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FERRO - KINETIC STUDIES IN A VARIETY OF HAEMATOLOGICAL DISORDERS, ACUTE PORPHYRIA AND SCURVY.

A THESIS PRESENTED TO THE FACULTY OF MEDICINE, UNIVERSITY OF THE WITWATERSRAND, IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF MEDICINE.

BY

SIDNEY KRAMER.

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TO MY WIFE

AND

CHILDREN

PREFACE

The work on which this thesis is based was carried out in the Department of Medicine at the Witwatersrand University from 1958 to 1960 while I was a tutorial registrar. I wish to acknowledge the considerable help and guidance given to me by Dr. T. H. Bothwell, who first inspired me to take an interest in the field of iron metabolism. Throughout I received constant encouragement from Professor G. A. Elliot. To them I owe much.

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Finally I would like to acknowledge that in Chapter II, Fig. 1, 2 and 3, were taken from the following articles:

Fig. 1. Rimington C. (1959), Brit. Med. Bull. 15, 19.

Fig. 2. Gray, C. H. (1954-5), The Chemistry of the Porphyrias. Brit. Postgraduate Medical Federation. Lectures on the Scientific Basis of Medicine, IV. p. 74.

Fig. 3. Waldenstrom J. (1957), Amer. J. Med. 22, 758.

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C H A P T E R I

INTRODUCTION

INTRODUCTION

Since changes occur in the size, shape and haemoglobin content of red cells in disease a classification of the anaemias based on the morphology of the red cell has been widely used (Wintrobe, 1956). While such a classification has a limited usefulness from the diagnostic and therapeutic approach it has two serious defects. Firstly, there is a large group of anaemias which are morphologically indistinguishable from the normal, both having normochromic normocytic red cells. Secondly, a descriptive classification gives little insight into the mechanism of production of a particular anaemia. When the red cell was found to have a finite life span it was realised that with the exception of blood loss all anaemias could be explained on an imbalance between production and destruction of red cells and a variety of tests were evolved to assess both of these processes.

The indices which have been used to determine the rate of red cell destruction are the determination of the red cell life span and the measurement of daily stool urobilinogen excretion. Reticulocyte counts, estimation of erythroid-myeloid ratio, plasma iron turnover and the rate of incorporation of radio-iron into haemoglobin have been employed to measure the rate of red cell production (Giblett et al, 1956).

RED CELL LIFE SPAN

The Ashby technique of differential agglutination has established the normal erythrocyte life span as 100-120 days (Mollison, 1951). Compatible cells which are nevertheless of a different blood group are transfused into the recipient. In samples of blood taken at intervals from the recipient the number of transfused red cells can be counted after agglutinating out the red cells of the recipient. The slope of the graph so obtained gives the life span of the cells. This method has been used for the study in normal subjects of the survival of red cells with an intrinsic defect and the survival of normal cells in patients with a random haemolytic process (Mollison, 1951). It is, however, impossible to utilise this technique to study the survival of the patient's cells in his own circulation. Radioisotopes (^{51}Cr ^{59}Fe) can be employed for the study of the survival of the subject's own red cells in his circulation. Using ^{59}Fe, however, there is the problem of re-utilisation (Finch et al, 1949a) and when ^{51}Cr is employed there is the problem of elution, i.e. when normal cells are labelled with ^{51}Cr the rate of disappearance of the ^{51}Cr (allowing for radioactive decay) is faster than the known rate of disappearance of the cells. There is elution of ^{51}Cr from the red cells (Ebaugh et al, 1953).

UROBILINOGEN

In normal subjects Heilmeyer and Oetzel (1931) and Watson (1936) reported a daily excretion of urobilinogen in the faeces of approximately 148mg per day and 180mg per day respectively.

The porphyrin moiety exclusive of iron makes up 3.5 per cent by weight of the haemoglobin molecule (Wintrobe, 1956). Since protoporphyrin and bilirubin have practically the same molecular weight, destruction of one gram of haemoglobin should make available 35mg of bilirubin. On an average 6.25G of haemoglobin are destroyed daily, so 220mg of bilirubin will be formed. The amount actually recovered (148 - 180mg) is only about three-quarters of the total pigment produced. Furthermore, at least 11 per cent of the total stool urobilinogen is not related to red cell destruction (London et al, 1950); therefore the amount of pigment recovered represents less than 2/3 of that produced by red cell destruction.

While urobilin excretion is useful in detecting increased rates of blood destruction values frequently exceed other measurements of rate of red cell breakdown (Giblett et al, 1956). Also, low values are occasionally found in patients who undoubtedly have a haemolytic anaemia (Giblett et al, 1956). Low values are found in patients to whom antibiotics have been administered (Sborov et al, 1951). It is presumed that antibiotics may so alter the bacterial flora that for several weeks there may be low urobilinogen excretion. There are thus many features which limit the use of urobilinogen as a quantitative index of blood destruction.

ERYTHROID : YELOID RATIO

Examination of the marrow appears to provide a good indication of erythropoiesis. In practice erythroid elements

are compared with myelocytic elements, which are assumed to be normal when the circulating neutrophil count is within normal limits. Several factors which were particularly applicable to this thesis detracted from the value of the erythroid myeloid ratio as a measure of erythropoiesis.

(1) In a variety of conditions to be studied, e.g.scurvy, there are moderate changes in the peripheral neutrophil count (Vilter et al, 1946).

(2) Measurements when there is marrow infiltration by foreign cells cannot be considered valid.

(3) Technical difficulties and counting errors arise when marrow particles are diluted by peripheral blood.

RETICULOCYTE COUNT

The merit of the reticulocyte count is its simplicity. This index can be a measure both of red cell destruction and production. If the patient is in red cell equilibrium it may be a quantitative expression of the rate of red cell destruction when expressed as a percentage of the circulating red cells (Giblett et al, 1956). To assess the rate of red cell production the absolute reticulocyte count, or the number of reticulocytes expressed as a percentage of the normal red cell count, must be employed (Giblett et al, 1956), e.g. the normal number of reticulocytes is 1.3 per cent of 5,000,000 red cells per cubic millimeter of blood = 65,000 reticulocytes. If a patient has a 3 per cent reticulocyte count with a red cell count of 2,500,000 he will have 75,000 reticulocytes, i.e. his production is just slightly greater than normal. A reticulocytosis of 3 per cent of 2,500,000 red cell blood cells "corrected" is

1.5 per cent for a normal red cell count.

There are several drawbacks to the use of the reticulocyte count as a measure of erythropoiesis.

(1) Appreciable fluctuations occur in the daily reticulocyte count in patients with haemolytic anaemia (Giblett et al 1956). In the absence of changes in other measurements of red cell production or destruction, these fluctuations are thought to result from the variable release of young reticulocytes into the peripheral blood from the large reticulocyte pool demonstrated in the marrow (Heir,1953; Heiff et al, 1958).

(2) Uncompensated haemolytic anaemias show a disproportionately high reticulocyte count when the rate of haemolysis is measured by other indices, and conversely in pernicious anaemia low estimates of blood production (as measured by the reticulocyte count) are found in comparison with the erythroid myeloid ratio (Giblett et al, 1956).

PLASMA IRON TURNOVER AND RED CELL UTILISATION

In the normal adult male the total body iron amounts to between 4 and 5 grams (Wintrobe, 1956). In the plasma iron is in transit from the reticulo-endothelial system to the erythropoietic tissue and circulates as a complex with beta-1-globulin (Schade and Caroline, 1946). This fraction of iron, however, constitutes only 0.1 per cent of the total body iron (3 mg.). Hence for reliable tracer studies it is essential to use radio-iron of high specific activity. In the earlier studies the amount of radio-iron given was not tracer (Greenberg et al, 1947; Finch et al, 1949b) and significantly altered the total amount of plasma iron. When these studies were repeated with true tracer doses, different results were obtained and different

conclusions drawn (Bush et al, 1956).

The intravenous iron turnover test as a specific diagnostic aid for the study of haemoglobin production was first described by Huff et al in 1950. He found that in normal subjects when plasma is labelled with radioactive iron of high specific activity there is an exponential disappearance of the radio-iron from the plasma with a half time of about 90minutes. Such a curve plotted on semi-logarithmic paper will give a straight line. This is known as the radio-iron plasma clearance rate.

Improved counting techniques over longer periods of time have shown that some of the points do not lie on a straight line. The line obtained may be approximated by the sum of two or more exponential functions and various mathematical models have been constructed for their refined interpretation (Sharney et al, 1954; Pollycove, 1958). In most series, however, (Giblett et al, 1956; Bothwell et al, 1957) 80 per cent of the radioactivity is cleared as a straight line and such mathematical models are not essential. Loeffler (1956), in a series of 700 cases, found very few disappearance rates which were not almost straight lines.

The measure of the radio-iron plasma clearance rate alone has been employed to characterise erythropoiesis (Wasserman et al,1952; Wetherley-Mein et al, 1956). While it is true that the clearance rate is usually lengthened in hypoplastic anaemias and accelerated with increased erythropoiesis, it is also dependent on the level of plasma iron (Bothwell et al, 1957); the higher the serum iron, the slower the rate of clearance. Hence, for the correct analyses

of Plasma iron clearance rates, plasma iron turnovers were calculated from the rate of disappearance of radioactivity from the plasma and the concentration of the plasma iron (Huff et al, 1950; Bothwell et al, 1956; Bothwell et al, 1957; Bush et al, 1956). This is a semi-quantitative measure of the amount of iron passing through the plasma. In normal subjects 30 - 40 mg. of iron are cleared through the plasma per day. As the plasma contains only 3 - 4 mg. of iron at any one time, this has to be replaced ten times a day. Since 21mg of iron are required by the marrow, it could be anticipated that the plasma iron turnover is predominantly related to haemoglobin synthesis. That this is so is confirmed by counting at numerous sites over the body after the intravenous administration of radioiron; maximum uptake occurs over the marrow and subsequently 70 - 90 per cent of the radioactive iron reappears in the red cells (Elminger, 1953; Loeffler, 1956).

In the interpretation of changes in the plasma iron turnover it is important to know to what extent the plasma iron turnover may be influenced by changes in the iron metabolic cycle. Iron is transported by the plasma from the reticulo-endothelial system, gut and stores for utilisation by the marrow and tissues. Iron is poorly excreted and excess iron is stored. Iron is released from the haemoglobin molecule of effete red cells in the reticulo-endothelial system. This completes the iron metabolic cycle. (Fig. 1, p. 8)

IRON METABOLIC CYCLE

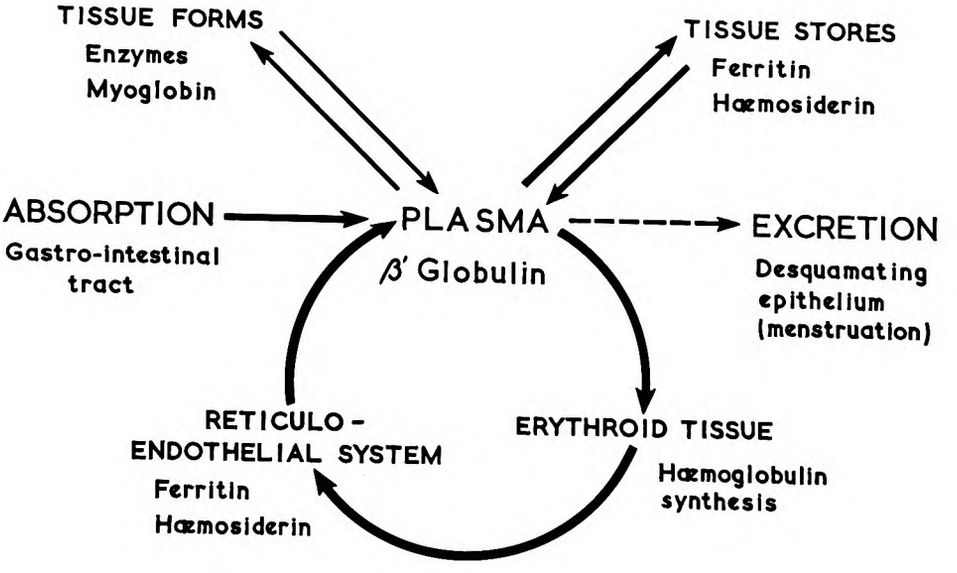


Figure 1 - Iron Metabolic Cycle

THE EFFECT OF VARIOUS CHANGES IN THE IRON METABOLIC CYCLE ON THE PLASMA IRON TURNOVER.

1. Effect of increased red cell destruction without any change in red cell production.

Bothwell et al (1957) injected non-viable, but compatible red cells into one dog and two normal volunteers. Preliminary studies had shown that 50 per cent of blood stored in citrate for 21 days disappeared from the circulation within two hours of transfusion. While the iron released from haemoglobin breakdown caused a prompt rise in serum iron, the plasma iron clearance rate decreased; the result was no appreciable alteration in the plasma iron turnover. It was apparent that red cell destruction did not significantly alter the plasma iron turnover.

2. Effect of increased body iron stores on the plasma iron turnover.

To assess this, patients with increased body iron stores (haemochromatosis) were studied (Bothwell et al, 1957). A mean increase in the plasma iron turnover of 67 per cent was noted. It was concluded that excess body iron stores could alter the plasma iron turnover, but only to a moderate degree.

3. The effect of changes in marrow activity on the plasma iron turnover.

The most marked changes in the plasma iron turnover result from changes in marrow activity. Experimentally stimulation of the marrow by blood loss increases the plasma iron turnover three times normal (Bothwell et al, 1957; Finch et al, 1959). This is confirmed by clinical experience (Loeffler, 1956).

Stimulation of the marrow over a long period increases the plasma iron turnover six times normal, e.g. congenital spherocytic anaemia, while depression of marrow experimentally (blood transfusions, exposure to whole body irradiation), or clinically, results in a moderate decrease in the plasma iron turnover (Bothwell et al, 1957).

4. Effect of iron absorption on the plasma iron turnover.

Iron absorption amounts to 1-1.5 milligrams a day (Callender, 1959). Since 30 - 40mg of iron are transported through the plasma a day, 1-1.5mg can only affect the plasma iron turnover to a slight degree. With the possible exception of iron deficiency anaemia, increased absorption could only have a slight effect on the plasma iron turnover, since increased absorption is broughtabout predominantly by increased marrow activity and the effect of increased erythropoiesis on the plasma iron turnover would mask any effect brought about by the increased iron absorption.

Because of all the above findings it has been concluded (Bothwell et al, 1957):-

i) that changes in the plasma iron turnover are predominantly related to marrow activity

ii) that increased body iron stores increase the plasma iron turnover only to a moderate degree ($\pm \frac{1}{3}$ times normal).

One of the significant advantages of the plasma iron turnover is that it is a rapid method of measuring marrow activity and furthermore changes in erythropoiesis are immediately reflected by changes in the plasma iron turnover (Loeffler, 1956). This

has proved of value in assessing the effects of different doses of radiation and cytotoxic drugs on the marrow. In polycythemia vera, if therapy is followed by a normal plasma iron turnover, it could be anticipated that the treatment has been adequate and that the haemoglobin will fall. If the plasma iron turnover is still increased, or returns to the previous raised level, therapy has been inadequate or relapse is imminent.

While the plasma iron turnover is not a reliable quantitative measure of red cell production, the method is clearly able to differentiate between blood diseases associated with varying degrees of erythroid hyperplasia from those in which there is erythroid hypoplasia (Bothwell et al, 1957).

PERCENTAGE RED CELL UTILISATION OF INTRAVENOUSLY INJECTED RADIOIRON.

The iron removed from the plasma by erythropoietic tissue is rapidly incorporated into haemoglobin and released back to the circulation in the red blood cells; 80 - 90 per cent of the iron cleared from the plasma is so utilised, the other 5 - 20 per cent going presumably to tissue systems or stores (Loeffler, 1956). The radioiron reappears in the circulation after the intravenous injection in as little as 3-12 hours and maximum reappearance occurs in 5-10 days.

From the plasma iron turnover and the percentage incorporated into red cells, various formulae have been calculated (Huff et al, 1950; Loeffler, 1956). However, there are certain limitations and errors in the calculation of the

percentage red cell utilisation of iron, and when there is rapid and random destruction of red cells, as in congenital spherocytosis, the sequestration of newly released radioactive erythrocytes will lower the percentage red cell utilisation (Bothwell et al, 1956).

DEGREE OF CORRELATION OF THE VARIOUS INDICES OF BLOOD FORMATION AND DESTRUCTION.

In the majority of anaemias in a state of haematological equilibrium, there is a good quantitative correlation between the various indices of blood formation and destruction. However, in Thalassemia major (Sturgeon and Finch, 1957), Thalassemia minor (Pearson et al, 1960), Addisonian anaemia (Finch et al, 1956) and in tropical sprue (Sheehy, 1960) there is a considerable increase in the plasma iron turnover, associated with marked erythroid hyperplasia and elevated faecal urobilinogen excretion. These signs of increased marrow activity are out of proportion to the rate of blood formation as judged by reticulocyte counts or red cell utilisation of iron and to the rate of red cell destruction as measured by survival studies. Such findings are taken to indicate a hyperactive marrow with failure of production of viable cells, i.e. defective cells are both produced and destroyed in the marrow and as a result a proportion of the plasma iron turnover is ineffective (Finch et al, 1956). The plasma iron turnover, like the erythroid myeloid ratio and urobilinogen excretion, measures total haemoglobin production. Coupled with a reticulocyte count or with the determination of red cell

utilisation of radioiron, it is possible to distinguish between the high plasma iron turnover of effective from ineffective erythropoiesis (Giblett et al, 1956).

OBJECTIVES OF THE THESIS

In the last ten years a considerable number of plasma iron turnover studies have been carried out and it has become apparent that the plasma iron turnover and the percentage red cell utilisation in association with the reticulocyte count, are particularly useful tools in the measurement of blood formation. These methods have not been applied to the study of blood disorders in South Africa. It therefore seemed worth-while to apply these measurements to patients suffering from a variety of blood disorders, including - iron deficiency anaemia, haemolytic anaemias, the anaemia of reticuloses and carcinoma and in polycythemia. A second objective was the collection of ferrokinetic data in white and non-white (Bantu) subjects with "Hepatic Porphyria", and on subjects with scurvy - still a common disease in South Africa. In neither of these conditions have ferrokinetic studies been carried out. It was decided to study iron metabolism in the porphyric group because of a chance observation that the plasma iron level is raised during acute episodes, which suggested that some derangement in iron metabolism may occur in this phase.

In scurvy, the position is equally obscure. Theoretically it seems probable that ascorbic acid through its reducing action may be involved at several levels in the transport of iron through the body. It was therefore proposed to measure the absorption of iron, the plasma iron turnover, the red cell

utilisation of iron, the passage of iron through reticulo-endothelial cells and the uptake of iron by erythroid marrow in subjects with untreated and treated scurvy! In this way, it was hoped to obtain a more complete picture of the relationship between ascorbic acid and iron in the body, with particular reference to the genesis of the anaemia of scurvy.

C H A P T E R II

METHODOLOGY

METHODS

THE CHOICE OF METHOD FOR INJECTING RADIOIRON INTRAVENOUSLY TO MEASURE THE PLASMA IRON CLEARANCE RATE.

Methods of administering radioiron have varied in different investigations.

1. It has been incubated with fresh plasma (Huff et al, 1950; Bush et al, 1956; Bothwell et al, 1955a). This requires the withdrawal of a large blood sample, centrifuging, removal of the plasma, incubation with radioactive ferric chloride (which immediately prior to use has been buffered to a pH of approximately 6) and re-injection; all of which must be done under sterile conditions.

2. The preparation of radioiron bound to Cohn's fraction IV-7 (which contains beta-1-globulin) suitable for use in patients is tedious and the results difficult to reproduce (Elminger et al, 1953; Sharney et al, 1954) and it has recently been shown that the rate of clearance of radioiron tracer bound to Cohn's fraction IV is slow in comparison with other methods (Bothwell, 1957). The finding by Jandl et al (1959) that in the preparation of Cohn's fraction IV-7 some of the protein is denatured could explain the slow rate of clearance of radioiron bound to Cohn's fraction IV.

