin are shown in Table 13, and it can be seen that the numbers of them in South Australia comprise approximately 8% of the total numbers of these people in Australia as a whole. This is roughly the proportion that the total population of South Australia comprises of the total Australian population, and it would therefore seem that similar problems to those encountered in this survey must occur in the other Australian centres.

The numbers of expected thalassaemia minor cases in these people are also shown in Table 13; these figures are based upon the percentage of cases regarded as proven in the haemoglobin A_2 survey: i.e. 2.0% of the Italians and 5.9% of the Greeks. It is not suggested that this approximation can be completely accurate, but it does give some indication of the size of the problem which is being encountered. With the dissemination of these genes by intermarriage, the actual incidence in the Greek and Italian population will probably fall, but it will then become a problem of

differential diagnosis in the Australian population as a whole. That this spread has already started to take place is shown by the cases described in patients of Australian birth who may, by this time, have names of Greek, Italian, Anglo-Saxon or any other origin; so it is obvious even now that the name of the patient will be increasingly less helpful in suggesting the possible diagnosis of thalassaemia minor. Emphasis must therefore be placed on the more specific methods of laboratory diagnosis.

CONCLUSION.

The more detailed aspects of this work are dealt with in the summaries for each relevent section, and further repetition of these points seems unnecessary for the purpose of this conclusion. It will simply be stated here, therefore, how the work embodied in this thesis is considered to advance medical knowledge and practice.

This study at the outset was based upon the desire to set in proportion and to elucidate the various forms of anaemia of pregnancy occurring in South Australia. At that time thinking was largely based upon the published reports from overseas, in particular those from the United Kingdom where the iron deficiency and megaloblastic types of anaemia form a problem of great importance among the anaemias of pregnancy.

This work, which has been dominated throughout by the practical problems of diagnosis and treatment of these anaemias, has illustrated that the lessons of overseas experience are not necessarily applicable to the Australian conditions, owing to factors attributable to variations in the general dietetic habits and the migration of peoples from the Mediterranean area.

Before emphasis could be placed on the peripheral red cell indices, it was necessary to determine whether variations in the blood volume affected the reliability of these indices; and it has been shown that although there may be some slight variations due to dilution, the accuracy of blood determinations is largely effected by the parameters of body weight or surface area on which these determinations are based. It has been concluded that the peripheral haemoglobin and haematocrit values still remain the best index of the haematological status of the patient.

Evidence has been produced to suggest that many of the patients

with haemoglobin levels of less than 12.5 g. per 100 ml. of blood are suffering from a mild degree of iron deficiency. Intensive investigation has been conducted into the problems of diagnosis of mild degrees of iron deficiency in pregnancy, and it has been shown that the M.C.V. is a better parameter for its diagnosis than the M.C.H.C.

Evidence has also been produced which suggests that the serum iron level does not fall until after the onset of delay in the erythrocyte maturation which is due to iron deficiency.

The incidence of the frankly megaloblastic anaemias of pregnancy in Australia is found to be lower than the incidence recently reported from the United Kingdom; and this is attributed to the superior dietetic standards prevalent in Australia.

During this survey a major problem has been created by the interaction of thalassaemia minor with pregnancy, resulting in many additional cases in whom there were initial diagnostic difficulties. This has accounted for 18% of the total number of cases of anaemia of pregnancy seen.

For the purposes of elucidation of the difficulties of differential diagnosis of the above condition, a new method for the quantitative electrophoresis of haemoglobin A_2 has been described. It is claimed that this method possesses advantages over previous techniques in that it is comparatively easy to establish, and gives accurate and reproducible results. Extensive trials with this technique have been undertaken, and it has been shown that raised haemoglobin A_2 values have a high degree of specificity for thalassaemia minor.

Other aspects of the differential diagnosis of thalassaemia minor have been investigated, and it has been found that the specificity of the foetal haemoglobin is relatively low, in that many cases of

thalassaemia minor have low values, and raised foetal haemoglobin values are occasionally encountered in patients who are not thalassaemic.

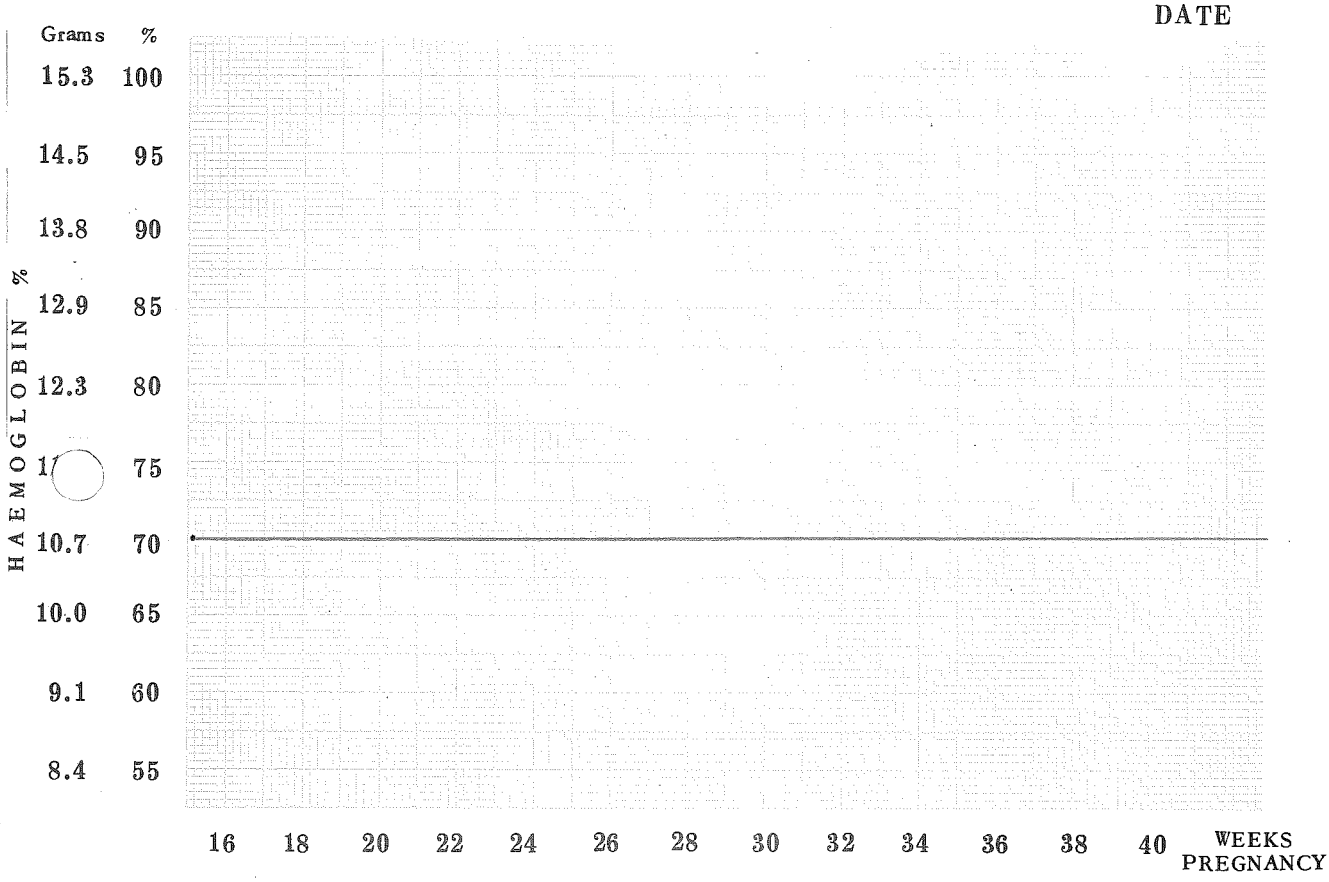
A survey of the incidence of thalassaemia minor in the Greek and Italian populations has been undertaken by the haemoglobin electrophoretic method described; and it has been found that 2% of the Italian population, and 6.8% of the Greek population, have raised haemoglobin A_2 values. Additional confirmatory evidence of thalassaemia minor has been found in the majority of these patients with raised haemoglobin A_2 values. This type of survey of the incidence of thalassaemia is believed to be unique at the time of writing. The interaction of thalassaemia with pregnancy has been intensively investigated and it has been shown that this condition usually manifests itself as an anaemia, presumably due to the increasing blood volume during pregnancy. No evidence of any comparable studies of the interaction of thalassaemia with pregnancy has been found in the literature.

I believe that thalassaemia minor now presents a major problem in the differential diagnosis of iron deficiency of pregnancy in Australia, and that the described method for the quantitation of haemoglobin A_2 has contributed to the elucidation of this problem.

HOSP. No. BIRTH PLACE DIAGNOSIS No.

NAME L.M.P. E.D.D. Delivered B.L.

DATE



INVESTIGATIONS

DATE						TYPE	DATE	RESULT
R.B.C. x 10^6						Serum I.		
Haemoglobin %						Foetal Hb.		
Haemoglobin G.						Sickling		
R.C. Diam.						Paper Elect.		
PCV						Starch "		
MCV						Bilirubin		
MCH						Occult B.		
MCHC						Urine		
Platelets								
Retics								
Leucocytes								
Icteric I.								
Aniso.								
Poik.								
Target Cells								
Polychromasia								
Punctate Bas.								