3. Radioiron may be given intravenously as ferric citrate (Loeffler et al, 1955; Loeffler, 1956). While it has been shown by in-vitro experiments (Bothwell et al, 1956) that citrate may interfere with the binding of iron to globulin, iron transfers sufficiently rapidly at pH 7 from citrate to the iron-binding protein to permit the use of this method. In vivo results are no different when ferric citrate is given alone or after binding in vitro to fresh plasma (Bothwell et al, 1957). However, whereas ferric citrate is apparently completely bound in normal subjects with available unsaturated iron binding protein, in one of two

cases with nearly complete saturation of the iron binding protein approximately half of the ferric citrate was immediately lost from the circulation (Bothwell et al, 1957). The clearance rate, however, as compared to ^{55}Fe bound in vitro to the plasma was identical, when both were injected concurrently. Hence reliable clearance rates could be obtained by giving the radioiron intravenously as ferric citrate, even when some was immediately lost from the circulation, and this technique was employed because of its simplicity and convenience as most studies were done at outlying hospitals. ^{59}Fe of high specific activity (1-2μc/ug) was available in a sterile solution as ferric chloride and 500uc were contained in a volume of 5ml at a pH of approximately 1.5 and 4-7uc were added to 0.1 per cent (W/V) sodium citrate in 5cc of normal sodium chloride. A weighed amount was injected into the subject and a weighed aliquot was taken as a standard. Immediately preceding the injection of radioiron, blood was drawn for serum iron estimation, according to the method of Bothwell and Mallet (1955) and for the serum iron binding capacity (IBC) by the method of Bothwell et al (1959). The serum iron estimation was repeated at the end of each study. Blood samples for radioactive determinations were drawn at intervals (10, 30, 40, 60, 90 and 120 minutes) after injection. When a rapid rate of clearance of radioiron was anticipated, six blood samples were drawn at ten minute intervals. 4ml plasma samples were counted. Conversely, when a slow rate of clearance

was anticipated, additional samples of blood were drawn at 180 and 240 minutes respectively. Radioactivity was measured using a well-type sodium iodide thallium activated crystal with a du Pont photomultiplier tube operated at 1100 volts. A straight line for the exponential fall in activity was obtained in all but two cases. Because of the difficulty in analysis of such curves, these two cases were excluded from this thesis.

Huff et al (1950) originally expressed plasma iron turnovers in terms of total mg/day, or mg/kg/day. The former does not permit comparison between individuals since it does not allow for variation in size and therefore in erythropoietic iron requirements. It further requires an estimate of blood volume. The plasma iron turnover may be related to body weight, but the blood volume must still be estimated and an additional error is introduced since red cell mass, and therefore iron requirements for erythropoiesis, does not correlate perfectly with body weight. It might be expected that a constant amount of iron would normally be required to provide the iron for the red cells in 100ml blood. Furthermore, whole blood volume does not change significantly in anaemia, i.e. a decrease of perhaps 10-15 per cent if acute haemorrhage, burns and salt depletion are excluded. For these reasons Bothwell et al (1957) related the plasma iron turnover to 100ml of whole blood as a standard of reference. The plasma iron turnover was calculated from the modified formula of Huff and associates as follows :-

$$\text{Plasma iron turnover (mg/day/100ml plasma)} = \frac{\text{Plasma iron (mg/100ml)} \times 0.693 \times 1440}{T\frac{1}{2} \text{ (minutes)}}$$

where 0.693 = natural logarithm of 2

This is the actual amount cleared when on a semi-logarithmic scale 50 per cent of the radioactivity has disappeared from the blood. 1440 = minutes per day and $T\frac{1}{2}$ = time for clearance of 50 per cent of the radioactivity from the plasma.

Since 0.693 x 1440 = approximately 1000 the expression was further simplified to read :-

$$\text{Plasma iron turnover (mg/day/100ml plasma)} = \frac{\text{Plasma iron (ug/100ml)}}{T\frac{1}{2} \text{ (minutes)}}$$

To convert this expression to plasma iron turnover per 100ml whole blood per day, the following formula was used:-

$$\text{Plasma iron turnover (mg/day/100ml whole blood)} = \frac{\text{Plasma iron (ug/100ml)}}{T\frac{1}{2} \text{ (minutes)}} \times \frac{\text{100-haematocrit}}{100}$$

Turnover rates were related to normal standards as follows :-

$$\text{Production Index or Erythropoietic Index (percentage of normal)} = \frac{\text{Subject's turnover/100ml whole blood} \times 100}{\text{Average normal turnover/100ml whole blood}}$$

The above formulae were adopted in this thesis. The total body haematocrit was used in the above formula = P.C.V. x correction factor .91. (Chaplin et al, 1953).

PERCENTAGE RED CELL UTILISATION CURVE OF INTRAVENOUSLY INJECTED RADIOIRON.

Blood samples were drawn on alternate days after the intravenous injection of radioiron to determine the percentage utilisation. This was calculated as follows:-

$$\text{Percentage red cell utilisation} = \frac{\text{counts/min/ml whole blood} \times \text{total volume of blood}}{\text{Total number of counts injected}}$$

A standard was always counted on the day of injection of radioiron

and on the days thereafter that the samples were counted to correct for radioactive decay. For this formula it was necessary to calculate the blood volume, which was calculated from the haematocrit and the plasma volume. The latter was found by extrapolation of the graph back to zero time. The number of counts injected divided by the number in one millilitre at zero time gave the plasma volume. Freireith et al (1957) found that the plasma volume calculated by extrapolation of the graph back to zero agreed closely with that derived from Evan's blue (± 3 per cent). This method has also been used by Norden (1960) to determine plasma volume.

When the serum iron is high some of the radioiron injected as citrate may be immediately lost from the circulation (Bothwell et al, 1957). This loss of radioiron would invalidate the plasma volume derived from extrapolation of the graph back to zero. The result would always be a plasma volume greater than the true plasma volume. (Fig.2, p. 21).

If the radioiron lost from the circulation was not available for haemoglobin synthesis the percentage red cell utilisation would be normal theoretically since the percentage lost would be corrected by a corresponding "increase" in the plasma volume, e.g. if 50 per cent is immediately lost and all that bound to beta-1-globulin is incorporated into red cells there would be a 50 per cent utilisation, but plasma volume as calculated from the extrapolation of the rate of clearance back to zero would be twice normal (Fig.2, p. 21), hence the

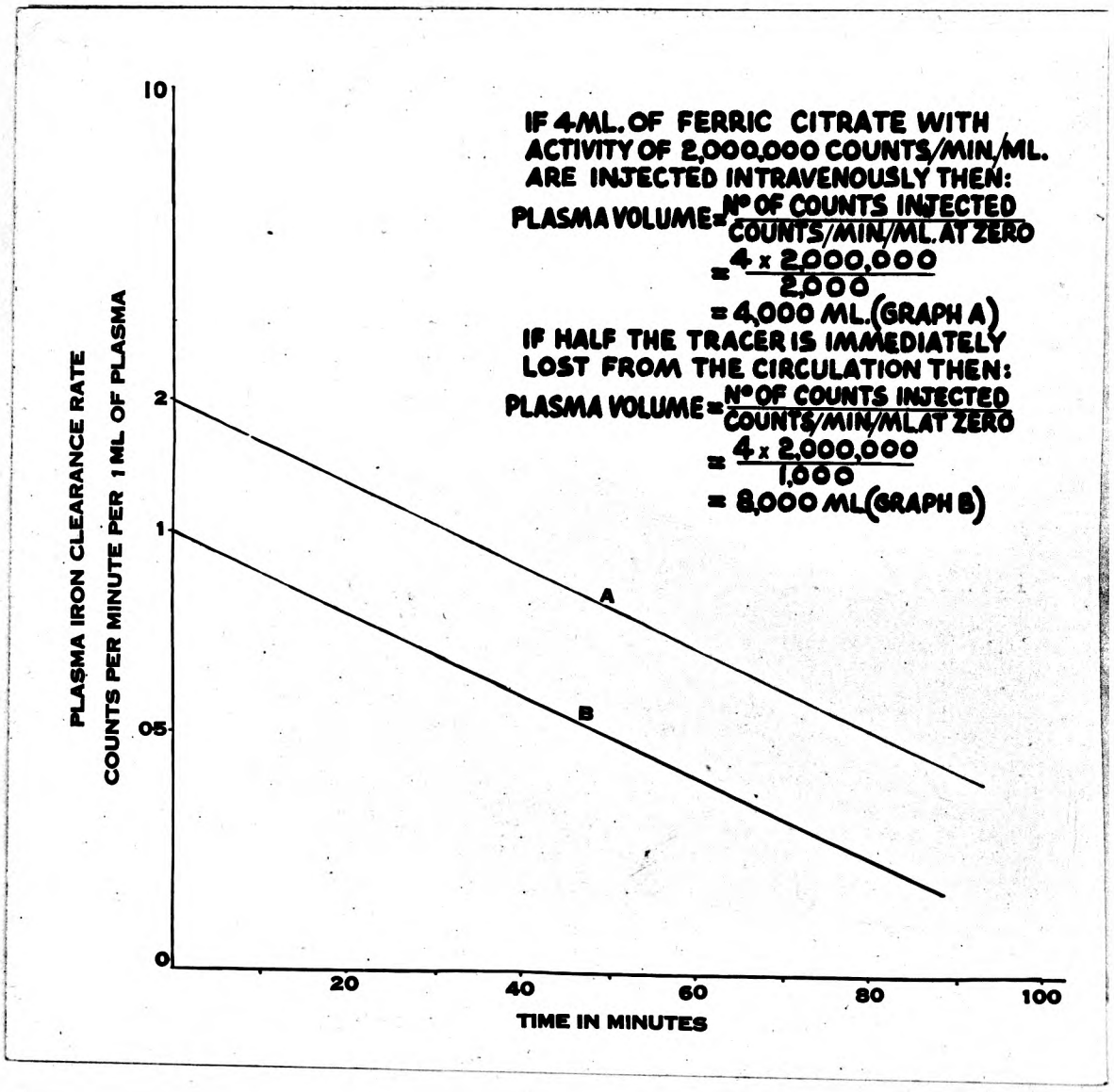


Figure 2 - The effect of calculating the plasma volume from extrapolation of the plasma clearance rate back to zero when all the radioiron injected intravenously is not bound to siderophilin.

percentage red cell utilisation would be 100 per cent. It has been shown, however, that intravenously injected radioiron immediately lost from the circulation may subsequently be utilised for erythropoiesis (Finch, 1949b). Hence, in the present study, if some of the radioiron was immediately lost from the circulation it would in all probability be utilised for haemoglobin production. Under such circumstances the percentage red cell utilisation curve would be greater than the true percentage red cell utilisation curve. Hence, conclusions derived from low percentage red cell utilisation curves would be justified, but a normal, or greater than normal, percentage red cell utilisation curve was only included in this thesis if the blood volume calculated from extrapolation of the graph back to zero was equal to or less than that derived from body weight (Ellis, 1953). It was then presumed that all the radioiron had been bound to beta-1-globulin. In view of these considerations only the patterns of red cell utilisation curves as compared with the normal were studied. In three cases the tracer was bound to plasma to obviate any of the above inaccuracies. Twenty millilitres of fresh plasma were drawn into a sterile syringe and 4-7 microcuries of radioiron at pH 6 were added. The mixture was left at room temperature for 30 minutes and the procedure thereafter was as for ferric citrate.

PLASMA OR SERUM IRON (Bothwell, T.H. and Mallett, B., 1955). All reagents were made up in glass-distilled water.

Reagents:

1. 2N hydrochloric acid (Analar).
2. Twenty per cent (W/V) trichloracetic acid.
3. Saturated solution of sodium acetate (Analar).
4. One hundred milligrams 4:7 diphenyl 1:10 phenanthroline were weighed into a boiling tube and 0.5ml chlorosulphonic acid added. The mixture was boiled for 30 seconds over the pilot flame of a Bunsen. After cooling, 10ml glass-distilled water was cautiously added and the tube placed in a water bath until the precipitate had completely dissolved. After adding sufficient distilled water to bring the volume to 100ml., 1ml thioglycolic acid was added.
5. Stock standard solution of Iron (Hawke et al, 13 Ed). Seven hundred and two milligrams reagent grade ferrous ammonium sulphate (Mohr's salt) was dissolved in 100ml of water. 5ml concentrated sulphuric acid was added, the solution was warmed slightly and a concentrated solution of potassium permanganate ($KMnO_4$) added drop by drop until one drop produced a permanent pink colour. The solution was made accurately up to 1 litre and mixed thoroughly. Such a solution contains 0.1mg/cc ferric iron and is stable indefinitely.
6. Working Iron Standard.
   A 1 in 10 dilution was made of the stock solution using glass-distilled water; 0.2ml of this solution contained 2 gamma ferric iron.
7. Thioglycolic acid.

Precautions:

In order to render all apparatus iron-free, it was soaked in chromic acid or concentrated hydrochloric acid for several hours, then washed thoroughly in distilled water and finally with glass-distilled water. The needles for collecting blood were washed in a similar way and then autoclaved. Blood was

allowed to drip from the needle directly into a clean iron-free tube to obviate the use of a syringe.

Method:

Two ml. 2N hydrochloric acid (HCl) were added to 4ml plasma or serum in a centrifuge tube, and the mixture was stirred with a glass rod. Two ml. trichloracetic acid were then added and the final mixture stirred vigorously for 1-2 minutes. After centrifuging for 20 minutes the supernatant fluid was decanted. Five ml. of this fluid was then added to a tube containing 0.5ml sulfonated 4:7 diphenyl 1:10 phenathroline solution plus 2.5ml saturated sodium acetate solution, and the mixture thoroughly stirred.

Blank -- this was prepared in a similar way. 2.5ml distilled water, plus 1.25ml trichloracetic acid, plus 1.25ml 2N HCl. To this was added colour-reagent (4:7 diphenyl 1:10 phenathroline) and 2.5ml saturated sodium acetate and the mixture thoroughly stirred.

Standard -- 2.3ml water was added to 0.2ml of the working standard iron solution, and then the other reagents added as for the blank. Colours were estimated in the EVELYN photocolorimeter using a 520mu filter.

Calculation:

$$\frac{\text{Reading of test (L value)}}{\text{Reading of standard (L value)}} \times 2 \times \frac{8}{5} \times \frac{100}{4} = \text{gamma iron/100ml serum or plasma}$$

$$= \frac{\text{Test}}{\text{Standard}} \times 80 = \text{gamma iron/100ml plasma or serum.}$$

Normal values:- 50 - 150 gamma per cent.

This method has been extensively used in the Department of Medicine at the Witwatersrand University and found to give reproducible results. In this laboratory 25 serum irons were analysed by this method and that described by Ramsay (1957). The mean difference was 4.7ug/100ml., hence the above method only has been used in this thesis.

P.C.V. -- Citrated whole blood was spun in a haematocrit tube for 30 minutes at 3000 r.p.m. This speed produced a force of 2260 x gravity, (Wintrobe, 1956).

Haemoglobin (Evelyn, 1936) -- 0.02ml blood was added to 10ml of 0.4 per cent ammonium hydroxide and read with a 540mu filter in the Evelyn electrophotometer. The haemoglobin was read against a blank of 10ml of 0.4 per cent ammonium hydroxide. The amount of haemoglobin was calculated as follows:-

$$\frac{(2 - \log G \text{ of reading}) \times 100}{\text{Factor of Evelyn}} = \text{Hb. in grams per 100ml blood.}$$

Leishman's stain was used for the histological examination of blood and marrow slides. Blood film slides were covered with twenty drops of Leishman's stain and left for two minutes, while bone marrow slides were left for four minutes. Then an equal volume of distilled water was added and mixed adequately by blowing. The blood smears were left for fifteen minutes and the bone marrow smears for twenty-five minutes. The slides were then washed with distilled water and allowed to dry.

To stain for reticulocytes a few drops of blood were incubated at 37°C for ten minutes in normal saline containing 1 per cent brilliant cresyl blue and 0.4G per cent sodium citrate.

Smears were then made and 500 red cells examined to estimate the percentage reticulocytes (Wintrobe, 1956).

Marrow films were stained for iron and sideroblasts as outlined by Douglas and Dacie (1953). After the films had been dried in air and fixed in methyl-alcohol for ten minutes, they were covered for ten minutes with a freshly prepared mixture of a 2 per cent solution of potassium ferrocyanide (Analar) and 2 per cent HCl in distilled water. The mixture was first warmed at 56°C for one to two minutes and then poured on to the slides. The slides were left for ten minutes and then washed in running water for a further ten minutes and counter-stained for three to four seconds with 0.1 per cent safranin. The smears were graded as to the amount of iron present as follows (Modified from Rath and Finch, 1948):-

No tissue iron stores	0	
Slight amount of tissue iron stores	+	(Normal)
Moderate " " " "	++	(Increased)
Heavy " " " "	+++ / ++++	(Markedly increased).

C H A P T E R III

FERROKINETIC STUDIES IN A VARIETY OF HAEMATOLOGICAL DISORDERS

CHAPTER III

In this chapter the results are recorded of radioiron studies done in a variety of conditions in which there was disordered haemoglobin synthesis, or red cell formation. These conditions consisted mainly of various types of anaemia and polycythemia. The anaemias were grouped together morphologically and further subdivided as far as possible on an aetiological basis (Wintrobe, 1956). Patients with polycythemia were also classified on an aetiological basis (primary, secondary and relative polycythemia). In addition, plasma iron turnover studies were done in three patients with cytosiderosis to see whether this test could clearly distinguish between cytosiderosis and haemochromatosis.

Iron Kinetic Studies in Normal Subjects.

Fifteen haematologically normal patients were selected as controls. There were ten Bantu and five European subjects.

Bantu Subjects (Table I, p. 28 : Fig.3, p. 29)

The haematocrit in the ten Bantu subjects ranged from 43-54 per cent (mean 48 per cent) and the plasma iron from 40 - 142 micrograms per 100ml of plasma (mean 81.7µg per cent). The rate of clearance of the radioiron ranged from 40 - 110 minutes (mean 72 minutes) and the range of the plasma iron turnover was 0.43 - 0.93 milligrams per 100ml of whole blood per day (mean 0.65 ± 33%) The data from the European subjects are recorded in Table II, p. 30 : Fig.3, p. 29).

TABLE I

Plasma Iron Turnover in Ten Haematologically Normal Bantu Subjects.