IMFERON FROM TO TOTAL DOSE Mg

B_{12} FROM TO TOTAL DOSE μg

FOLIC ACID FROM TO TOTAL DOSE Mg

APPENDIX 2

BLOOD VOLUME AND RED CELL MASS IN A SERIES OF PREGNANT PATIENTS

Case No.	Haematocrit	Total Blood Volume (ml)	Red Cell Mass ml/Kg Body Wt	Red Cell Mass ml/Kg Ideal Wt	Red Cell Mass ml/sq. metre
113	26.8	6,523	29.1	32.5	1,113
	33.7	6,997	30.0	43.0	1,474
136	29.5	5,628	29.1	33.0	1,064
	31.4	4,885	27.6	34.4	1,067
118	31.8	5,726	33.0	37.9	1,203
	36.6	5,730	36.1	43.2	1,353
I.D.	22.6	5,490	20.6	24.2	771
	33.3	5,643	30.3	36.5	1,147
131	23.7	4,503	19.7	20.3	714
	36.6	4,958	32.7	34.7	1,215
152	30.6	5,938	26.9	29.9	996
	33.6	7,099	30.6	39.3	1,239
R.O.I.	30.1	4,353	21.4	23.5	780
	34.1	5,502	27.3	30.7	1,009
124	30.9	4,854	27.3	31.8	1,007
	37.0	5,016	33.0	39.4	1,229
R.O.I.	33.1	5,131	30.9	36.4	1,152
	34.8	5,595	31.1	38.7	1,194
238 (6)	29.5	6,142	35.7	40.4	1,294
	31.0	5,110	29.8	35.4	1,112
	36.5	5,570	37.6	45.4	1,422
116	31.0	7,157	24.7	35.5	1,199
	36.6	6,475	25.8	36.9	1,224
I.D.	28.4	6,651	25.6	30.2	1,086
	31.8	7,579	34.3	38.6	1,409
262 (6)	29.2	5,001	32.4	31.0	1,035
	26.8	4,952	28.3	30.3	903

Case No.	Haematocrit	Total Blood Volume (ml)	Red Cell Mass ml/Kg Body Wt	Red Cell Mass ml/Kg Ideal Wt	Red Cell Mass ml/sq. metre
132	27.9	6,025	32.0	35.7	1,144
235	26.5	6,177	18.6	24.3	844
	35.8	6,646	26.8	35.2	1,220
129	27.0	6,466	25.0	31.3	1,021
	32.6	6,628	29.7	38.9	1,245
I.D.	29.1	6,260	27.9	32.7	1,056
	30.0	6,143	27.6	33.0	1,059
133	25.6	5,755	24.8	30.4	944
	33.1	6,419	35.0	43.8	1,345
130	31.0	5,075	28.9	31.3	1,028
	32.2	5,569	31.4	35.6	1,142
122	28.3	4,974	24.8	23.7	926
	36.0	5,127	26.8	31.1	1,119
126	26.7	5,326	25.5	28.3	917
199 (4)	32.1	5,487	26.6	29.7	1,087
H.H.	26.3	6,434	31.5	30.6	1,121
R.T.	31.1	6,469	22.2	38.3	1,032
R.O.I.	27.4	5,756	29.0	32.5	1,048
I.D.	27.6	5,787	27.9	29.7	1,037
I.D.	28.0	5,438	23.6	28.0	896
I.D.	29.5	5,804	30.1	35.3	1,112
195(4)	23.6	6,346	22.2	26.9	810
134	26.6	6,849	25.2	32.7	1,125
	37.3	5,150	30.4	34.5	1,179
I.D.	32.7	6,480	33.6	39.4	1,329
110	30.1	5,739	24.4	29.1	1,034
119	37.3	5,901	35.7	41.8	1,406
112	26.9	4,572	22.6	26.3	823

Case No.	Haematocrit	Total Blood Volume (ml)	Red Cell Mass ml/Kg Body Wt	Red Cell Mass ml/Kg Ideal Wt.	Red Cell Mass ml/sq. metre
117	29.1	5,860	27.9	35.2	1,083
R.O.I.	31.1	5,019	24.3	29.7	979
H.H.	32.4	4,689	28.1	32.9	1,062
	34.1	4,689	29.0	33.1	1,047
128	25.3	4,191	27.8	23.7	855
245 (6)	32.4	6,485	37.7	40.0	1,338
220 (5)	27.7	5,965	30.8	36.9	1,155
R.O.I.	30.0	6,241	27.2	33.6	1,061
138	28.9	8,001	30.5	35.2	1,277
I.D.	30.7	6,592	22.2	31.7	1,054
N.A.	32.9	6,571	32.9	40.2	1,314

NOTES TO APPENDIX 2

Case Numbers refer to appendix 3, with exception of figures indicated by parentheses e.g. 199 (4) indicates case no. 199 in appendix 4

R.O.I.	indicates	that the patient responded to oral iron.
I.D.	indicates	insufficient data for final diagnosis of the type of the anaemia.
H.H.	indicates	Holly's hypoplastic anaemia.
R.T.	indicates	refractory (Toxic) anaemia.
N.A.	indicates	Patients haemoglobin greater than 11.0 G/100 ml, therefore not included in anaemia survey.

APPENDIX No. 3

PATIENTS RESPONDING TO PARENTERAL IRON THERAPY

No.	Min. Hb.	M.C.V.	M.C.H.	M.C.H.C.	% RETIC.	S.I.	T.I.B.C.	% SAT.	TOTAL Hb. RISE	DAILY Hb. RISE
1	10.6	88	30.5	34	3.2	-	-	-	0.765	.022
2	9.0	70	21	30	5.0	-	-	-	1.99	.047
3	9.3	104	33	32	2.8	-	-	-	1.22	.035
4	9.3	78	22.5	29	3.4	-	-	-	1.99	.047
5	10.3	87	26.5	31	3.1	-	-	-	1.84	.065
6	10.3	98	31.5	33	2.6	-	-	-	1.38	.049
7	10.3	-	-	-	-	-	-	-	1.38	.060
8	8.5	70	21	30	1.4	-	-	-	2.45	.058
9	10.3	-	-	34	-	-	-	-	1.38	.039
10	10.0	-	-	-	-	-	-	-	1.68	.031
11	10.6	92	30	33	1.2	-	-	-	1.22	.025
12	10.3	98	31.5	32.5	-	-	-	-	1.53	.024
13	9.8	-	-	-	-	-	-	-	2.60	.031
14	10.3	-	-	-	-	-	-	-	0.92	.019
15	10.3	70	22	31.5	1.0	-	-	-	0.92	.044
16	10.8	-	-	-	-	-	-	-	0.92	.040
17	9.5	-	-	-	-	-	-	-	2.45	.066
18	10.6	-	-	-	-	-	-	-	1.22	.029
19	10.0	-	-	-	-	-	-	-	1.68	.034
20	9.3	85	24	28.5	3.6	-	-	-	1.68	.054
21	9.5	79	24	30.5	2.8	-	-	-	2.14	.051
22	10.3	82	24.5	30	0.4	-	-	-	0.76	.016
23	9.5	-	-	-	-	-	-	-	1.38	.033
24	10.0	80	26	32.5	3.4	-	-	-	2.60	.12
25	9.8	88	27.5	30.5	2.4	-	-	-	1.22	.044
26	8.8	77	22.5	29.5	9.0	-	-	-	2.60	.062
27	10.3	-	-	-	-	-	-	-	1.53	.033
28	10.0	88	28.5	32.5	2.4	-	-	-	1.38	.049
29	10.0	-	-	-	-	-	-	-	1.07	.031
30	10.3	85	26.5	31	2.1	59	-	-	1.38	.066

No.	Min. Hb.	M.C.V.	M.C.H.	M.C.H.C.	% RETIC.	S.I.	T.I.B.C.	% SAT.	TOTAL Hb. RISE	DAILY Hb. RISE
31	10.6	71	22	31.5	1.2	-	-	-	1.22	.058
32	9.7	86	26	30	2.2	53	-	-	2.45	.029
33	10.8	-	-	-	-	-	-	-	0.92	.022
34	10.6	100	33	33.5	3.4	80	-	-	1.53	.024
35	9.8	94	30	32	1.0	101	-	-	1.84	.031
36	10.0	79	23	29.5	0.02	-	-	-	1.07	.051
37	10.0	86	25	28.5	1.4	-	-	-	1.07	.022
38	9.5	92	27	30	0.04	71	-	-	3.06	.065
39	10.3	92	31	33.5	2.6	118	-	-	1.38	.034
40	9.5	85	28	33	2.0	12	-	-	1.07	.019
41	10.0	72	23.5	33	2.2	109	-	-	2.14	.051
42	10.0	88	29.5	33.5	0.2	29	-	-	2.60	.084
43	8.8	-	-	-	-	88	-	-	2.14	.058
44	9.6	-	-	-	-	56	-	-	2.60	.031
45	10.3	95	28	29.5	1.8	-	-	-	0.76	.036
46	10.3	82	27	33.5	2.4	-	-	-	1.38	.039
47	10.0	100	31	31	3.8	94	419	22	2.45	.059
48	10.8	-	-	-	-	-	-	-	1.38	.022
49	10.0	74	24.5	33.5	6.2	53	463	11	3.21	.055
50	10.3	-	-	-	-	71	431	16	2.29	.050
51	9.8	78	24.5	31.5	2.4	-	-	-	1.07	.019
52	10.6	100	33	33	0.6	-	-	-	1.84	.052
53	10.3	-	-	-	-	-	-	-	1.38	.022
54	10.8	68	22	32	1.8	-	-	-	2.45	.068
55	10.8	85	29	34.5	1.0	94	419	22	0.92	.026
56	10.6	-	-	-	-	137	387	35	1.68	.027
57	10.0	85	25	31	2.4	38	738	5.1	2.60	.062
58	9.7	88	29	33	5.2	62	532	12	2.14	.044
59	9.0	-	-	-	-	59	354	16	1.99	.026
60	10.3	88	29.5	33.5	3.2	-	-	-	0.76	.012
61	10.8	-	-	-	-	29	439	7	1.53	.061
62	10.3	96	32	33	4.8	83	378	22	1.22	.028
63	10.0	71	24	33.5	3.6	44	709	6.2	2.60	.062
64	10.3	-	-	-	-	-	-	-	1.84	.044
65	9.5	-	-	-	-	6	436	1.4	2.60	.074
66	10.5	72	23	32	1.2	35	475	7.3	2.60	.058
67	9.0	74	23.5	32	2.8	67	667	10.1	1.99	.046