Case No.	Diagnosis	Sex	Age	PCV	% Reticulocytes	Plasma Iron ug/100ml	Time taken for radioiron to reach half concentration (minutes)	U.I.B.C.	Plasma Iron Turnover mg/day/100ml plasma	Plasma Iron Turnover mg/day/100ml whole blood	Erythropoietic Index	Marrow Haemosiderin
1	Syphilitic Aortic incompetence	M	42	51	2.2	60	44		1.36	0.75	115	++
2	Idiopathic Heart Failure	M	50	51	0.5	62	56	200	1.11	0.60	92	+
3	? Coronary Thrombosis	M	50	44	1.4	118	84		1.40	0.85	131	++
4	Idiopathic Heart Failure	F	47	54	1.6	51	60	297	0.85	0.43	66	+++
5	Mental Confusion	F	38	44	1.2	83	110	146	0.76	0.45	69	++
6	Fractured R. Femur	M	32	47	1.2	40	39	214	1.03	0.59	91	+
7	Fractured R. Tibia and Fibula	M	28	51	0.8	119	84	84	1.42	0.77	118	+
8	R. Inguinal Hernia	M	36	49		142	86		1.65	0.93	143	++
9	Fractured R. Humerus	M	57	43	1.5	44	46	210	0.96	0.59	91	+
10	Old Fract. R. Tib. Fib.	F	51	47	1.3	98	110	106	0.93	0.54	83	+
	Mean Values and Range			48.1 43-54	1.3 0.5-2.2	81.7 40-142	71.9 39-110	180 84-297	1.15 0.76-1.65	0.65 0.43-0.93	100 66-143	

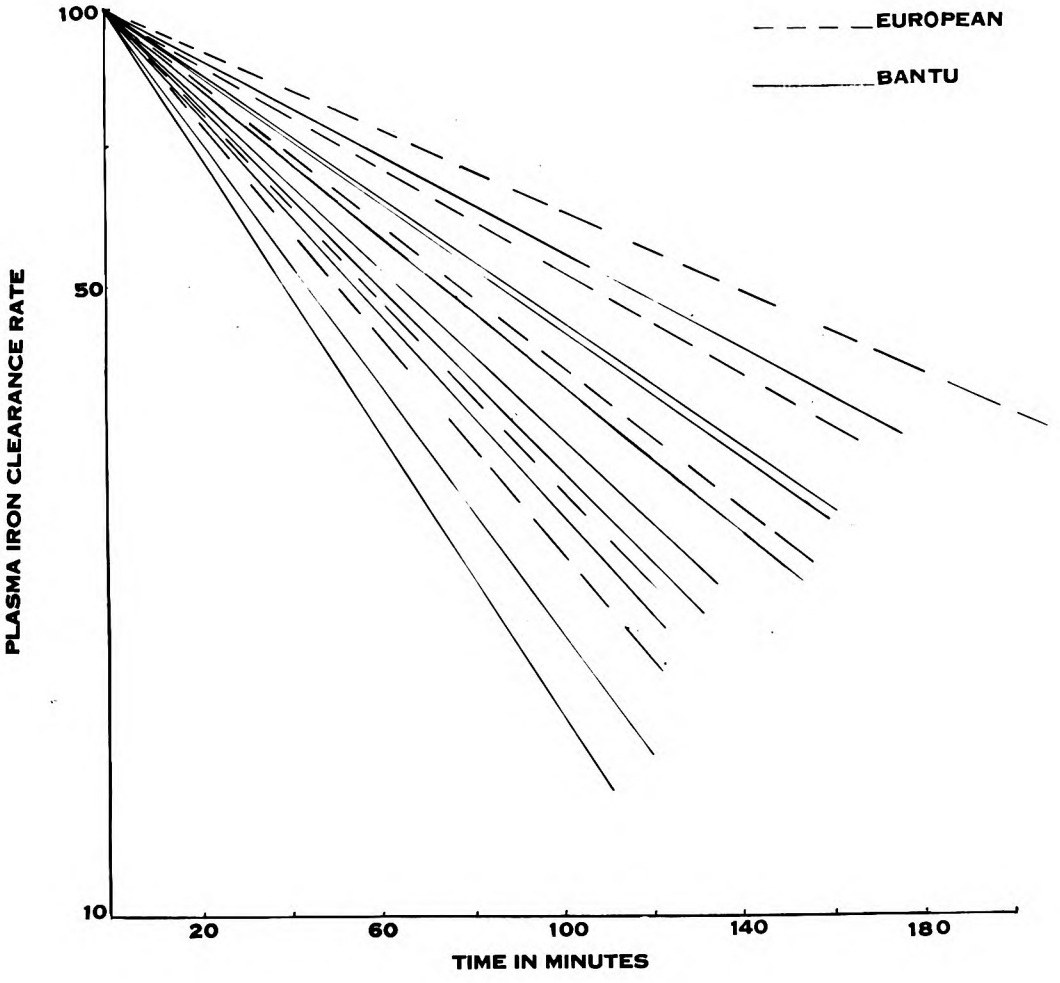


Figure 3 - Plasma Iron Clearance Rates in Fifteen Haematologically Normal Patients. (Two Bantu patients had the same clearance rates.)

TABLE II

Plasma Iron Turnover in Five Haematologically Normal European Subjects

Case No.	Diagnosis	Sex	Age	PCV	% Reticulocytes	Plasma Iron ug/100ml	Time taken for radioiron to reach half concentration (minutes)	U.I.B.C.	Plasma Iron Turnover mg/day/100ml plasma	Plasma Iron Turnover mg/day/100ml whole blood	Erythro-poietic Index
1.	Angina	M	62	56	1.5	85	75		1.13	0.58	98
2.	Fractured R. Ulna	F	29	51		72	58		1.24	0.68	116
3.	Progressive Muscular Atrophy	M	44	55	1.2	77	75	180	1.03	0.56	96
4.	Anxiety	F	40	47	2.0	126	132	129	1.05	0.60	102
5.	Psycho-neurosis	M	36	44	0.69	96	102	220	0.94	0.54	92
	Mean Values and Range			50	1.3	91	88	143	1.08	0.59	100
				44-56	0.6-2.0	72-126	58-132	129-220	0.94-1.24	0.54-0.68	92-116
	Mean Values and Range of all haematologically normal Bantu and European Patients.			48.7	1.3	84.8	77.3	168.6	1.1	0.63	
				43-56	0.5-2.2	40-142	39-132	84-297	0.76-1.65	0.43-0.93	

The data, both as regards the mean value and range, is comparable with that described by others (Bothwell et al, 1957 ; Loeffler, 1956) and corresponds with a range of 25 - 40 milligrams of iron cleared through the plasma per day (Huff et al, 1950; Huff et al, 1952; Baldini, et al, 1959).

The rate of clearance of radioiron from the plasma was inversely related to the serum iron level (Fig.4, p.32), i.e. the higher the serum iron level the slower the rate of clearance. Hence, as noted by Bothwell et al (1957) the radioiron plasma clearance rate alone is of limited value and the plasma iron turnover must be calculated to give a quantitative measure of the amount of iron passing through the plasma.

Due to the acute bed shortage the percentage red cell utilisation curves were measured in only four Bantu subjects (Fig.5, p.33). However, even in this small number of subjects it was apparent that the pattern and degree of incorporation was similar to that described by others (Huff et al, 1950; Loeffler, 1956). (In all subsequent graphs the range in normal subjects for the plasma iron clearance rate, or percentage red cell utilisation of radioiron has been shaded in).

It is of importance to consider what the findings would be if plasma iron turnovers were done in other parts of South Africa. The only known environmental factor which affects the plasma iron turnover, is altitude. Huff et al (1952) showed that on ascending to high altitudes (15,000 feet) the plasma iron turnover significantly increased. They surmised that if the

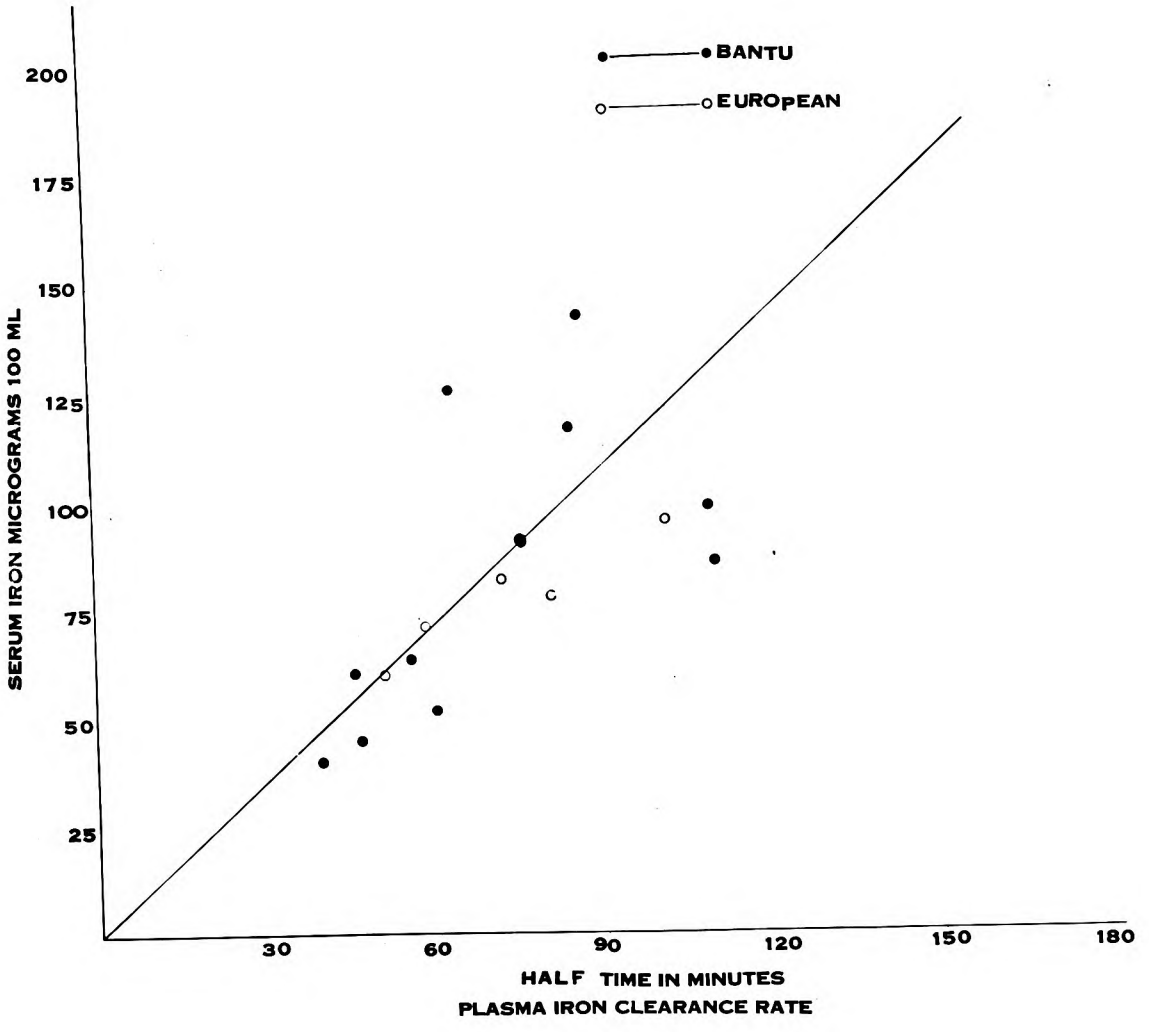


Figure 4 - The Relationship Between the Serum Iron Level and the Clearance Rate of Radioiron from Plasma.

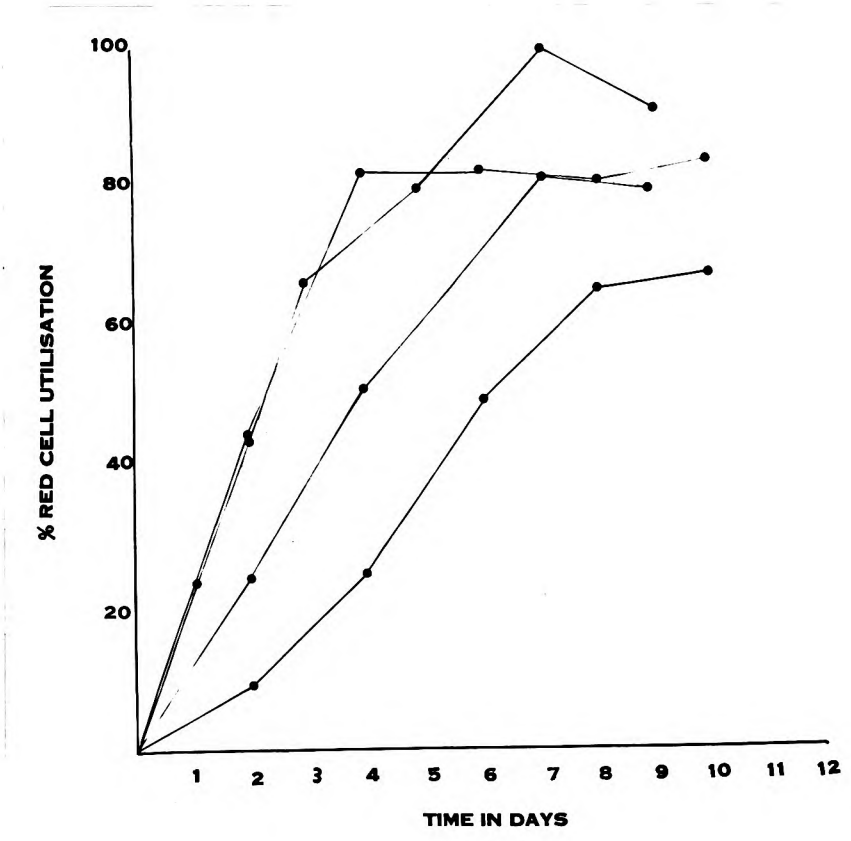


Figure 5 - Percentage Red Cell Utilisation Curves in Four Haematologically Normal Bantu Patients.

subjects were to remain at a high altitude the plasma iron turnover would equilibrate at one and a half times normal to maintain the increased red cell mass (secondary polycythemia). Conversely, there is a significant fall in the plasma iron turnover in subjects brought from a high altitude to sea-level (Raynafarje et al, 1959). These changes in the plasma iron turnover are brought about by changes in red cell production secondary to changes in oxygen saturation. There is only a small decrease in oxygen saturation at 5,740 feet (Hurtado et al, 1948) and Ellis (1953) has noted no significant difference between the red cell volume in subjects from Johannesburg as compared with those at sea-level. Hence there should be no significant difference between plasma iron turnovers done in Johannesburg as compared with those done in the other larger centres (Durban and Capetown) which are at sea-level.

In all studies and the subsequent calculations it is assumed that figures obtained for the plasma iron turnover at any one time reflect accurately the pattern of the plasma iron turnover throughout a twenty-four hour period. Bothwell and Mallett (1955b) found no consistent change in eight normal subjects in whom the plasma iron turnover was repeated within 12-14 hours. (The plasma iron turnovers were done during the morning and in the evening). However, Paterson (1957) in three normal subjects found that during sleep there was a delay in the clearance of radioiron from the plasma. The plasma iron was measured at the same time, and a significant decrease in the plasma iron turnover was noted during

sleep. Paterson suggested that the increased half-time and diminished iron turnover were secondary to a diurnal variation in the rate of synthesis of haemoglobin which in turn was dependent on the sleep rhythm.

In three patients (Table III, p. 36) the plasma iron turnover during sleep was compared with that done during the day. One patient, in spite of heavy sedation (phenobarbitone gr.6 orally) was restless and vaguely aware of the plasma iron turnover done during the night (Case No.2, Table III). The data in the remaining two cases support the observation of Paterson (1957) that there is a decrease in the plasma iron turnover during sleep.

Because of the fairly wide range in normal subjects and the diurnal variation, the plasma iron turnover can only be regarded as a semi-quantitative test. Furthermore, since in this thesis the calculation of the percentage red cell utilisation may be inaccurate, it was felt that formulae derived from the plasma iron turnover and percentage red cell utilisation of radioiron, e.g. the red cell iron turnover, would have had little additional value and have, therefore, been excluded.

Iron Kinetic Studies in Patients with Various Haemolytic Anaemias

1. Congenital Spherocytosis

Six patients with congenital spherocytosis were studied (Table IV, p. 37; Fig. 6, p. 38). All had microspherocytosis and increased excretion of stercobilinogen in the stools. The osmotic fragility of the red cells was greatly increased in all and a

TABLE III

Diurnal Plasma Iron Turnovers in Three Haematologically Normal Subjects

Case No.	Diagnosis	Sex	Ages	Time when the plasma iron turnovers were done	PCV	% Reticulocytes	Plasma Iron ug/100ml	Time taken for radioiron to reach half concentration (minutes)	Plasma Iron Turnover mg/day/100ml plasma	Plasma Iron Turnover mg/day/100ml Whole blood	Erythro-poietic Index
1	Psychoneurosis	M	36	12.30p.m. 4.7.59 (D)	44	0.8	96	102	0.94	0.54	86
				1.00a.m. 5.7.59 (N)	44		78	110	0.71	0.43	68
2	Anxiety Neurosis	F	47	12.30a.m. 7.7.59 (N)	46	2.0	95	188	1.08	0.60	95
				11.30a.m. 7.7.59 (D)	46	1.7	126	132	1.05	0.61	97
3	Progressive Muscular Atrophy	M	55	12.30a.m. 12.7.59 (N)	51	1.2	77	106	0.73	0.39	62
				11.30a.m. 12.7.59 (D)	61	1.3	77	75	1.03	0.56	89

TABLE IV

Plasma Iron Turnover Studies in Ten Patients with Haemolytic Anaemia

Case No.	Diagnosis	Sex	Age	PCV	% Reticulocytes	Plasma Iron ug/100ml	Time taken for radioiron to reach half concentration (Minutes)	Plasma Iron Turnover mg/day/100ml plasma	Plasma Iron Turnover mg/day/100ml whole blood	Erythropoietic Index
1A	Congenital Spherocytosis	F	58	28	2.1	75	29	2.68	2.0	317
B	"			30	2.4	102	21	4.86	3.96	628
2	"	M	30	35		68	24	2.83	1.93	306
3	"	F	37	37	11.8	167	26	6.42	4.27	678
4	"	F	23	35	2.8	152	36	4.22	2.88	457
5	"	M	19	41	6.0	98	37	2.65	1.67	265
Mean Values and Range				34.3 28-41	5.1 21-11.8	110 68-167	28.7 21-37	3.9 2.68-4.86	2.8 1.67-4.27	442 317-678
6	Congenital Spherocytosis (partial recovery from aplastic crisis)	M	17	30	7.3	40	28	1.43	1.06	180
7	Chronic Idiopathic Haemolytic anaemia	M	47		6.0	71	23	3.09	2.14	383
8	Acute Haemolytic anaemia (possibly due to APC tablets+)	M	37	35	3.0	72	37	1.95	1.23	195
9	Acute Haemolytic anaemia (possibly due to pyridium or APC tabs.)	M	34	25	17.5	96	30	3.15	2.43	386
10	Hypersplenism complicating "pre-cirrhotic cholangio-hepatitis"	M	33	40	1.6	52	23	2.26	1.44	229

+ APC = Aspirin, Phenacetin and Codeine Tablets.

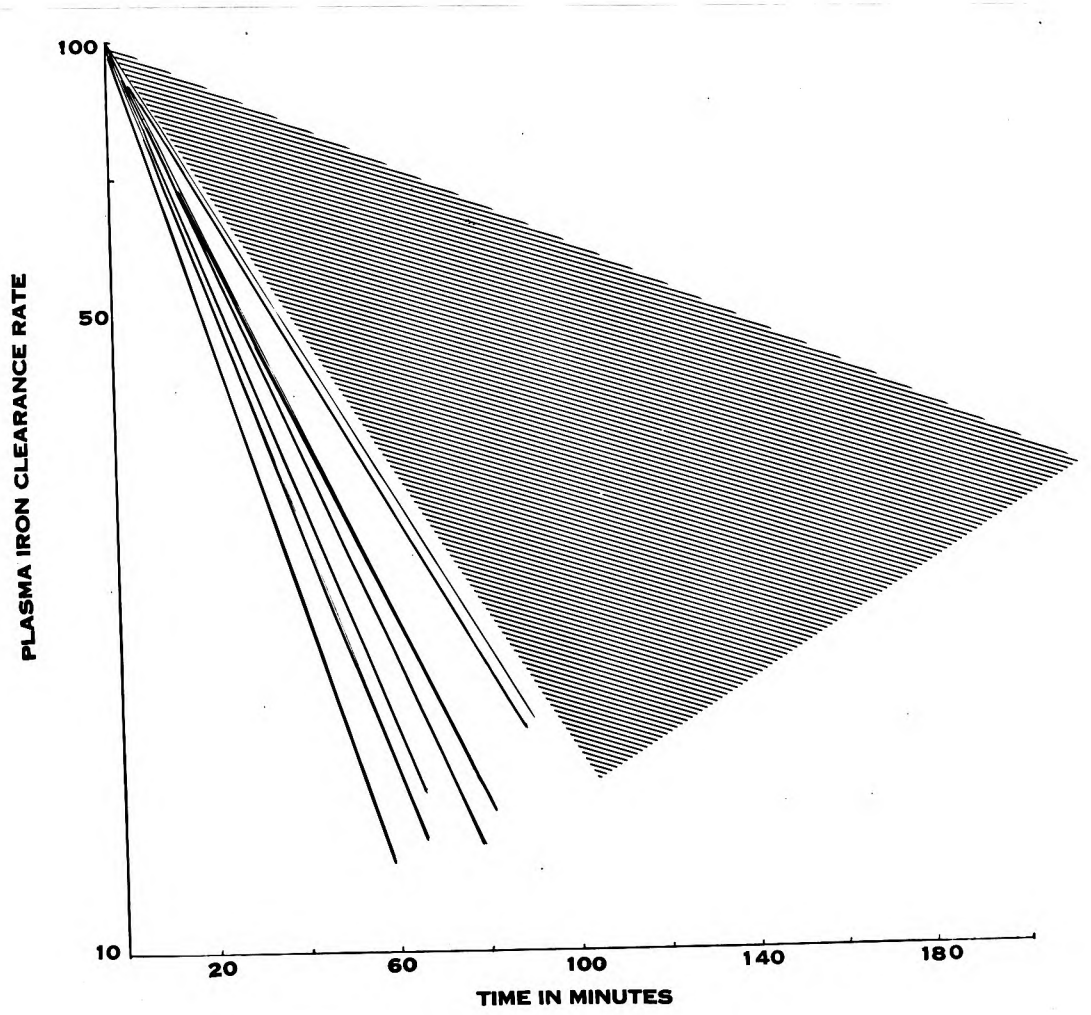


Figure 6 - Seven Plasma Iron Clearance Rates in Six Patients with Congenital Spherocytosis.

positive family history was present in five of the six patients studied.