No.	Min. Hb.	M.C.V.	M.C.H.	M.C.H.C.	% RETIC.	S.I.	T.I.B.C.	% SAT.	TOTAL Hb. RISE	DAILY Hb. RISE
68	10.7	97	32.5	33.5	1.2	138	553	26	1.07	.027
69	10.5	94	32	34	1.6	103	433	24	1.68	.034
70	10.7	84	28	33	5.4	61	406	15	2.75	.053
71	10.7	64	20	31.5	2.4	115	410	28	0.92	.018
72	9.8	68	21	31.5	1.4	-	-	-	1.84	.065
73	10.0	82	27	33.5	3.0	-	-	-	1.07	.031
74	10.8	93	32	33	1.0	-	-	-	1.53	.069
75	10.5	76	26.5	35	1.6	30	490	6	1.38	.049
76	8.3	65	18	27.5	3.0	6	386	1.6	3.67	.066
77	10.3	-	-	-	-	-	-	-	1.84	.037
78	10.7	106	34	32.5	2.2	-	-	-	0.92	.016
79	10.6	92	30	33	5.4	-	-	-	0.76	.019
80	10.0	84	29	34.5	3.2	97	-	-	0.92	.016
81	10.7	98	35	36	2.4	-	-	-	1.53	.035
82	10.7	104	33.5	32	3.6	109	409	27	1.99	.040
83	10.7	92	31	32	2.4	-	-	-	0.76	.016
84	10.7	106	34	31.5	5.6	-	-	-	1.53	.023
85	10.5	92	31	34	3.0	-	-	-	0.92	.021
86	10.2	86	30	35	3.0	-	-	-	2.60	.039
87	10.7	89	28.5	31.5	4.0	-	-	-	1.22	.022
88	10.2	94	31	33	6.2	-	-	-	2.60	.093
89	10.0	84	28	33	0.8	-	-	-	1.99	.024
90	10.0	94	31	33.5	3.8	-	-	-	1.99	.025
91	10.7	92	31	34	2.8	-	-	-	2.29	.065
92	10.5	82	26	32	4.6	-	-	-	0.92	.024
93	10.7	77	27.5	35.5	3.6	-	-	-	0.92	.019
94	10.5	76	23.5	31	2.8	-	-	-	2.45	.050
95	10.5	76	28.5	31.5	4.2	-	-	-	1.22	.029
96	10.7	80	26	32	1.4	-	-	-	2.29	.062
97	10.2	82	27	33	4.8	-	-	-	2.29	.082
98	10.2	94	30	32	3.0	-	-	-	1.99	.033
99	10.7	88	31	34	2.2	-	-	-	1.84	.035
100	10.2	82	27	33	4.6	-	-	-	2.14	.038
101	10.2	65	21.5	33	4.0	-	-	-	0.76	.022
102	10.7	82	26	32	2.2	-	-	-	1.84	.052
103	10.5	95	29	31	2.2	-	-	-	1.99	.057
104	10.5	100	33	33	3.2	-	-	-	1.99	.030

No.	Min. Hb.	M.C.V.	M.C.H.	M.C.H.C.	% RETIC.	S.I.	T.I.B.C.	% SAT.	TOTAL Hb. RISE	DAILY Hb. RISE
105	10.5	90	29	33.	5.2	-	-	-	1.68	.040
106	10.7	104	35	33	4.0	-	-	-	1.53	.044
107	10.4	72	24	33	4.0	-	-	-	0.76	.020
108	10.7	106	33.5	32	5.2	-	-	-	1.53	.033
109	10.5	104	34	34	7.2	-	-	-	0.92	.016
110	10.0	88	29	32	3.2	-	-	-	0.76	.022
111	10.7	92	30	32.5	3.8	95	470	20	1.53	.036
112	9.0	72	22.5	31	5.2	11	575	2	2.14	.061
113	9.5	68	21.5	31.5	1.2	-	-	-	1.84	.044
114	10.5	82	27	33	5.2	-	-	-	2.45	.039
115	9.0	70	21	30	4.8	-	-	-	1.38	.076
116	10.7	74	25	33.5	3.0	-	-	-	1.22	.035
117	9.2	79	24	31	1.4	42	-	-	1.53	.036
118	10.7	95	31	31.5	4.4	-	-	-	1.84	.044
119	7.0	60	17.5	29	5.0	16	576	3	2.4	.057
120	10.5	82	25	31	2.6	-	-	-	1.38	.016
121	10.5	72	23	31.5	4.0	31	-	-	1.68	.065
122	9.6	80	25	32	4.2	39	-	-	2.75	.086
123	10.3	100	31	31	5.2	-	-	-	1.99	.047
124	10.7	92	31	33	3.2	-	-	-	1.07	.025
125	10.5	110	38	34	3.4	-	-	-	1.68	.032
126	9.7	104	35	33.5	1.6	-	-	-	0.76	.012
127	10.5	94	31	34	5.8	-	-	-	0.92	.019
128	9.5	100	31	31.5	2.4	-	-	-	0.76	.014
129	10.7	98	31.5	32	2.6	41	-	-	0.92	.016
130	10.7	98	31	31	1.2	92	-	-	0.76	.016
131	7.0	70	16	23.5	4.0	-	-	-	5.91	.098
132	9.5	74	21	31	3.8	-	-	-	3.21	.092
133	9.2	66	21	31	3.0	-	-	-	1.84	.047
134	8.0	68	18	27	3.0	-	-	-	3.67	.193
135	10.0	63	19.5	31	2.6	14	589	2	2.29	.057
136	10.5	87	27.5	32	2.0	-	-	-	2.14	.063
137	10.5	89	29.5	33	3.8	127	-	-	0.92	.029
138	8.7	68	20	30	2.4	19	-	-	2.45	.047
139	9.0	83	26.5	32	2.4	29	-	-	1.99	.057
140	10.0	92	27.5	31	2.4	-	-	-	2.75	.079
141	10.7	84	29	34.5	1.3	97	-	-	1.84	.073

No.	Min. Hb.	M.C.V.	M.C.H.	M.C.H.C.	% RETIC.	S.I.	T.I.B.C.	% SAT.	TOTAL Hb. RISE	DAILY Hb. RISE
142	10.5	81	26.5	33	2.4	-	-	-	2.60	.046
143	10.5	89	29.5	33	3.6	-	-	-	2.29	.066
144	9.0	75	22.5	30	3.8	-	-	-	2.60	.124
145	10.0	86	29	33	1.8	-	-	-	0.32	.076
146	10.7	82	26	32	0.9	29	464	6	0.76	.022
147	10.2	72	21	29.5	4.1	32	-	-	2.29	.027
148	10.5	81	25.5	33	3.4	68	-	-	0.76	.028
149	10.7	92	29	31.5	2.4	-	-	-	0.76	.022
150	10.0	96	30	31	3.8	-	-	-	2.68	.076
151	11.2	84	27	32	4.4	27	582	4.6	2.29	.082
152	10.7	68	23.5	34	3.6	-	-	-	1.22	.029
153	9.2	84	25.5	30.5	2.2	59	634	9	2.45	.070
154	10.0	77	24	31.5	1.6	-	-	-	1.68	.060
155	9.5	79	24.5	31	1.8	35	435	8	2.60	.074
156	10.5	86	28.5	32	1.2	127	367	35	1.38	.039
157	10.5	86	28	32	1.0	103	-	-	1.53	.055
158	10.7	87	28.5	32.5	4.6	47	-	-	2.45	.047

Figures quoted for the red cell indices, etc. are pretreatment values.

APPENDIX 4

THALASSAEMIC CASES DIAGNOSED DURING THE GENERAL SURVEY OF ANAEMIAS OF PREGNANCY.

No.	Origin	Min. Hb.	M.C.V.	M.C.H.	M.C.H.C.	Retic.	A_2	F	S.I.	T.I.B.C.	% SAT.	% Fragil.	Parent.	Iron
159	Gk.	7.2	76	24	28	5.8	-	-	150	380	40	R	-	
160	Gk.	9.8	67	21.5	31.5	2.3	++C	4.2	-	-	-	-	yes	
161	Gk.	9.3	71	23	31	1.1	++C	0.0	-	-	-	-	yes	
162	It.	9.3	69	21	31	1.9	-	4.0	194	464	42	-	yes	
163	Gk.	8.7	67	19.5	29	5.4	+++C	6.9	-	-	-	-	-	
164 (d)	Gk.	9.8	72	22	30	3.8	Neg. C	4.0	-	-	-	-	yes	
165	Gk.	9.5	74	24	32	3.1	++I	2.4	-	-	-	-	yes	
166	It.	9.4	75	22.5	30	-	-	5.0	-	-	-	-	-	
167	Gk.	10.3	73	22	30.5	3.7	+C	0.4	77	-	-	-	yes	
168 (e)	It.	10.0	68	20	29.5	1.8	+++I	1.7	-	-	-	-	-	
169	Cyp.	9.6	64	18.5	28.5	3.1	+I	1.0	115	415	28	R	yes	
170	Gk.	9.8	77	24	31	3.9	-	5.5	150	-	-	-	yes	
171 (f)	Gk.	10.6	68	21.5	31.5	5.6	Neg. C	1.0	-	-	-	-	yes	
172	It.	8.7	70	21	29	5.6	++I	1.6	80	-	-	-	yes	
173 (a)	Gk.	8.6	70	21.5	31	1.4	-	0.4	77	-	-	-	yes	
174 (M)	It.	9.8	62	19.5	31	7.4	++I	1.3	56	-	-	-	-	
175	Gk.	9.5	67	20.5	31	2.1	-	3.7	80	-	-	-	yes	
176	Cyp.	10.2	73	23	31	3.6	++I	0.0	50	-	-	-	yes	
177	It.	9.6	77	23	31	1.6	+++I	2.7	118	408	29	-	yes	
178	It.	10.6	72	22	31	5.0	++I	0.8	112	312	36	-	yes	
179 (L)	Gk.	8.5	71	20.5	30.5	4.6	+++I	3.7	74	299	25	-	-	
180	It.	9.5	65	20	31	4.7	++I	1.6	185	375	49	-	yes	
181	It.	10.7	74	25	31.5	5.6	++I	0.8	235	490	48	-	yes	
182 (g)	It.	10.6	63	20	31.5	4.4	+++I	0.0	100	440	23	-	yes	
183	Gk.	10.7	78	23.5	30	2.1	-	1.2	112	-	-	-	yes	
184	It.	10.6	76	24	32	5.9	8.7	0.0	132	492	26	-	yes	
185	It.	10.0	68	21	30.5	3.7	-	0.6	53	443	15	-	yes	
186	It.	9.7	73	26	31	3.2	7.9	0.5	82	472	17	-	yes	
187 (h)	It.	10.0	77	25	31	4.7	-	1.9	200	515	39	-	yes	