The serum iron was normal in four patients and slightly raised in two. In every patient the rate of clearance of the radioiron from the plasma was rapid (Fig.6, p.38). The plasma iron turnover in five patients (Cases No.1-5, Table IV, p.37) ranged from 1.67 to 4.27mg/100ml whole blood per day (mean 4.1mg per cent). This represents an increase in marrow activity of three to seven times normal. The plasma iron turnover was repeated in one patient (Case No.1, Table IV, p.37) after an interval of six months. The initial plasma iron turnover was 3 times normal; the repeat plasma iron turnover 6.5 times normal. The reason for this difference was, however, not apparent. The range of the plasma iron turnover in these patients with congenital spherocytosis is similar to that reported by others (Giblett et al, 1956; Bothwell et al, 1956; Bothwell et al, 1957). In the sixth patient, a plasma iron turnover was done shortly after a pyrexial episode associated with a fall in haemoglobin and was only 1.8 times normal. There was a reticulocyte count of 7 per cent. These findings are compatible with partial recovery from an "aplastic" crisis (Owren, 1948).

The erythropoietic activity of the marrow can increase six to eight times normal (Crosby and Akeroyd, 1952). This increase is seen mainly in cases of chronic haemolytic anaemia, e.g. congenital spherocytosis (Crosby and Akeroyd, 1952; Giblett et al, 1956; Bothwell et al, 1956; Bothwell et al, 1957) and

sickle cell anaemia (Bothwell et al, 1957). With acute blood loss the increase in marrow activity is 2-3 times normal (Loeffler, 1956; Bothwell, 1957; Finch et al, 1959). It is the expansion of erythroid tissue in chronic haemolytic anaemias which accounts for the further increase in erythropoiesis. This is supported by the finding that after the intravenous injection of radioiron the radioactivity over the sacrum is less in chronic haemolytic anaemias than in the acute varieties. In the former, with the increase in erythroid tissue, there is a wider distribution of the radioiron and thus a dilution of the radioactivity, (Huff et al, 1951). In four patients studied the percentage red cell utilisation curve was reduced. This has been noted by others and is said to be due to the random destruction of red cell with prompt reutilisation of the radioiron which will give a blood radioactivity reasonably constant over many red cell life spans (Giblett et al, 1956; Bothwell et al,1956; Bothwell et al, 1957),(Fig. 7 p. 41).

A further four patients with haemolytic anaemia were studied, (Table IV, p. 37 ; Fig.8, p. 42). Two of these patients had acute haemolytic anaemia (Cases No.8 and 9, Table IV). Their plasma iron turnovers were 1.23 and 2.43mg per 100ml of whole blood per day respectively. The percentage red cell utilisation of radioiron was measured in only one patient in whom it was rapid and complete (Case No.9, Fig.9, p. 43). The two chronic patients are discussed in detail.

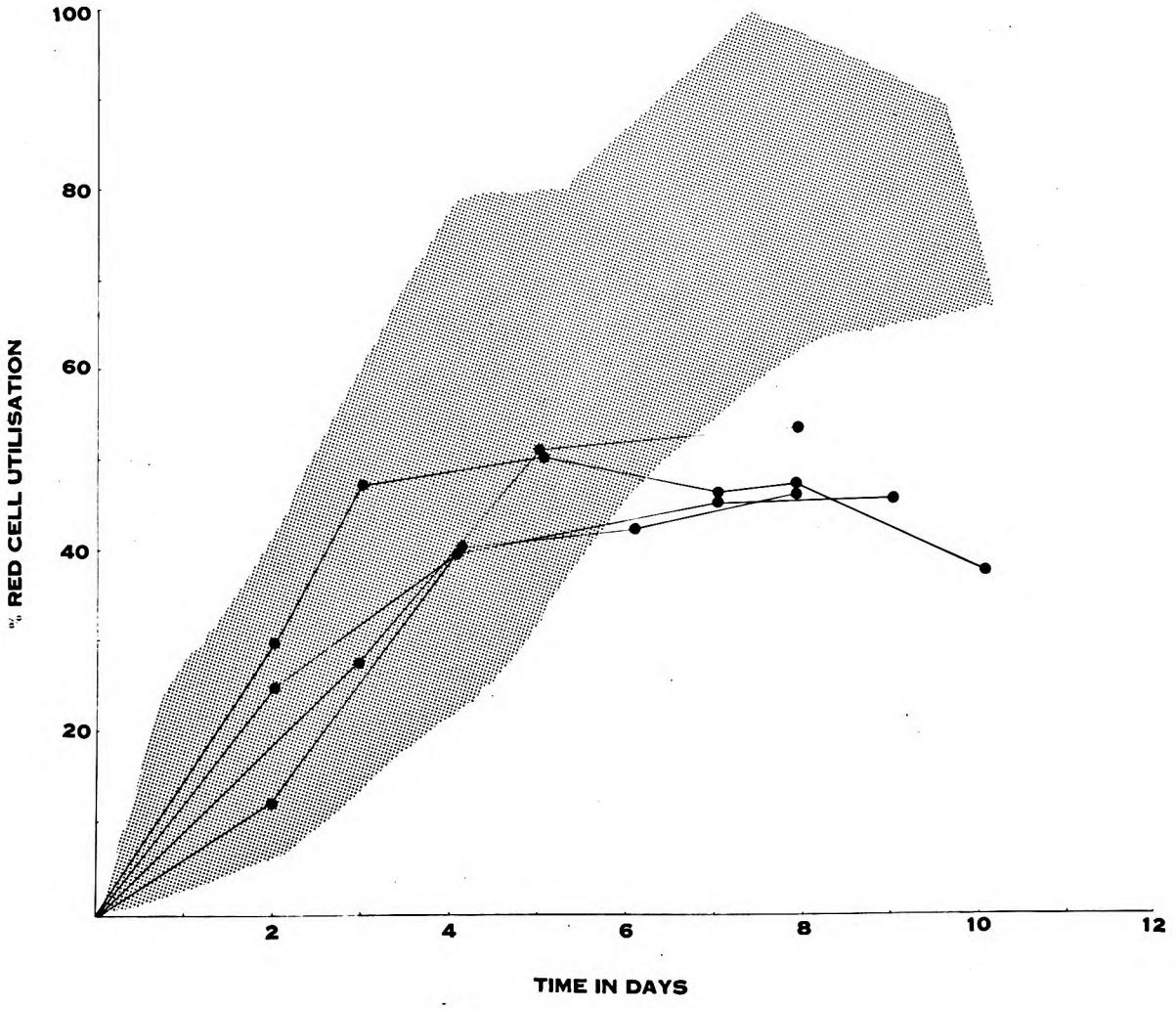


Figure 7 - Percentage Red Cell Utilisation Curves in Four Patients with Congenital Spherocytosis.

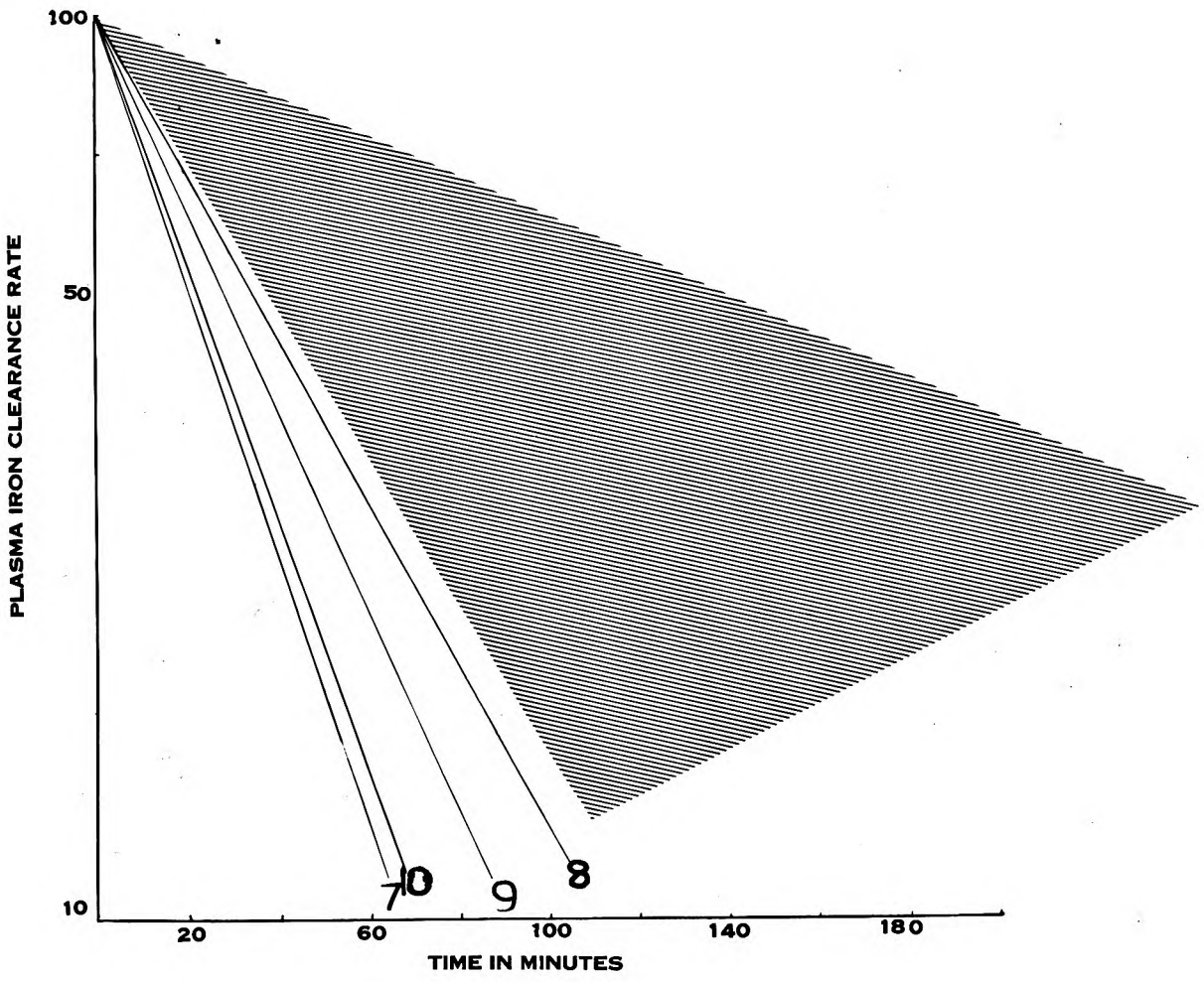


Figure 8 - Plasma Iron Clearance Rates in Four Patients with Haemolytic Anaemia. (Two patients had acute and two chronic haemolytic anaemia)

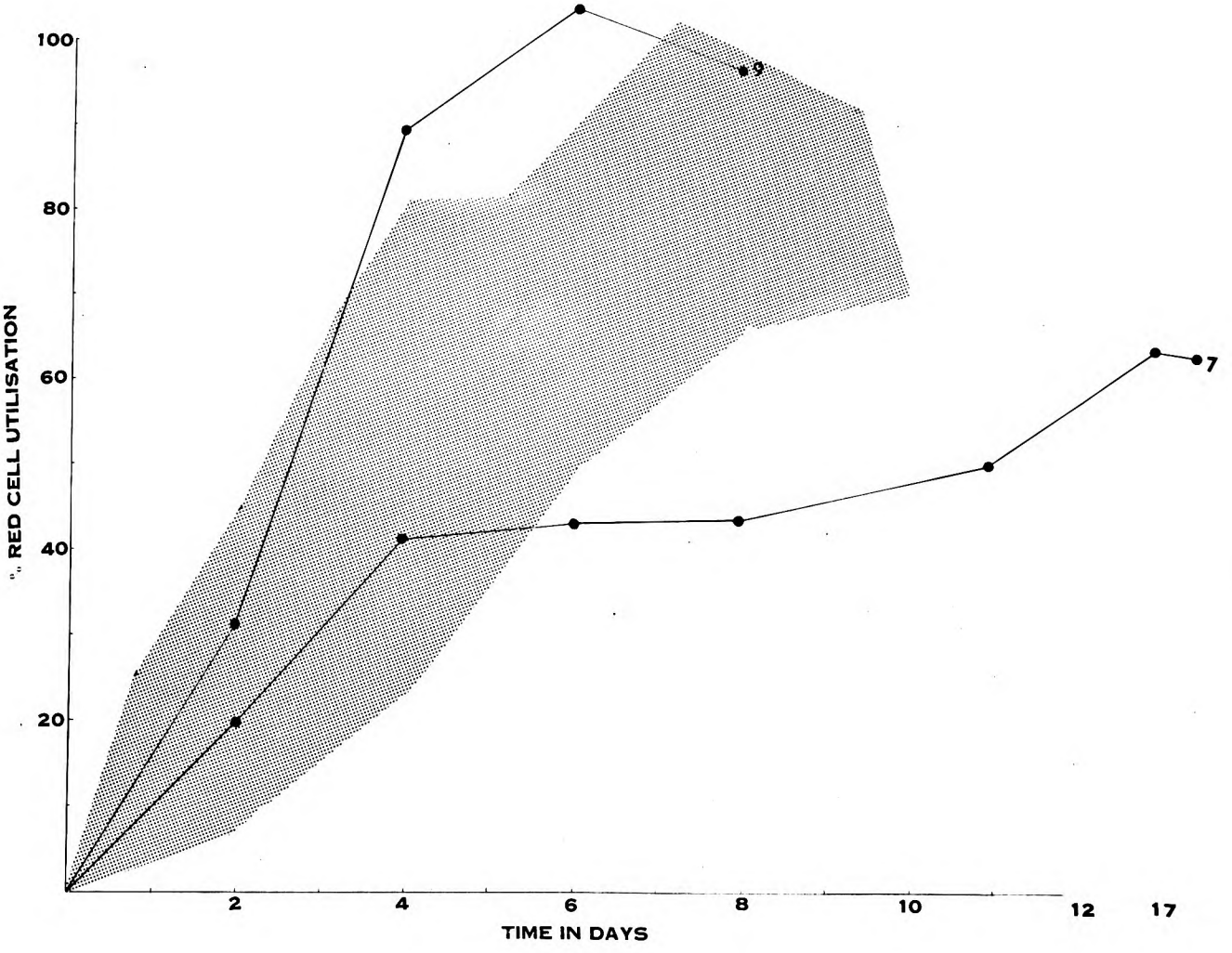


Figure 9 - The Percentage Red Cell Utilisation Curves in One Patient with Acute Haemolytic Anaemia (No.9) and One Patient with Chronic Haemolytic Anaemia. (No.7).

Case No.7 (Table IV)

For several years Mr.A.W. had been treated with Artane (Trihexyphenidyl) 4mg daily, in an attempt to control the tremor and rigidity of paralysis agitans. Pallor was noted in October 1958 and he was admitted for investigation. But for the pallor and the neurological manifestations of paralysis agitans, the general examination was negative. There was no jaundice, no splenomegaly or lymphadenopathy and no purpura.

	Haemoglobin	9.8G%
	Leucocytes	3,000/c.mm.
Differential Count	Neutrophilic leucocytes	30%
	Monocytic leucocytes	7%
	Eosinophilic leucocytes	3%
	Lymphocytic leucocytes	60%
	Reticulocytes	6%
	Platelets	300,000/c.mm. (normal in number)

Bone Marrow - "normoblastic erythroid hyperplasia".

The serum bilirubin level was not raised, but there was excess urobilinogen in the urine and stool. The Coombs' and Schumm's tests were negative, as were the Ham's and the Donath Landsteiner tests. The screening test for osmotic fragility of the red cell was negative. Repeated examinations for "L.E" cells were negative. The serum iron was 71 micrograms per cent. The rate of clearance of the radioiron was rapid and the plasma iron turnover markedly increased, 2.41mg per 100ml of blood (Table IV, Case No.7, p.37). The red cell utilisation curve was similar to that seen in congenital spherocytosis (Fig.9, Case No.7, p.43). It was thought at the time that he had a drug

induced haemolytic anaemia. However, he denied using drugs other than artane, and there is no report of a haemolytic anaemia due to this particular agent. Artane was stopped, but there was no clinical remission. There was a slight increase in the uptake of radioiron after prednisolone (8mg, q.i.d.) followed by a slight rise in haemoglobin. The patient was observed for 18 months. But for an occasional blood transfusion and the development of an ischio-rectal abscess, which responded to massive antibiotic therapy, his clinical picture remained unchanged.

Case No.10

Mr. V.V., aged 33 years, was admitted to the Johannesburg General Hospital in January 1959. His past illnesses included unexplained jaundice in February 1947. In 1958 he was involved in a motor-car accident and for two months afterwards bled intermittently from the nose, ears and mouth. Since the accident the left side of his face was paralysed. He was admitted following two small haematemeses. On examination there was a lower motor neuron lesion and old scarring on the left side of the face. The spleen was grossly enlarged and a caput medusa was present. The rest of the examination was non-contributory.

Haemoglobin	13.8G%
Haematocrit	42.0%
Mean Corpuscular Haemoglobin Concentration	32%
Leucocytes	3,300/c.mm.
Neutrophilic leucocytes	55.5%

Monocytic leucocytes	11%
Lymphocytic Leucocytes	31%
Eosinophilic lymphocytes	2.5%
Reticulocytes	1.6%
Platelets	68,000/c.mm. (diminished in number).

The bleeding and clotting times were within normal limits, 5 and 6 minutes respectively. Hess' Test was negative.

Liver function tests

Thymol turbidity	6.5 units	(normal 2 units)
Thymol flocculation Test	++	" 0
Colloidal red Test	+++	" 0
Cephalin cholesterol flocculation test	++++	" 0
Takata Ara	+++	" 0
Zinc sulphate turbidity	18.6 units	" 15.8
Total bilirubin	0.6mg%	" 1mg%
direct	0.2mg%	.5mg%
Total protein	5.4G%	(normal range 6.0-7.5G%)
Albumin	3.0G%	(" " 3.5-4.5G%)
Globulin	3.24G%	(" " 2 - 3 G%)
Alpha I	0.22G%	
Alpha II	0.42G%	
Beta	0.62G%	
Gamma	1.9 G%	
Total lipid	640mg%	

Alkaline Phosphatase 7.4 King Armstrong Units (normal 15).

No bilirubin, but excess urobilin was present in the urine. Repeated examinations for "L.E." cells were negative. The direct and indirect Coombs' tests and Schumm's test were negative, as were the Ham's and Donath Landsteiner tests. Stools were negative for occult blood.

The red cell osmotic fragility test was within normal limits. On bone marrow examination myelopoiesis and platelet genesis were normal. There was a slight normoblastic erythroid hyperplasia. Oesophageal varices were demonstrated on barium swallow and splenography revealed large dilated splenic and portal veins. A plasma iron turnover was done shortly after admission (Table IV, Case No.10, p.37). The haematocrit was 40 per cent, the serum iron 52 micrograms per 100ml of plasma. The rapid clearance of radioiron (half-time 23 minutes) (Fig.8, p.42) gave a plasma iron turnover of 1.44mg per 100ml of blood per day (2.5 times normal). These findings were all in keeping with a compensated haemolytic process, possibly due to the enlarged spleen. Hypersplenism was supported by the low white cell count and thrombocytopenia. Counting techniques to estimate the degree of red cell sequestration and destruction within the spleen were not available. After a further haematemesis laparotomy was followed by splenectomy, but no spleno-renal shunt was done as the pressure in the splenic and portal veins was not raised. The pathological report on a liver biopsy was "pre-cirrhotic cholangio-hepatitis". There was marked bile duct proliferation with acute inflammatory cell infiltration. The plasma iron turnover was not repeated, but a rise after splenectomy in the platelet count to 417,000/cmm, and the leucocyte count to 12,000/cmm (69 per cent polymorphonuclear leucocytes), supported the original diagnosis of hypersplenism.

PERNICIOUS ANAEMIA

Only one patient with pernicious anaemia was studied (P.C.V. 28%, M.C.V. 120c.u., Histamine fast achlorhydria, marrow grossly megaloblastic erythropoiesis). A serum iron of 126ug% was associated with a rapid rate of clearance of radioiron (half-time 40 minutes) with the result that the plasma iron turnover was nearly four times normal (2.36mg/day/100ml whole blood. Erythropoietic index 375). The reticulocyte count was disproportionately low (1.7 per cent). The patient was given vitamin B.12, 1000μμg intramuscularly, so the percentage red cell utilisation curve in an untreated patient with pernicious anaemia was not recorded.