No.	Origin	Min. Hb.	M.C.V.	M.C.H.	M.C.H.C.	RETIC.	A_2	F	S.I.	T.I.B.C.	% SAT.	% Fragil.	Parent. Iron
188	Gk.	10.0	79	25	31	3.2	8.6	-	115	370	31	-	yes
189 (e)	Gk.	9.6	67	24	30	2.3	7.5	2.0	156	431	36	-	yes
190	Gk.	10.0	63	20	32	5.8	-	-	120	505	24	-	yes
191 (b)	Gk.	9.6	68	20	29	3.7	6.1	1.7	178	393	45	-	yes
192	It.	8.7	70	22	31	2.4	-	0.0	103	498	21	-	yes
193	Gk.	9.5	72	22.5	31	6.0	8.5	0.7	121	396	31	-	-
194	Gk.	10.7	68	20.5	30	2.8	4.7	2.3	111	481	23	-	yes
195	Gk.	8.7	73	21	30	4.0	7.2	5.3	114	344	33	97	-
196 (K1)	Gk.	10.7	70	21	30.5	3.6	6.5	1.4	-	-	-	95	-
197 (K2)	A.It.	10.7	71	22	30.5	3.1	5.6	1.2	83	-	-	-	-
198 (f)	Gk.	11.4	-	-	-	-	6.4	4.0	-	-	-	88	-
199 (i)	A.Gk.	10.7	73	23	31	3.7	5.2	6.4	144	529	27	72	-
200 (j)	A.It.	10.0	75	23	31	3.7	6.3	1.5	90	360	25	-	-

APPENDIX 5

PATIENTS WITH RAISED HAEMOGLOBIN A_2 VALUES FOUND AS A RESULT OF THE HAEMOGLOBIN ELECTROPHORESIS SURVEY

No.	Origin	Min. Hb.	M.C.V.	M.C.H	M.C.H.C.	RETIC.	A_2	F	S.I.	T.I.B.C.	% SAT.	% Fragil.	Parent. Iron
201	Gk.	10.0	69	21	30	2.9	4.6	0.4	80	-	-	79	-
202	Gk.	11.7	83	25.5	30.5	3.4	5.3	1.1	-	-	-	100	-
203	Gk.	9.5	81	24	31	2.8	5.5	7.3	-	-	-	83	-
204	It.	10.5	70	21	30	6.4	5.6	2.6	167	-	-	-	-
205	It.	10.0	64	21	31	4.4	5.5	0.7	-	-	-	90	-
206	Gk.	10.2	73	21.5	30	3.0	6.0	1.3	-	-	-	97	-
207	Gk.	10.0	87	32	35	3.8	6.0	-	-	-	-	-	yes
208 (g)	Gk.	10.7	77	23	30.5	1.8	5.8	1.8	-	-	-	-	-
209 (c)	It.	9.7	70	21	29	6.3	5.8	1.9	156	-	-	88	-
210 (d)	Gk.	10.5	73	22	30.5	4.0	6.2	2.2	112	-	-	85	-
211	Gk.	11.6	-	-	-	-	7.4	-	-	-	-	-	-
212	Gk.	10.0	69	21.5	31.0	1.3	7.7	-	125	-	-	78	-
213 (c)	Gk.	10.5	80	24	30	3.1	7.4	3.3	127	-	-	69	-
214	Gk.	9.5	66	20.5	31.0	6.1	6.4	1.1	106	-	-	75	-
215	Gk.	10.8	85	26	30	4.0	6.5	1.2	105	365	29	82	-
216	Gk.	9.7	84	25	29.5	3.0	6.4	2.7	-	-	-	66	yes
217 (h)	It.	10.2	76	23	31	3.9	6.4	2.7	-	-	-	92	yes
218	Gk.	9.2	85	26.5	30	5.7	5.8	0.7	-	-	-	70	-
219	It.	10.0	69	21	30	6.8	5.5	1.4	126	-	-	88	-
220	Cyp.	9.7	83	25.5	30.5	2.7	5.3	2.0	95	-	-	93	yes
221	It.	10.0	74	22	30	4.0	6.2	0.8	265	265	100	86	-
222	Gk.	10.0	68	20.5	30.5	3.2	5.1	1.5	-	-	-	67	-
223	It.	10.7	67	21	31	4.1	6.3	0.7	332	582	57	54	-
224	Gk.	10.7	82	26	31	3.6	6.3	0.2	144	594	24	86	-
225	It.	10.5	77	24	30.5	4.7	5.9	7.4	112	-	-	92	-
226	Cyp.	9.5	77	23	30	5.7	4.8	0.9	-	-	-	93	-
227	Gk.	10.2	70	21	30	6.5	5.9	1.0	-	-	-	89	-
228	Gk.	9.7	64	19	30	1.7	5.8	2.0	144	-	-	85	-

No.	Origin	Min. Hb.	M.C.V.	M.C.H.	M.C.H.C.	RETIC. %	A_2	F	S.I.	T.I.B.C.	% SAT.	% Freeil.	Parent. Iron
229	It.	10.2	69	21.5	31	4.0	5.5	1.4	235	395	60	96	-
230	Gk.	10.5	74	24	32	6.3	5.0	1.9	144	324	44	94	-
231	Gk.	11.7	82	25.5	31	2.1	6.6	1.7	-	-	-	89	-
232	Gk.	12.4	-	-	-	-	5.1	-	-	-	-	-	-

4 cases in this series were given parenteral iron by the patients own physician

Notes

The values quoted for the M.C.V., M.C.H., M.C.H.C., Retics, A_2, F and Fragilities are the mean values for all estimations made during the pregnancy.

Case No.	Symbol	Meaning
	+	Raised A_2 value by semiquantitative starch gel electrophoresis.
	NEG.	Normal A_2 value by semiquantitative starch gel (& Tiselius) electrophoresis.
	C	Starch gel electrophoresis carried out by Dr. Curtain.
	I	Starch gel electrophoresis carried out by [illegible]. Ibbotson.
	R	Resistance to osmotic fragility (quantitative method) Figure 26.
173	a	Thalassaemia major child.
191	b	Thalassaemia major child in previous pregnancy.
189	c	Subsequent pregnancy in Case no. 213 appendix 5.
164	d	Subsequent pregnancy in Case no. 210 appendix 5.
168	e	Subsequent pregnancy in Case no. 209 appendix 5.
171	f	Subsequent pregnancy in Case no. 198 appendix 4.
182	g	Subsequent pregnancy in Case no. 208 appendix 5.
187	h	Subsequent pregnancy in Case no. 217 appendix 5.
199	i	Australian birth of Greek parents.
200	j	Australian birth, adopted child, father believed to be an Italian.
196	k_1	Blood not taken at first visit to antenatal clinic.
197	k_2	Blood not taken at first visit to antenatal clinic, Australian birth.
179	L	Serum iron taken at 20 weeks gestation.
174	M	Miscarriage at 19 weeks gestation.

For the analysis of incidence figures, each pregnancy is regarded separately. Many of the non-thalassaemic cases also had repeat pregnancies and the figures are therefore regarded as comparable.

APPENDIX 6

PATIENTS RESPONDING TO FOLIC ACID OR B_{12} THERAPY

No.	MIN. Hb.	M.C.V.	M.C.H.C.	% RETIC.	S.I.	T.I.B.C.	% SAT.	MARROW APPEARANCE	DAILY Hb. RISE	MAX. RETIC. COUNT
233 (N)	8.2	93	32.5	1.8	-	-	-	Megaloblastic	(T)	8.2
234 (O)	8.5	100	34	0.1	62	382	16	Megaloblastic	-	24.4
235 (P)	9.5	104	34.5	0.4	-	-	-	Megaloblastic	.092	16.4
236 (Q)	8.5	85	37	0.6	-	-	-	Megaloblastic	(T)	10.4
237 (R)	9.2	104	35	1.0	232	232	100	Megaloblastic	.077	17.0
238 (S)	11.0	118	32	1.2	22	427	5	(S)	.052	13.2
239	9.8	96	30	1.2	-	-	-	Normoblastic	.017	5.2
240	9.8	100	35	1.2	98	383	26	Normoblastic	.020	-
241	10.0	87	33.5	3.6	-	-	-	Normoblastic	.049	-
242	10.6	94	33.5	0.8	-	-	-	Normoblastic	.044	6.8
243	10.5	110	35	3.0	-	-	-	Normoblastic	.027	5.0
244	11.0	100	33	3.4	-	-	-	-	.036	13.2
245	10.7	97	32	5.2	-	-	-	Normoblastic	.020	-
246	10.3	90	33	3.2	203	-	-	-	.026	-
247	10.0	79	31	6.6	125	-	-	-	.036	-

NOTES

Figures quoted for the M.C.V., M.C.H., M.C.H.C. and % RETIC. are pretreatment values.
(T) indicates transfusion.

Case No. 238 (S) Partial gastrectomy 1956, Transitional megaloblasts in the marrow smears. Patient included in calculation of the incidence of megaloblastosis.

233 (N) Aboriginal patient.
234 (O) Hyperemesis gravidarum; partial response to F.A.; response to B_{12} therapy.
235 (P) Aboriginal patient.
236 (Q) Erythroblastosis foetalis.
237 (R) On dilantin therapy for epilepsy.

The Figures for the M.C.H. have been omitted from this Appendix owing to limitation of space.

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PUBLICATIONS.

A paper dealing with the quantitative determination of haemoglobin A_2 has been published by the Journal of Clinical Pathology. A copy of this paper is included within the binding of this thesis, and abstracts from the paper are inserted where they are relevant to the general discussion.

It is anticipated that further papers, based on some of the data included herein, will be submitted for publication.

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QUANTITATIVE DETERMINATION OF HAEMOGLOBIN A_2 USING PAPER ELECTROPHORESIS

BY

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