The plasma iron turnover is markedly increased in pernicious anaemia (Finch et al, 1956; Bothwell et al, 1957; Giblett et al, 1956). This is in keeping with the degree of erythroid hyperplasia of the marrow and the excess amount of urobilinogen in the stools (Finch et al, 1956; Giblett et al, 1956). In this anaemia the reticulocytosis is slight and the percentage red cell utilisation of radioiron reduced (Finch et al, 1956). The latter two findings indicate that a normal and not an increased number of red cells are delivered to the blood. The discrepancy between the marked erythroid hyperplasia and the number of red cells released into the blood stream indicates a large turnover of iron through defective cells which are either destroyed in situ or survive only a day or two in the peripheral blood, i.e. a large fraction of total erythropoiesis is

ineffective. Plasma iron turnovers have been repeated in patients with pernicious anaemia after therapy (Finch et al, 1956). There is no quantitative change. There is, however, a fall in the serum iron with a marked rise in the incorporation of radioiron into red cells and a significant reticulocytosis, i.e. total erythropoiesis is now effective. Similar results have been reported in patients with Cooley's anaemia (Sturgeon and Finch, 1957), in tropical sprue (Sheehy et al, 1960) and in a patient where effective erythropoiesis was blocked by an iso-antibody (Giblett et al, 1956a).

DECREASED MARROW ACTIVITY

A hypocellular marrow with no evidence of increased red blood cell destruction were the diagnostic criteriae for hypoplastic anaemia. The results of radioiron studies in three cases are summarised in Table V, p. 50 ; Fig.10, p. 51). All patients were being maintained on repeated blood transfusions. There was a significant elevation of the plasma iron in only one patient. The time required for the disappearance of the injected radioiron was prolonged in every patient (Fig.10, p. 51). The percentage red cell utilisation was reduced in all cases (Fig.11, p. 52). The pattern of iron kinetics in anaemia secondary to hypoplasia of the marrow is well illustrated by the following patient (Case No.1, Table V, p. 50):

Mrs. P., aged 30 years, was seen elsewhere in January 1958 complaining of tiredness and excessive bleeding from the gums. In August 1957 she had a pyrexial illness which was diagnosed as

TABLE V

Plasma Iron Turnovers in Three Patients with Marrow Hypoplasia

Case No.	Diagnosis	Sex	Age	PCV	% Reticulocytes	Plasma Iron ug/100ml	Time taken for radioiron to reach half concentration (minutes)	U.I.B.C.	Plasma Iron Turnover mg/day/100ml plasma	Plasma Iron Turnover mg/day/100ml whole blood	Erythropoietic Index
1	Exposure to several insecticides	F	33	19	0.3	180	400	145	0.45	0.38	60
2	Hypopituitarism and Steatorrhoea	M	38	26	1.0	96	154	134	0.63	0.43	68
3	Chloramphenicol toxicity	F	25	33	1.1	88	122	239	0.72	0.50	79
	Mean Values			26	0.8	121	225	173	0.6	0.44	69
4	Exposure to Radiation (1 curie of cobalt)	M	31	43		114	96	114	1.19	0.75	118

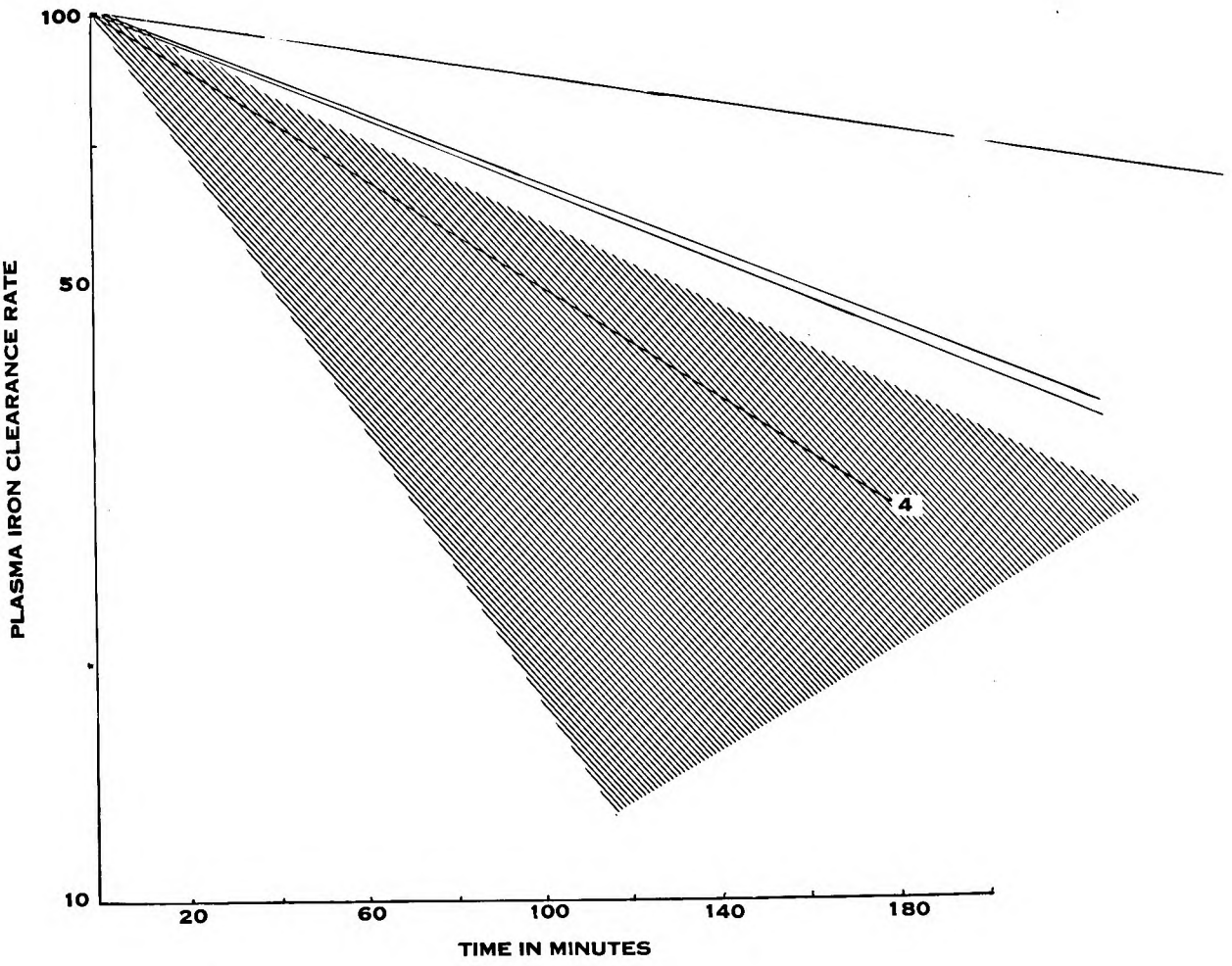


Figure 10 - Plasma Iron Clearance Rates in Three Patients with Hypoplasia of the Marrow.
Patient No. 4 -- Plasma Iron Clearance Rate in a Patient Six Days After accidental Exposure to 1 curie of Cobalt.

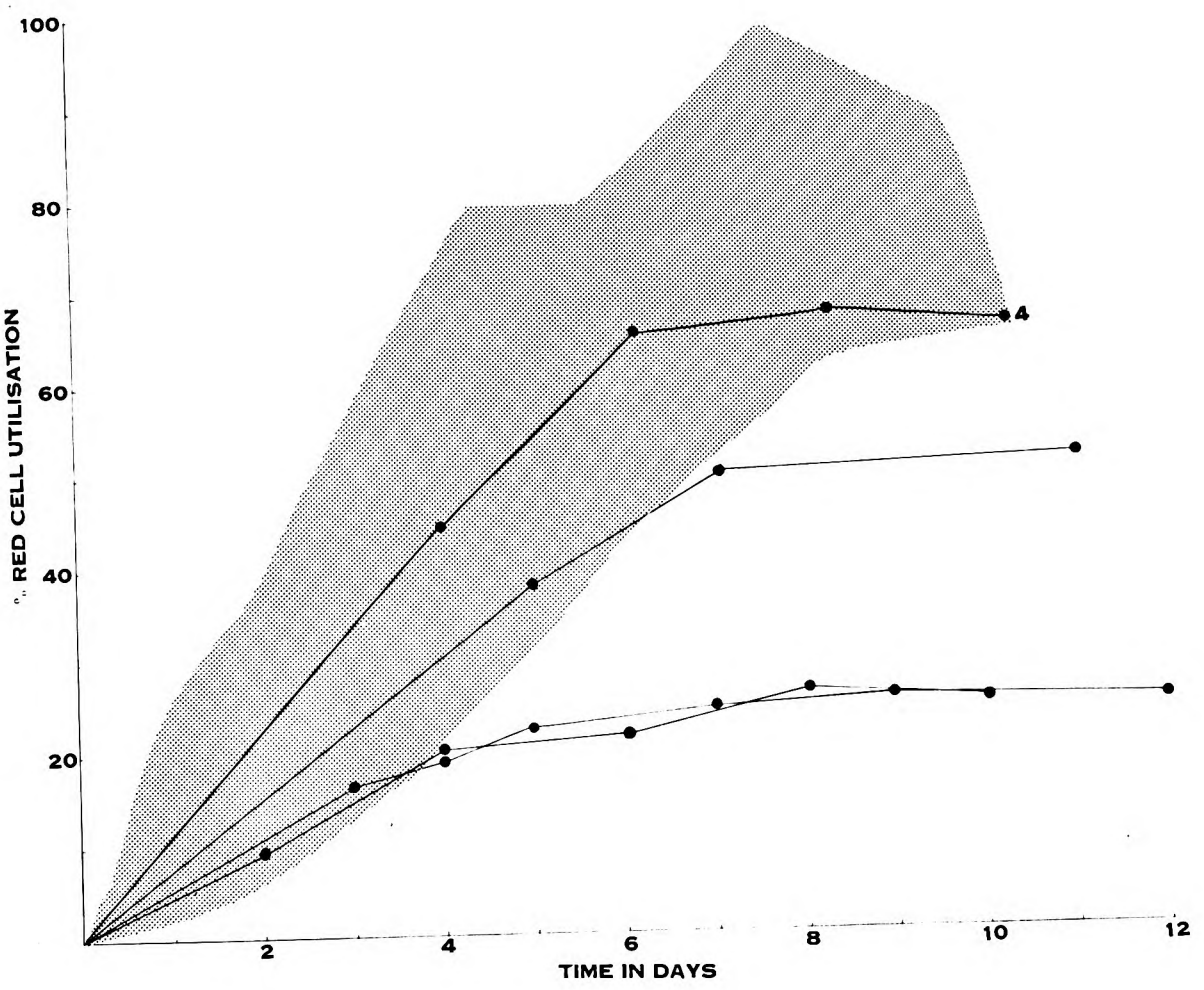


Figure 11 - Percentage Red Cell Utilisation Curves in Three Patients with Hypoplasia of the Marrow. Patient No.4 -- Percentage Red Cell Utilisation Curve in a Patient after Accidental Exposure to 1 Curie of Radioactive Cobalt.

"Asiatic Influenza". Recovery was prolonged. Review of the history disclosed that she had been exposed over long periods to "Pyagra", "D.D.T." and "Agricura". She was given a "sulpha" drug during the pyrexial illness and had taken aspirins on occasions. On examination at that time there was a severe degree of anaemia and a few petechiae on her chest. There was no evidence of hepatic or splenic enlargement and no lymphadenopathy.

Investigations:

Urine - no albumin, no sugar and no increased urobilinogen excretion. Stools were positive for occult blood. Bleeding time more than 15 minutes. Hess's test positive. Clotting time 8 minutes.

Haemoglobin 7.3grams%
Red Blood Cells 2.3 million per cu. mm.
Haematocrit 22%
Mean Corpuscular Volume 96 c.u.
Mean Corpuscular Haemaglobin Concentration 33%
Leucocytes 2,500/cu.mm.
Neutrophilic leucocytes 31%
Eosinophilic " 5%
Monocytic " 10%
Lymphocytic " 54%
Reticulocytes 1.1%
Platelets 22,000/c.mm.
Direct Coombs' test negative.
Gastric Analysis - free hydrochloric acid present.
Bone marrow smears were markedly hypocellular.
Erythropoiesis was normoblastic.
The patient was given therapeutic trials of Imferon, vitamin B. 12 and Presnisolone (30 milligrams a day), but continued to

require blood transfusions (1000 ccs.) every three weeks.

The patient was first seen on 30.7.58. Clinical findings and routine investigations were as follows:

Haemoglobin 7.4 grams%

Red Blood Cells 4.04 million per cu.mm.

Haematocrit 25%

Mean Corpuscular Haemoglobin Concentration 30%

Leucocytes 4,000/c.mm.

Neutrophilic leucocytes		36%
Lymphocytic	"	57.5%
Eosinophilic	"	1.5%
Monocytic	"	5%

Reticulocytes 2%.

Agglutination tests for brucellosis and typhoid were negative. The Brucella Coombs' was negative. No "L.E." cells were seen. There was hypoplasia of all elements on bone marrow examination. No megakaryocytes were seen in the smears. Lymphoid reticulum cells and tissue mast cells, both commonly found in hypoplastic states, were increased. No primitive or malignant cells were seen.

Her plasma iron was 180ug%. The half-time for the clearance of radioiron was 400 minutes. The plasma iron turnover was 0.376mg/100ml whole blood per day and the percentage red cell utilisation curve was markedly decreased (Fig.11, p. 52). Steroids (Prednisolone, 15mg q.i.d.) with antibiotic cover - penicillin (phenoxy-methyl-penicillin) 250mg. q.i.d. - failed to decrease the frequency of blood transfusions, and she was discharged on the above regime in the hope that a

remission might occur.

Discussion

The cessation of marrow function by the administration of cytotoxic drugs, radiation or blood transfusions, is followed by a marked slowing in the rate of clearance of radioiron from the plasma, a progressive rise in the serum iron and a plasma iron turnover of half normal which is probably due to the shift of the high serum iron to the liver and to the turnover of iron in tissues other than the erythroid marrow (Bothwell et al, 1957). The percentage red cell utilisation of radioiron is significantly reduced. The data in Case No.3, Table V, p.50 conforms to total marrow inactivity. Such a pattern, however, is uncommon in hypoplasia of the marrow. The findings in the remaining two patients were similar to those described by others, i.e. a normal or raised serum iron, delay in the clearance of radioiron from the plasma, a normal plasma iron turnover and a decrease in the percentage red cell utilisation of radioiron (Huff et al, 1950; Giblett etal, 1956; Bothwell et al, 1956; Bothwell et al, 1957). It is suggested that the finding of a normal plasma iron turnover with hypoplasia of the marrow is evidence of partial depression or of a degree of ineffective erythropoiesis.

While the plasma iron turnover has little accuracy as a quantitative measure of hypofunction of the marrow, qualitatively, however, it is of great value. It is generally agreed that a plasma iron turnover within normal limits in any anaemia other than iron deficiency anaemia, is almost certainly due to marrow

hypofunction (Bothwell et al, 1957).

A rise in the serum iron and a marked slowing in the rate of clearance of radioiron from the plasma are the earliest indications of erythroid arrest. Rubin et al (1958) observed these changes in five of fifteen subjects on chloramphenicol which could not be accounted for by the course of the underlying disease. They concluded that continued administration of the drug would have led to irreversible marrow aplasia and suggested serial plasma iron turnovers to detect impending marrow failure during treatment with a drug known to produce this effect (Rubin et al, 1960).

Radioiron studies were done in two patients to determine whether irradiation had caused any depression of erythropoiesis.

Mr. P.S., a traffic policeman, accidentally placed a capsule with 1 curie of cobalt 60 in his left thigh pocket where it remained for twenty minutes and for the next hour he was within 5-15 feet of it (Elliot, 1960). Three days later he developed radiation burns on the left thigh and there was intense generalised pruritus. Ferrokinetic studies on the sixth day indicated normal marrow activity (Case No.4, Table V, p. 50 ; Fig.10, p. 51). The bone marrow smear was normal and the subsequent uptake of radioiron into red cells was normal (Fig.11, p. 52). As anticipated no haematological sequellae developed.

Mr. P., a proven case of Hodgkin's disease, had been treated with several courses of deep x-ray therapy to the spleen, (Case.No.5, Table VIII, p.68). He subsequently developed an anaemia for which he required repeated blood transfusions. As he had a reticulocytosis of only 4 per cent and only a slight

increase in the amount of stercobilinogen in the stool, it was thought that bone marrow depression may have followed radiation therapy. While the serum iron was high (133 micrograms per cent) the rate of clearance of the radioiron was rapid (half-time 60 minutes) and the plasma iron turnover was 2.7 times normal. This excluded any marrow depression due to ionizing irradiation and indicated that the anaemia was secondary to Hodgkin's disease in which the plasma iron turnover may be markedly increased (Bergsagel and Giannopoulos, 1959).

SIMPLE CHRONIC ANAEMIAS

The anaemia associated with the majority of infections and chronic systemic diseases has been called simple because there is no very radical alteration from the normal blood picture. It is ordinarily mild in degree and overshadowed by the underlying disease (Wintrobe, 1956).

INFECTION

Four subjects with various types of infection were studied (Table VI, p. 59; Fig. 12, p. 60). Three of these patients had tuberculosis and the fourth pyelonephritis complicating cirrhosis of the liver. Although all were on therapy when the plasma iron turnover was done they were still ill. The plasma iron concentration was below normal in three. The rate of clearance of the radioactive iron was normal. The plasma iron turnover ranged from 0.35 mg. to 1.21mg. of iron per 100ml of blood per day (mean 0.68). The red cell utilisation curve was measured in only two patients, in both of whom it was normal (Fig. 13, p. 61). During this period the patients were still running a high temperature.

Ferrokinetic studies in infection have been reported in animals (Keiderling, 1958) and in man (Bush et al, 1956; Keiderling, 1957). The plasma iron turnover in animals exposed to various forms of infection was slightly above normal. In man, Keiderling (1957) found a mean plasma iron turnover of

TABLE VI

Plasma Iron Turnover Studies in Four Patients with Infection

Case No.	Diagnosis	Sex	Age	PCV	% Reticulocytes	Plasma Iron ug/100ml	Time taken for radioiron to reach half concentration (minutes)	Plasma Iron Turnover mg/day/100ml plasma	Plasma Iron Turnover mg/day/100ml whole blood	Erythropoietic Index
1	Pulmonary Tuberculosis & Diabetes	M	29	33	1.7	35	69	0.50	0.35	56
2	Cirrhosis of Liver & pyelo-nephritis	M	46	37	1.1	39	46	0.87	0.58	92
3	Cavitating Pulmonary Tuberculosis	F	22	34	2.0	37	40	0.93	0.64	102
4	Cavitating Pulmonary Tuberculosis	M	27	30	2.2	70	43	1.63	1.21	192
	Mean Values and Range			33.5	1.5	45.5	49.5	0.98	0.69	110.5
				30-37	1.1-2.2	35-70	40-69	0.5-1.63	0.35-1.21	56-192

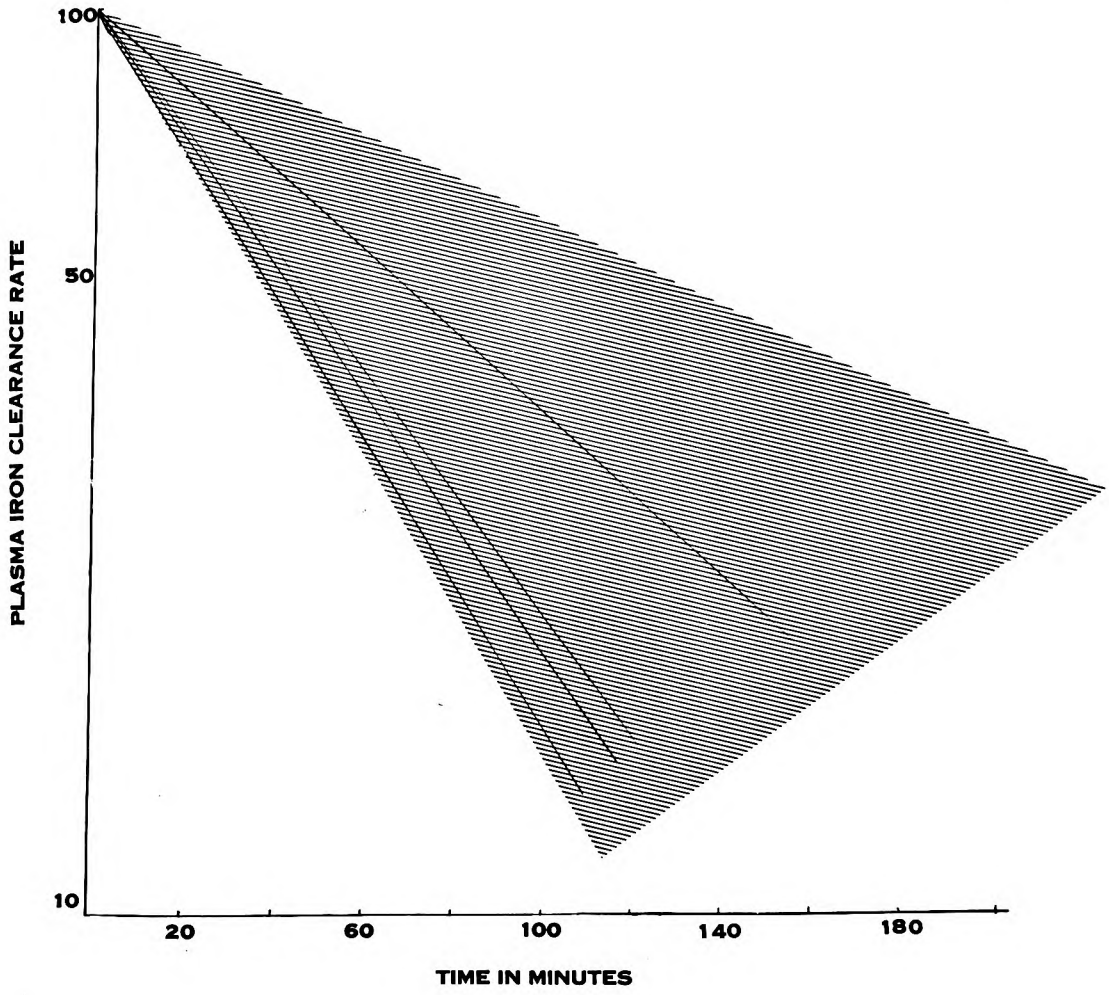


Figure 12 - Plasma Iron Clearance Rates in Four Patients with Infection.

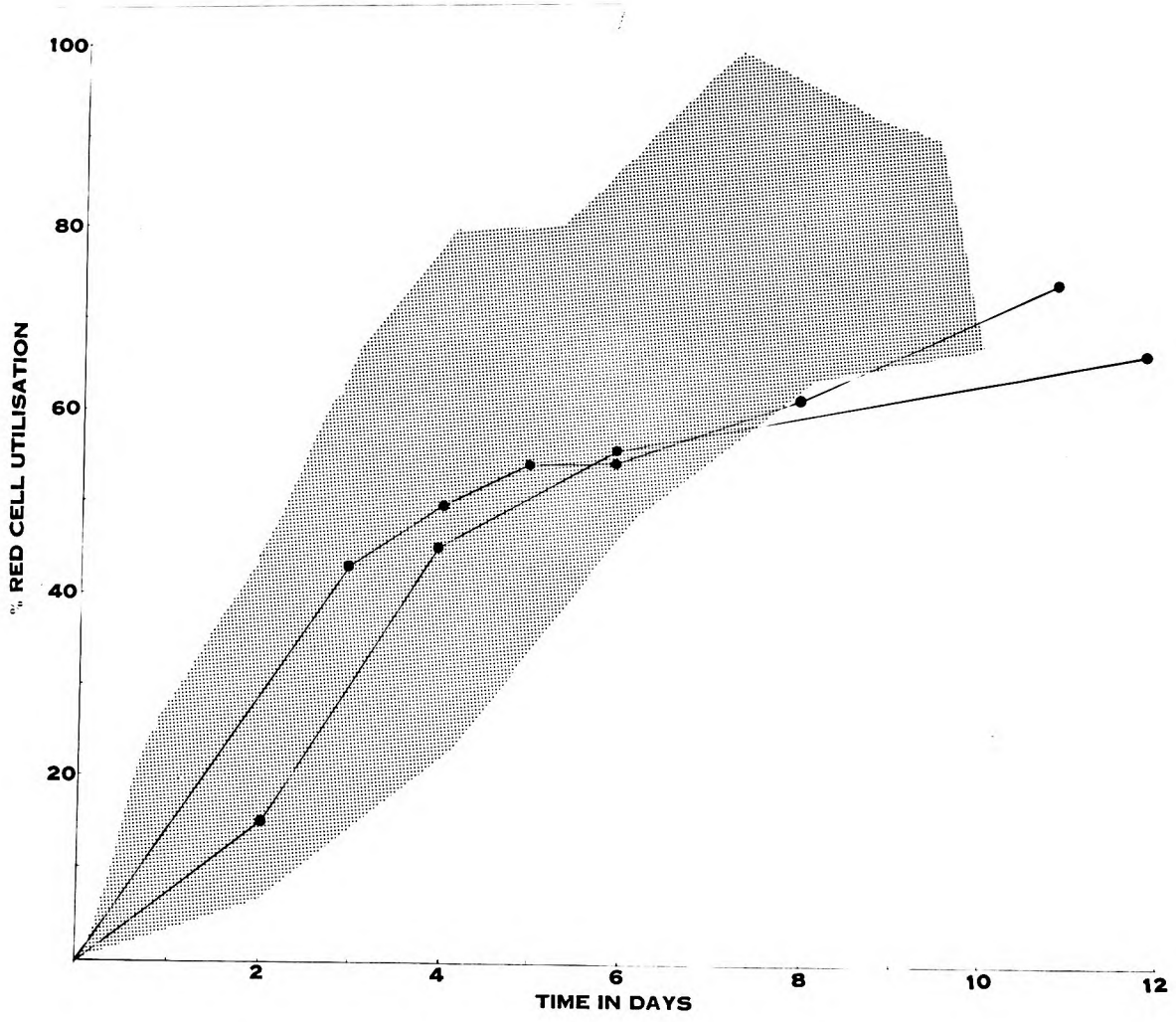


Figure 13 - Percentage Red Cell Utilisation Curves in Two Patients with Chronic Infection and Anaemia.

1.8 times normal, and in the study by Bush et al (1956) the mean plasma iron turnover was normal. In another study it lay between 0.9 and 1.4 times normal (Bothwell et al, 1956). In the presence of anaemia a normal plasma iron turnover indicates marrow hypofunction (Bothwell et al, 1957). If this were the only cause for the anaemia the percentage red cell utilisation should be reduced. Greenberg et al (1947) and Finch et al (1949) found in infection a profound lowering in the red cell utilisation of radioiron. However, such results were obtained with relatively large doses of radioiron. In animals Keiderling (1958) noted a slight fall (90-70%) in the percentage red cell utilisation. Using tracer amounts of radioiron Bush et al (1956) and Bothwell et al (1956) showed that the red cell utilisation of radioiron in infection was normal. These findings indicated that hypofunction of the marrow was not the only factor in the pathogenesis of the anaemia of infection. Although the usual evidence for increased blood destruction, increased urobilinogen excretion and elevated serum bilirubin concentration are lacking in infection, the erythrocyte life span is shortened (Keiderling, 1957; Bush et al, 1956). Normal cells may have a markedly shortened survival when transfused into patients with malaria, septicaemia or other acute infectious processes (Berlin et al, 1959). The survival of normal cells or of autologous ^{51}Cr labelled red cells in patients with chronic infectious processes is slightly reduced ($\pm$ 25 per cent), (Wintrobe, 1956). These various studies suggest that in

infection there is a marked inability of the bone marrow to compensate for the modest degree of shortening of the erythrocyte life span.

In infection the level of transferrin in the plasma is lower than normal and the plasma iron falls (Cartwright and Wintrobe, 1949). There is still, however, stainable iron in the marrow. This has suggested a block in the release of iron from the reticulo-endothelial system. It has been postulated that the failure to release iron from the reticulo-endothelial system may be a factor in the production of the anaemia in infection (Freireich et al, 1957a).

Iron kinetic studies in anaemic patients with rheumatoid arthritis are very similar to those in infection. Freireich et al (1957b) found a mean plasma iron level of 40 micrograms per 100ml of blood, a mean plasma iron turnover of 1.3 times normal and a normal percentage red cell utilisation. They concluded that the anaemia in rheumatoid arthritis was due to an inadequate marrow response to a mild degree of haemolysis. Weinstein (1959) also found in rheumatoid arthritis a slightly impaired red cell survival with inability of the marrow to adjust.

CHRONIC URAEMIA

The anaemia of chronic uraemia was studied in five patients (Table VII, p.64 ; Fig.14, p. 65). The plasma iron turnover was repeated in one patient after an interval of six months. The plasma iron concentration was very low in only one patient (25 micrograms per cent). The plasma iron turnover ranged from

TABLE VII

Plasma Iron Turnover Studies in 5 Patients with Chronic Renal Disease

Case No.	Sex	Age	Urea	PCV	% Reticulocytes	Plasma Iron ug/100ml	Time taken for radioiron to reach half concentration (minutes)	U.I.B.C.	Plasma Iron Turnover mg/day/100ml plasma	Plasma Iron Turnover mg/day/100ml whole blood	Erythropoietic Index
1A	M	38	200	29	2.7	110	108	205	1.02	0.75	119
B	M	38	190	29	1.0	48	71	270	o.65	0.49	78
2	F	35	240	24	2.0	96	140	129	0.69	0.54	86
3	M	49	225	25	1.1	41	56	162	0.74	0.57	90
4	M	33	280	18	0.6	25	38	363	0.67	0.56	89
5	F	34	190	25	0.8	71	65	160	1.09	0.80	127
Mean Values and Range				25	1.4	65	79.7	215	0.81	0.62	98
				18-29	0.6-2.7	25-110	38-140	129-363	0.65-1.09	0.49-0.80	78-127

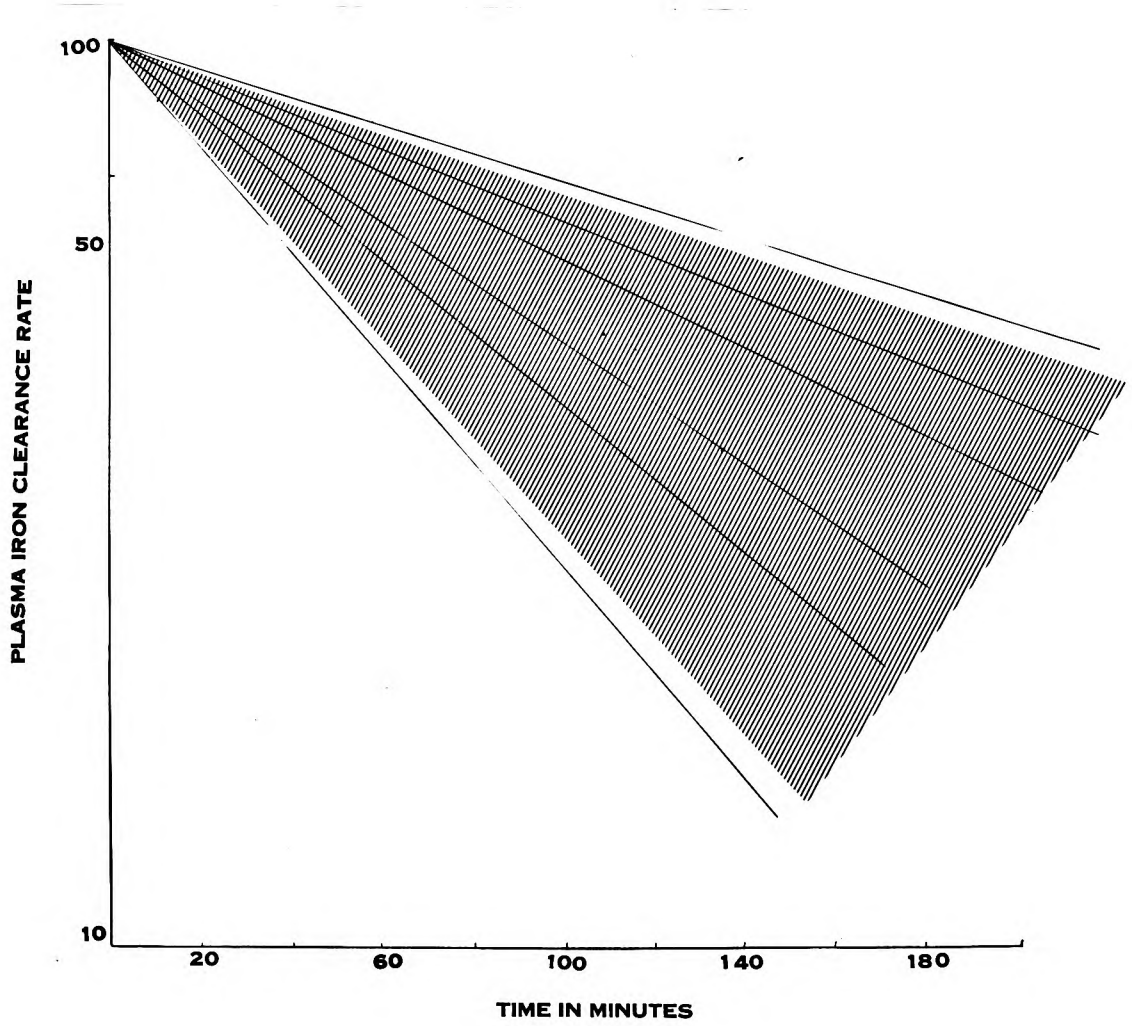


Figure 14 - Plasma Iron Clearance Rates in Five Patients with Uraemia. (The plasma iron clearance rate was repeated in one case after six months.)

0.49 to 0.8mg/100ml whole blood/day (mean 0.62mg/100ml blood/day). Three patients were transfused with packed cells, so their percentage red cell utilisation of radioiron was not estimated, but the percentage red cell utilisation curves in the remaining two were normal (Fig.15, p. 67). It is evident that radioiron studies in chronic renal disease are in many ways similar to those in infection, i.e. in the presence of anaemia there is a normal plasma iron turnover and percentage red cell utilisation curve. This suggests failure of the marrow to increase its activity in response to haemolysis. It has been shown that the survival of transfused red cells in patients with renal disease is shortened (Dock and Thedering, 1952). Red cells with a decreased ^{51}Cr half-life in their own circulation survived normally in normal recipients. This indicated that an extracorpuscular factor causes haemolysis in uraemia, (Berlin et al (1959)) and Ragen et al (1960) noted that the percentage red cell utilisation of radioiron was normal in two cases and decreased in three others. Joske et al (1956) also found a reduced percentage red cell utilisation in chronic renal disease. They concluded that the anaemia of chronic renal disease was primarily due to bone marrow depression. They found that red cell survival was decreased only in the late phase of chronic renal disease.

THE ANAEMIA IN VARIOUS NEOPLASTIC STATES

The diagnosis of malignancy was established, or strongly suspected in six patients (Table VIII, p. 68; Fig.16, p.69). The findings in the patients with lymphatic leukaemia and

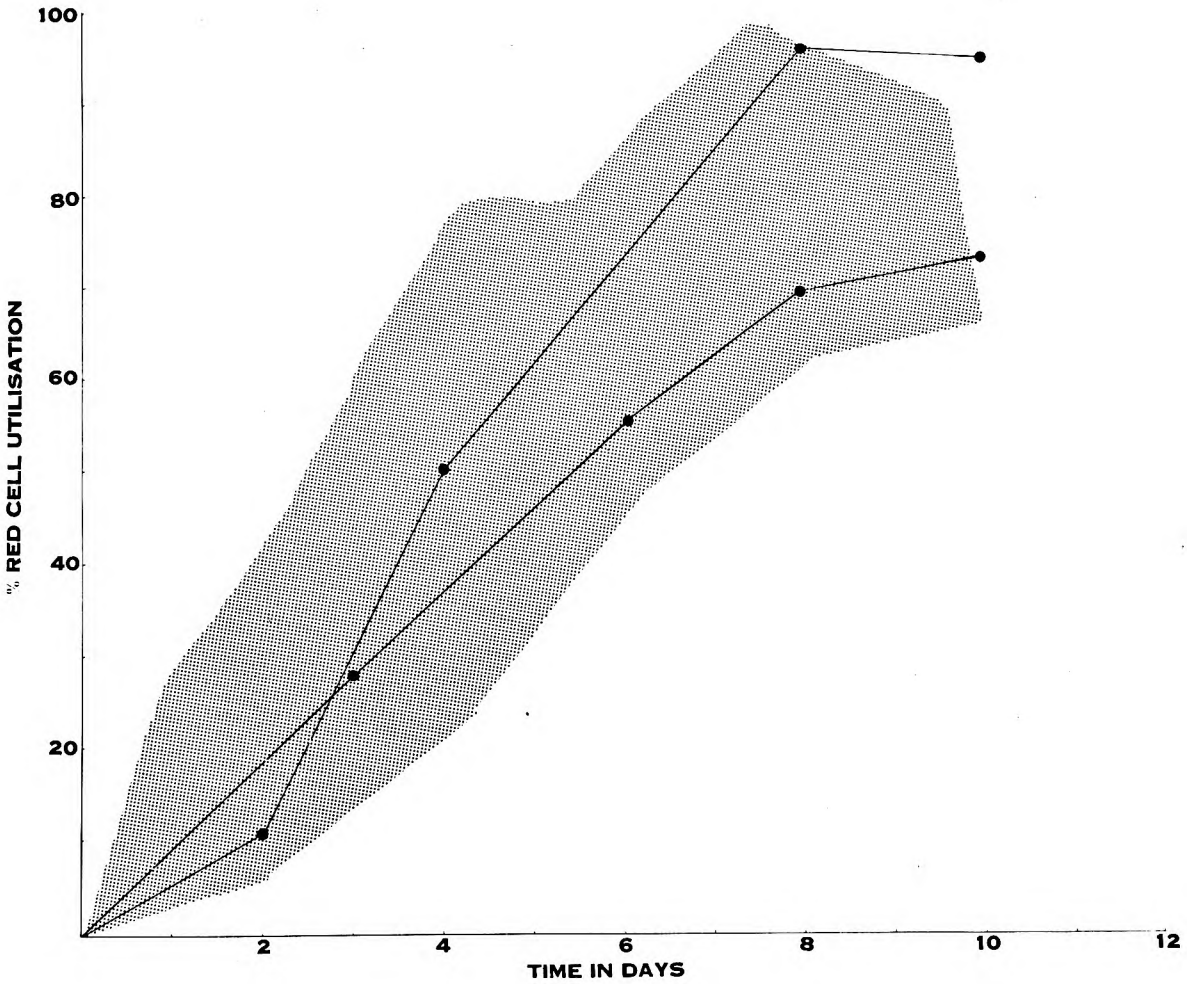


Figure 15 - The Percentage Red Cell Utilisation Curves in Two Patients with Uraemia.

TABLE VIII

Case No.	Diagnosis	Sex	Age	PCV	% Reticulocytes	"Corrected" % Reticulocytes	Plasma Iron ug/100ml	UIBC	Time taken for radioiron to reach half concentration (Minutes)	Plasma Iron Turnover mg/day/100ml plasma	Plasma Iron Turnover mg/day/100ml whole blood	Erythropoietic Index "Corrected" % Retic	P.I.T.
1	Neoplastic involvement of spine	M	69	39	4.0	3.4	49	202	27	1.81	1.2	261	192
2	Malignant lymphoma	M	39	34	2.8	2.1	106	175	51	2.08	1.44	161	229
3	Leuro-erythro-blastic anaemia in multiple myelomatosis	M	44	37	9.2	7.5	67	176	21	3.19	2.10	577	333
4	Carcinoma of liver	F	45	38	4.0.	3.4	82	122	40	2.05	1.31	261	208
5	Hodgkin's Disease	M	30	22	4.0	2.0	133	50	60	2.22	1.78	154	283
6	Lymphatic Leukaemia	M	57	20	0.5	0.2	273	0	220	1.24	0.87	15	138
Mean Values and Range				32 20-39	4.1 0.5-9.2		118 49-273	121 0-202	70 27-220	2.1 1.24-3.19	1.45 0.87-2.1		230 138-333

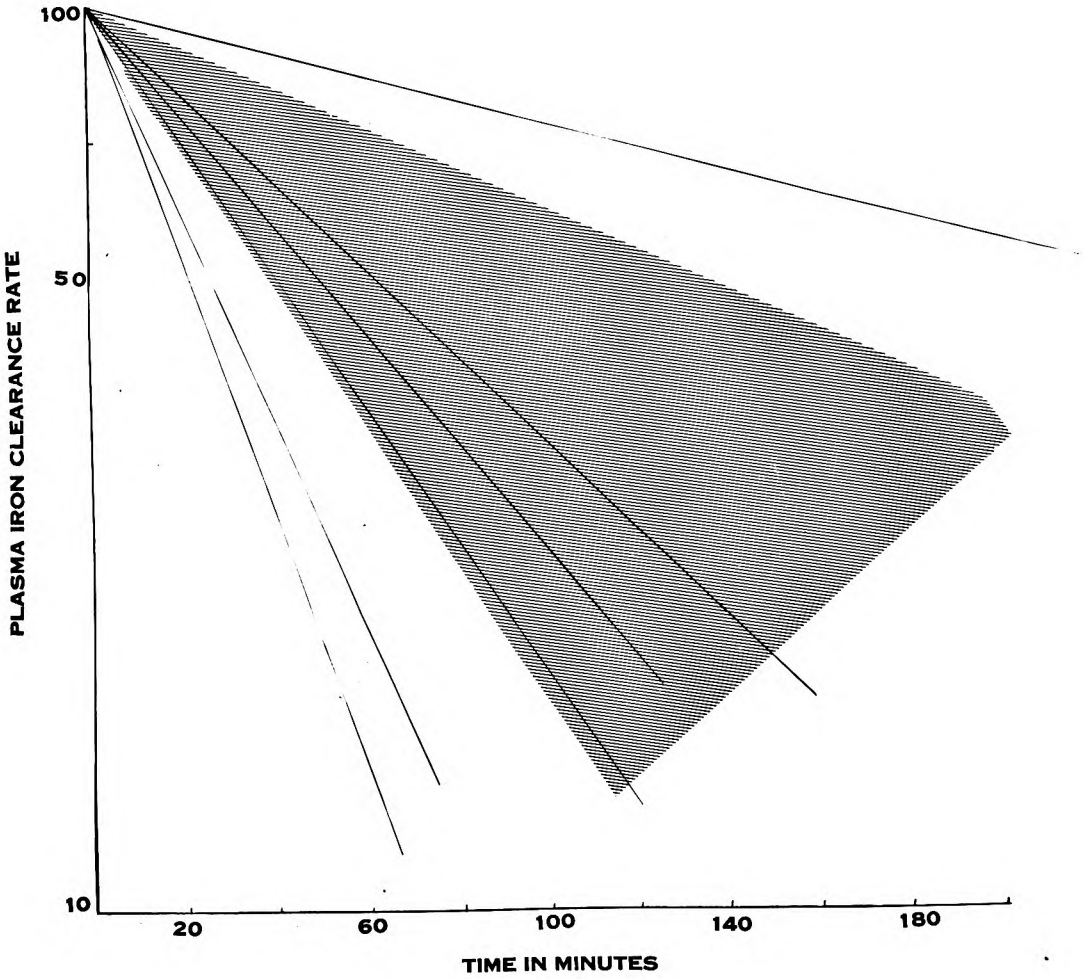


Figure 16 - Plasma Iron Clearance Rates in Six Patients with Neoplasm. A Slow Clearance Rate was Found in the One Patient with Lymphatic Leukaemia.

Hodgkin's disease corresponded to those of Bergsagel and Giannopoulos (1959). In the former (Case No.6, Table VIII, p.68) a raised plasma iron and a decrease in the fraction of iron removed per hour gave a normal plasma iron turnover. The percentage red cell utilisation curve in this patient was markedly reduced (Fig.17, p. 71). In the latter (Hodgkin's disease, Case No.5, Table VIII, p.68) the plasma iron turnover (1.78ml/100ml blood) was 2.7 times normal. In Hodgkin's disease hypoferraemia is the rule, but the serum iron was raised in this patient, probably as a result of numerous blood transfusions. The plasma iron turnover was increased in the remaining four patients, (1.21 - 2.1mg/100ml whole blood/day). In the absence of significant blood loss this indicated either haemolysis or ineffective erythropoiesis. There was no significant reduction of the percentage red cell utilisation curves in three of these four patients, in whom the corrected reticulocyte count was 3.4, 3.4 and 7.5 per cent respectively. These findings suggested haemolysis rather than ineffective erythropoiesis.

There have been several radioiron studies in patients with carcinoma, lymphomas or leukaemia. The plasma iron turnover was normal or slightly raised in 33 of 37 carcinomatous patients (Miller et al, 1956). In two patients it was reduced and in the remaining patients it was raised 2-4 times normal. In acute and subacute myelogenous leukaemias the plasma iron turnover has ranged from 0.4 times normal (Bothwell et al, 1956)

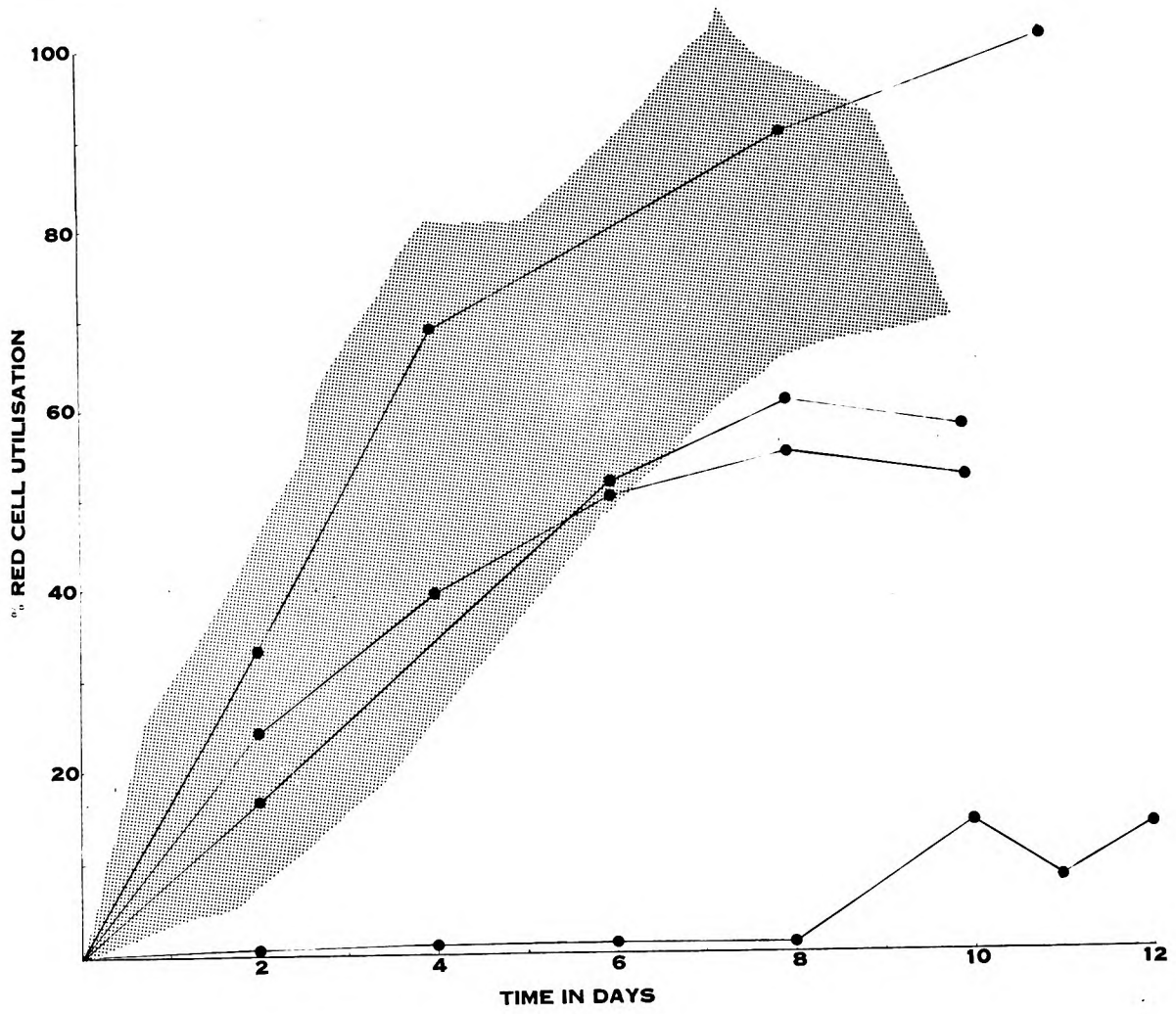


Figure 17 - Percentage Red Cell Utilisation Curves in Four Patients with Neoplasm. A markedly reduced percentage red cell utilisation was noted in the one case with lymphatic leukaemia.

to 1.3 times normal (Elminger et al, 1953). In chronic myelocytic leukaemia the average plasma iron turnover was 2.4 times normal with a 50 per cent red cell utilisation (Huff et al, 1950). Huff et al (1950) also reported a mean plasma iron turnover of 1.4 times normal (range 0.5 - 2.4) in lymphatic leukaemia and a plasma iron turnover of 5.4 times normal was found in one patient with lymphatic leukaemia and a haemolytic anaemia (Elminger et al, 1953). Bergsagel and Giannopoulos (1959) noted a difference in the plasma iron turnover in lymphatic leukaemia as compared with Hodgkin's disease. In the former the mean plasma iron turnover was 0.46mg per cent and the red cell utilisation of radioiron 37 per cent. In Hodgkin's disease the mean plasma iron turnover was 1.36mg/100ml whole blood and the red cell utilisation 72 per cent. Hypoferraemia was found in Hodgkin's disease (Bergsagel and Giannopoulos, 1959) and in 17 of the 37 cases of carcinoma studied by Miller et al (1956) there was a fall in the serum iron with progression of the disease.

Anaemia in neoplastic conditions may be due to -

1. depressed marrow function even in the absence of marrow infiltration with metastases (Miller et al, 1956)
2. increased blood destruction (Miller et al, 1956; Desforges et al, 1960)

or a combination of both.

The pattern of shortened red cell survival with failure of the marrow to increase its activity is common to infection, rheumatoid arthritis, neoplastic states, cirrhosis of the liver (Loeffler, 1956) and chronic nitrogen retention. In addition

in infection, rheumatoid arthritis (Weinstein, 1959), Hodgkin's disease and some cases of carcinoma, there is a block in the release of iron from the reticulo-endothelial system. It is doubtful whether this defect in mobilisation of iron from the reticulo-endothelial system contributes towards functional impairment of erythropoiesis since in Hodgkin's disease, although hypoferraemia is present, the plasma iron turnover is greater than twice normal. If in any of the above-mentioned conditions marrow hypofunction is the major factor in the production of the anaemia, the plasma iron turnover will be normal. Conversely, if the plasma iron turnover is significantly increased, haemolysis or ineffective erythropoiesis must be the principal component.

POLYCYTHAEMIA

The diagnosis of polycythaemia was made when the packed cell colume was greater than 57 per cent. The patients were then classified according to the type of polycythaemia (Table IX p.74 ; Fig.18, p. 76). There were five patients (Cases No.1-5, Table IX, p. 74) with polycythaemia vera and in four of these the spleen was palpated (Table X, p. 75). In two of these patients (one of whom had a significantly raised alkaline phosphatase) there was clinical and radiological evidence of emphysema, but this was almost certainly not the cause of the polycythaemia, as lung function studies were normal. The evidence for polycythaemia vera in the fifth patient was a significantly increased red cell mass (51.3ml/kilogram body weight)

TABLE IX

Plasma Iron Turnover Studies in Patients with Polycythemia

Case No.	Diagnosis	Sex	PCV	% Reticulocytes	Plasma Iron ug/100ml	UIBC	TIBC	Time taken for radioiron to reach half concentration (minutes)	Plasma Iron Turnover mg/day/100ml plasma	Plasma Iron Turnover mg/day/100ml whole blood	Alk. Phos.	Erythropoietic Index
1	Polycythemia Vera	M	70	2.0	90			43	2.10	0.76	15 30	121
2	"	F	64	2.4	86	193	279	34	2.53	1.06	228	168
3	"	M	63	1.1	39	355	394	20	1.95	0.80	150	127
4	"	M	73		46			23	2.00	0.68		108
5	"	M	62	1.8	114	225	339	50	2.28	0.96	12.5	152
	Mean Values and		66.4	1.8	75			34	2.17	0.85		135
	Range		62-73	1.1-2.4	39-114			20-50	1.95-2.53	0.68-1.06		108-168
6	Secondary Polycythemia	M	75	1.3	118	389	507	36	3.28	1.05		167
7	"	M	74	1.2	64	238	302	41	1.56	0.50	19.2	79
8	"	M	70	1.9	96			96	1.00	0.37		59
9	"	F	68	1.7	118			36	3.27	1.24	22.5	197
10	"	M	57	2.4	60	354		38	1.73	0.84		133
	Mean Values and		67	1.7	91	327		49	2.17	0.80		127
	Range		57-75	1.2-2.4	60-118	238-389		36-96	1.0-3.28	0.37-1.24		59-197
11	Relative Polycythemia	M	72		126			64	1.96	0.66		106
12	"	M	65		80	270	350	60	1.33	0.553		88
13	? Renal Polycythemia	M	65		154	125	279	71	2.17	0.933	17	148
14	Primary Polycythemia	M	56		34			18	1.89	0.91	89	144

TABLE X

History, Clinical Findings and Relevant Investigations in Five Cases with Polycythaemia Vera

Case No.	Sex	Age	History	Clinical Findings	Investigations
1	M	50	Admitted with a gripping chest pain of four hours duration.	Plethoric. B.P.130/90. Emphysema. 3-finger splenomegaly	Hb.21G%. W.B.C.10,000/cmm. Lung function studies - "No reason for developement of polycythaemia secondary to cardio-pulmonary disease." Alkaline phosphatase = 30 units.
2	F	50	Giddy spells and vomiting for 8 months. 44 lbs. weight loss and intense pruritus after a bath	Plethoric. 2-finger splenomegaly	Hb.22.6G%. W.B.C. 26,000/c.mm. Neutrophils 81% Albumin 4.12G% Alk.phosphatase 228 units.
3	M	60	Severe gripping chest pain accompanied by vomiting and sweating and lasting 4 hours. Past history of coronary thrombosis	Plethoric adult male. B.P. 160/100 3-finger splenomegaly	Hb.19.1G% W.B.C. 22,500/c.mm. Alk.phosphatase 150 units.
4	M	54	Black stools for one day. For 6 months had experienced intense generalised puritus after a bath.	Plethoric. B.P.150/80. Confused and a flapping tremor. Emphysematous. Uneventful recovery. 2-finger splenomegaly.	Hb.24G%. WBC 13,000/c.mm. Total protein 8G% Alb.3.53G%. Gamma Glob. 2.23G%. Bilirubin 2.0mg% Alk.phosphatase 75 units. X-ray chest - Emphysema Lung function studies n.a.d.
5	M	46	Plethora for 10 years. Tiredness for 5 years. Intense pruritus after a bath for 2 years.	Plethoric. B.P. 200/120. No splenomegaly.	1955- Hb.24.8G%. WBC 3,400/c.mm. Red Cell Mass 51.3ml/kilo (average normal 35.8; range 29-43) Lung Function studies - n.a.d. 6 phlebotomies 1956. P^{32} 1956 and 1957. Alk. phosphatase 12.5units.

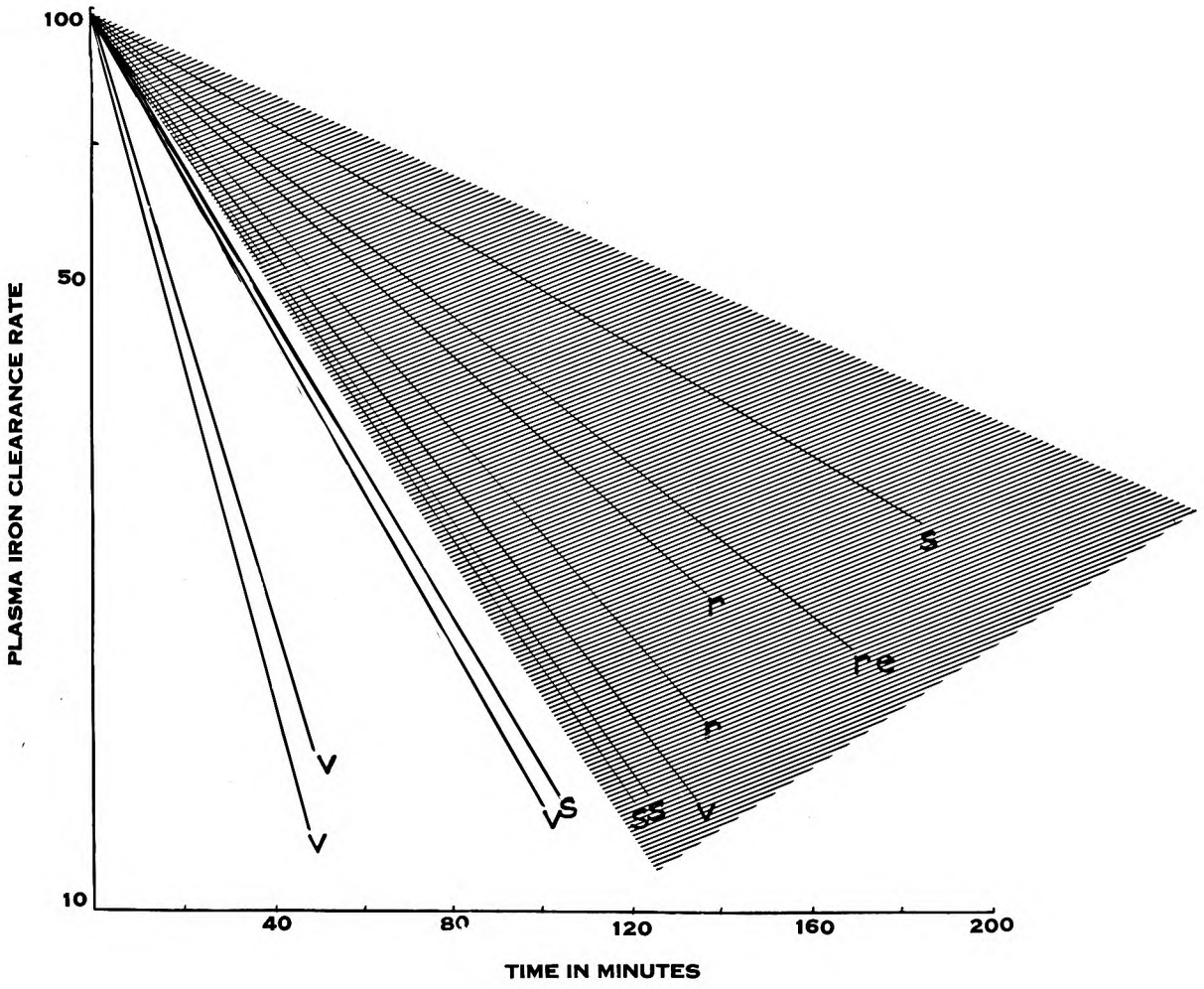


Figure 18 - Plasma Iron clearance rates in thirteen patients with Polycythaemia. Two patients with polycythaemia vera and two patients with secondary polycythaemia had the same clearance rates, 34 and 36 minutes respectively.

V = Polycythaemia vera.
S = Secondary Polycythaemia.
r = relative Polycythaemia.
re = Polycythaemia secondary to renal disease.

(Ellis, 1953) without any apparent cause. There was no splenomegaly nor was the alkaline phosphatase raised. The patient had, however, received intensive therapy with radioactive phosphorus (P^{32}) 18 months prior to investigation. There were five patients with secondary polycythaemia complicating severe respiratory disease (Cases No.6 -10, Table IX, p. 74). The plasma iron turnover in the patients with polycythaemia vera ranged from 0.68 - 1.06 mg/100ml blood/day (mean 0.85). In the patients with secondary polycythaemia the range was 0.37 - 1.24mg/100ml blood/day (mean 0.79). In all these patients the percentage red cell utilisation was rapid and complete (Fig.19, p. 78). Two patients (Cases No.11 and 12) had relative polycythaemia. Their haematocrits were greater than 57 per cent, but the red cell mass measured with Cr^{51} labelled red cells was normal (33 and 35 ml/kilogram respectively). The plasma iron turnover in each was normal. One patient (Case No.13 Table IX, p. 74) was difficult to classify:

Dr. G.L. was regarded as a mild case of polycythaemia vera. His red cell mass (estimated from Cr^{51} labelled red cells) was slightly raised (42ml per kilogram body weight; normal range 27 - 40ml per kilogram body weight). Splenomegaly, leucocytosis and thrombocythaemia were absent. His plasma iron turnover (0.93mg per 100ml whole blood) was at the upper limits of normal. In 1955 a calculus was removed from the pelvis of the right kidney. Polycythaemia is known to complicate renal pathology, including hydronephrosis (Gardner and Traymann, 1958; Jones et al, 1960).

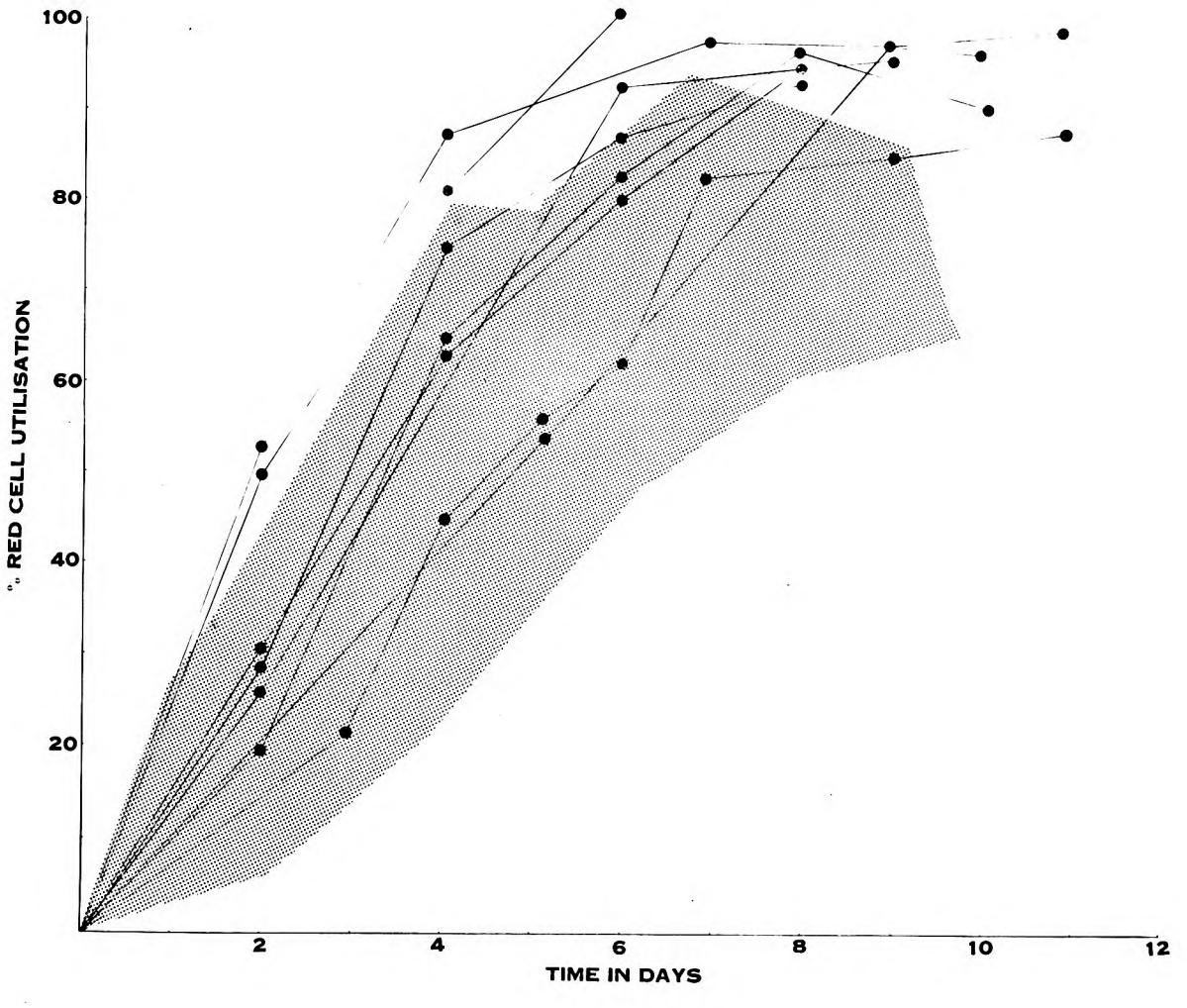


Figure 19 - Percentage Red Cell Utilisation Curves in Four Patients with Polycythemia Vera and Four Patients with Secondary Polycythemia.

It is possible that in this patient the calculus had caused hydronephrosis which was complicated by polycythaemia.

Huff et al (1950) reported a difference in ferrokinetics between polycythaemia vera and secondary polycythaemia. The mean plasma iron turnover in untreated patients with polycythaemia vera was 3.8 times normal, as compared with secondary polycythaemia where the increase in the plasma iron turnover was twice normal. They suggested the plasma iron turnover as an additional test to distinguish between polycythaemia vera and secondary polycythaemia.

To explain their results in polycythaemia vera these workers postulated a double red cell population, one with a normal and the other a shortened life span. The theory of a double red cell population in polycythaemia vera is supported firstly by the finding of two peaks of incorporation of C^{14} labelled glycine into haemoglobin (Berlin et al, 1956) and secondly by the recent finding of a bizonal pattern of erythrocyte distribution as judged by blood chromatograms (Riley, 1956). However, a normal red cell life span was found when N^{14} labelled glycine was incorporated into haemoglobin (London et al, 1949). The radioactive iron studies have also not been confirmed. In polycythaemia vera Wasserman et al (1952) reported a plasma iron turnover of 2.4 times normal. Sharney et al (1954) observed in two patients that the radioiron clearance rates when plotted over several hours deviated from a straight line. When these curves were analysed on a two component basis, the plasma

iron turnover was significantly lower than the extremely high rates obtained with calculations based on a single component. Their results were compatible with a single red cell population with a normal life span.

Eleven untreated patients with polycythaemia vera were studied by Huff et al (1950). The mean plasma iron turnover was 3.8 times normal, but in one patient the plasma iron turnover was as much as ten times normal. The maximum marrow capacity is only six to eight times normal. Employing the same method for iron determination, the same laboratory reported a mean plasma iron of 180 micrograms per cent in healthy natives in the Andes, a figure that they felt was probably due to a technical fault. In the method they employed for iron estimation (Kitzes et al, 1944) the plasma is heated. Heat will dissociate iron from haemoglobin and a haemolysed specimen of blood will give a higher than normal plasma iron estimation. This will increase the plasma iron turnover which could then be as much as ten times normal. The mean plasma iron turnover of the remaining ten cases was 3.1 times normal, a figure more in keeping with the increase in size of the red cell mass in patients with polycythaemia.

Parker and Lawrence (1959), state that the plasma iron turnover in polycythaemia vera was greatly increased, leading to a calculated daily haemoglobin synthesis sometimes several times more than could be consistent with a normal red cell life span. Although there was a statistical difference between

the groups of patients with polycythaemia vera and secondary polycythaemia, in the individual case the plasma iron turnover by itself was not of diagnostic significance. The present number of cases is too small to determine if there is any statistically significant difference in radioiron studies between patients with polycythaemia vera and secondary polycythaemia. The results agree with most authors in that in the individual case the plasma iron turnover cannot distinguish between these two forms of polycythaemia.

Plasma iron turnovers have been reported in three patients with polycythaemia secondary to renal disease (Gardner and Traymann, 1958; Jones et al, 1960). The plasma iron turnover was 3.5 times normal in one case. This figure returned to normal after nephrectomy. The plasma iron turnover in Dr. G.L. in whom the polycythaemia may have been secondary to hydronephrosis, was similar to that found in the other two patients (1.5 times normal) (Jones et al, 1960).

One patient (Case No.14, Table IX, p.74) with primary polycythaemia was not included for analysis. The plasma iron turnover was preceded by repeated venesections with the result that the iron stores were mobilised and there was a fall in the serum iron. A low serum iron may complicate the interpretation of radioiron studies in polycythaemia (Bothwell et al, 1956).

RADIOIRON STUDIES IN PATIENTS WITH INCREASED AND DECREASED BODY IRON STORES

CYTOSIDEROSIS IN THE BANTU

Three Bantu patients with cytosiderosis were studied (Table XI, p.83; Fig.20, p.84). In association with the high serum iron level heavy deposits of iron were demonstrated in the marrow in two patients and in the liver in the third. The rate of clearance of the radioiron was normal, or slightly delayed, with the result that the plasma iron turnover was significantly increased in two patients (1.4 and 1.7 times normal). The percentage red cell utilisation was reduced in two patients (Fig.21, p. 85). In the third patient, from the extrapolation of the graph back to zero, it was apparent that some of the injected radioiron was not bound (Methodology, p.21). Hence this percentage red cell utilisation curve was not measured.

The findings are the same as in haemochromatosis (Bothwell et al, 1955; Bothwell et al, 1957; and Case No.4, Table XI, p.83). In haemochromatosis the increase in the plasma iron turnover is due to the deposition of iron in the liver, and is shown by an increase in uptake of radioactivity over the liver. Jandl has demonstrated in vitro that with 60 per cent saturation of plasma transferrin iron is passively transferred to the liver (Jandl et al, 1959). Although the distribution of iron in cytosiderosis in hospital patients differs from that in haemochromatosis in that in the former the iron accumulates predominantly in the reticulo-endothelial system (Higginson, 1957), whereas in the latter it is found in the liver cell, the plasma iron

TABLE XI

IRON OVERLOAD

Case No.	Diagnosis	Sex	Age	PCV	% Reticulocytes	Plasma Iron ug/100ml	Time taken for radioiron to reach half concentration (minutes)	Plasma Iron Turnover mg/day/100ml Plasma	Plasma Iron Turnover mg/day/100ml whole blood	Erythropoietic Index	Iron Stores
1	Cytosiderosis	M	36	54	1.7	261	138	1.89	0.96	148	++++ (marrow)
2	Cataract Cytosiderosis	M	56	51	1.3	178	140	1.27	0.69	106	++++ (marrow)
3	Abdominal Pain	F	39	44		199	110	1.81	1.09	168	++++ (liver)
	Mean values			50		213	129	1.46	0.91	140	
4	Haemochromatosis	M	35	51	0.4	200	112	1.79	0.97	150	

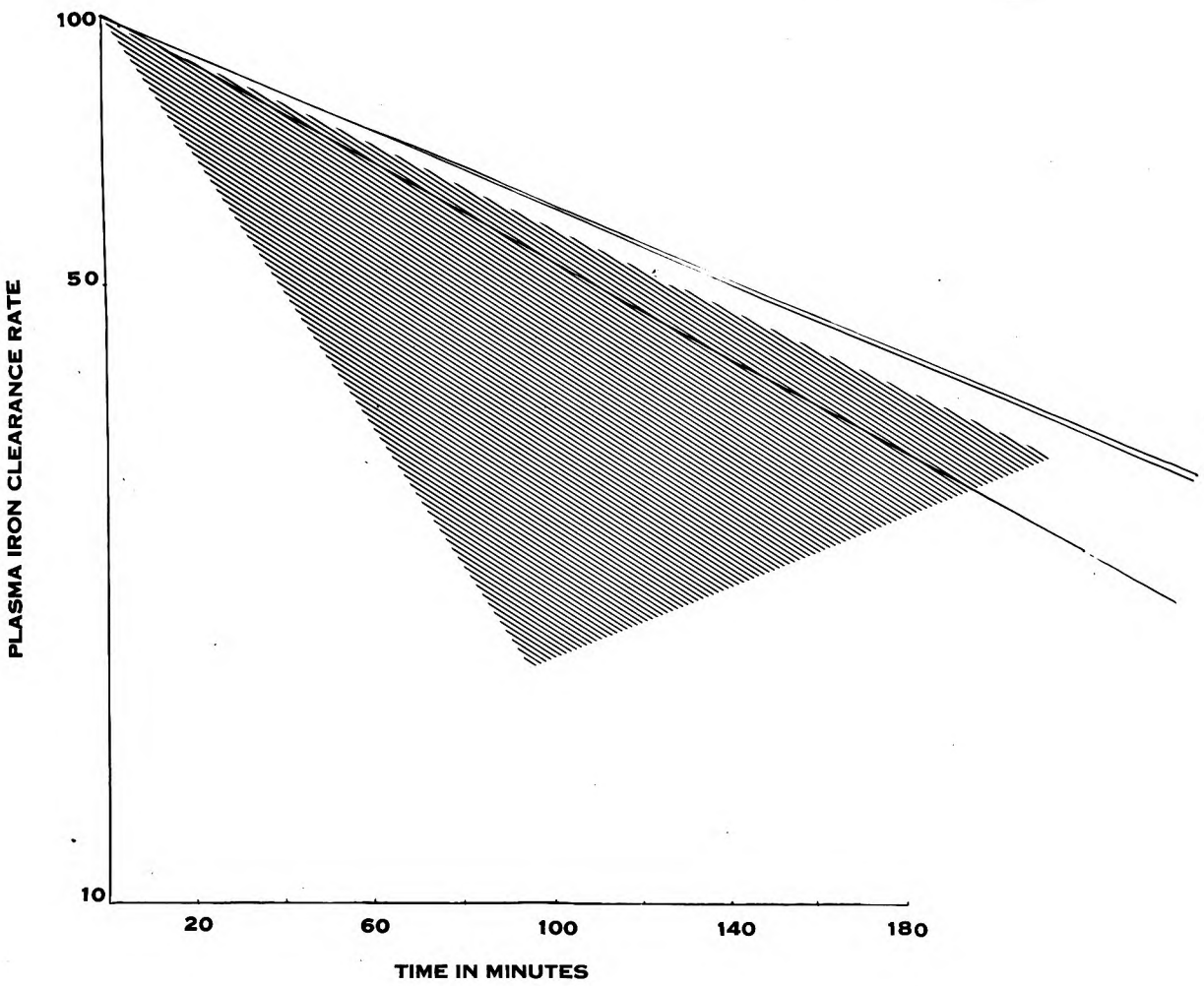


Figure 20 - Plasma Iron Clearance Rates in Three Patients with Cytosiderosis.

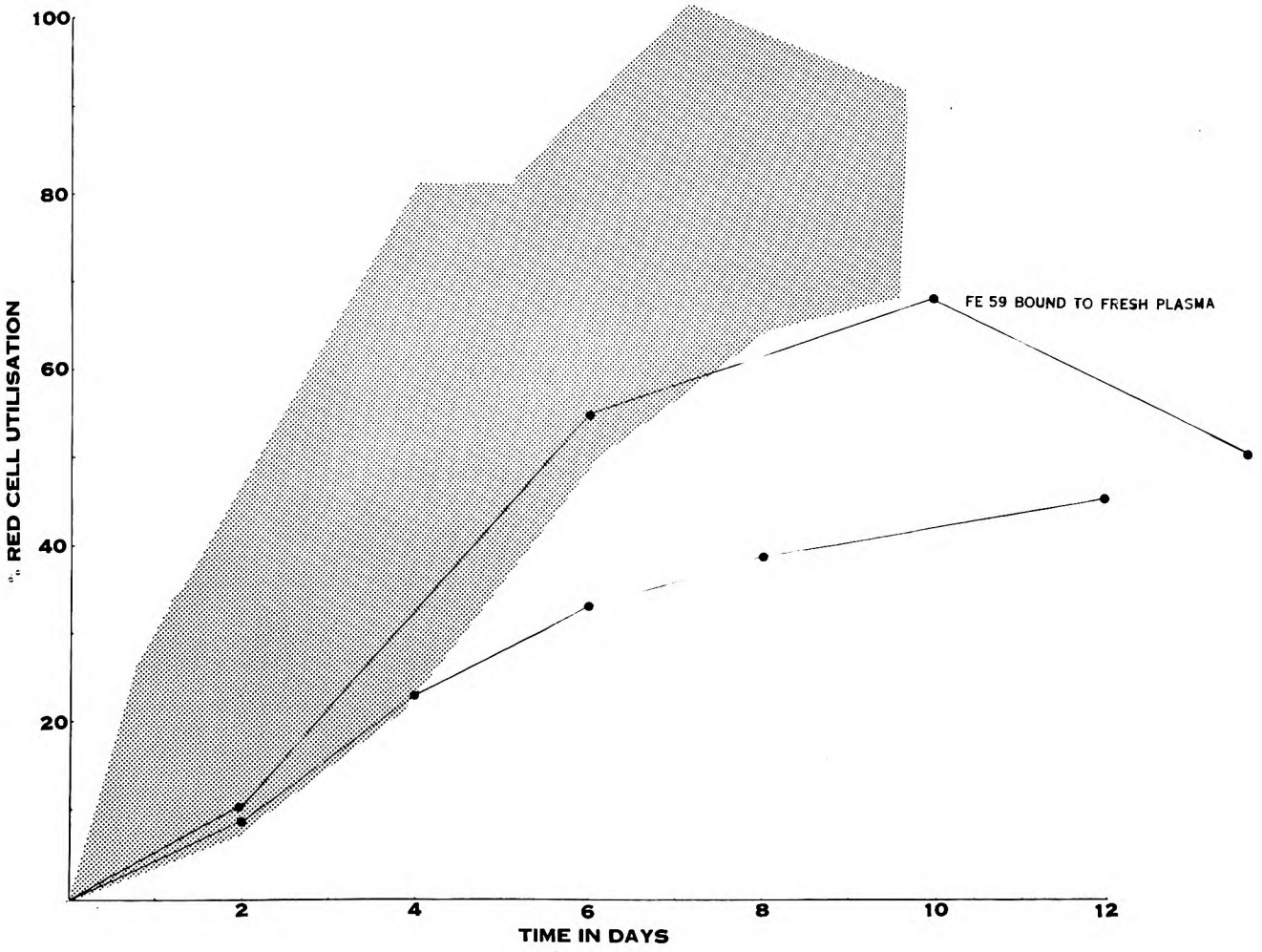


Figure 21 - Percentage Red Cell Utilisation Curves in Two Patients with Cytosiderosis.
In one case the radioiron prior to administration was bound to plasma.

turnover test and percentage red cell utilisation of radioiron are similar, and do not differentiate between them.

IRON DEFICIENCY ANAEMIA

Radioiron studies in seven patients with iron deficiency anaemia are recorded in Table XII, p. 87 . In six patients the plasma iron ranged from 14-22 micrograms per cent. In all patients the rate of clearance of iron was rapid (Fig.22, p. 88) and the plasma iron turnover per 100ml of whole blood per day was normal (mean 0.6mg per cent). The percentage red cell utilisation in the three patients studied was rapid and complete (Fig.23, p. 89). A normal plasma iron turnover with a 100 per cent red cell utilisation should produce a normal amount of haemoglobin. Similar findings have been reported before (Bothwell et al, 1956) and various explanations have been suggested for the presence of anaemia when haemoglobin production is apparently normal. 1) The indices that measure the plasma iron turnover may be inaccurate. It has been suggested (a) that the serum iron measurement may be inaccurate at such low levels in that pigments may be included in colorimetric estimation of the serum (Henry, 1959); (b) the rate of clearance is not a single exponential and that the analysis of the two components will give a plasma iron turnover in proportion to the haemoglobin level. 2) It is also possible that lack of iron is not the only factor in the production of anaemia in iron deficient subjects. Heiderling (1957) and Verloop et al (1960) using ^{51}Cr labelled red cells

TABLE XII

Plasma Iron Turnover Studies in Seven Patients with Iron Deficiency Anaemia

Case No.	Diagnosis	Sex	Age	PCV	% Reticulocytes	Plasma Iron ug/100ml	Time taken for radioiron to reach half concentration (minutes)	U.I.B.C.	Plasma Iron Turnover mg/day/100ml plasma	Plasma Iron Turnover mg/day/100ml whole blood	Erythropoietic Index
1	Menorrhagia	F	29	38	1.0	22	17	460	1.29	0.85	134
2	"	F	34	30		18	19	404	0.95	0.69	110
3	Emphysema, Bleeding Duodenal ulcer	M	42	35		18	16		1.13	0.76	121
4	Menorrhagia	F	28	33	0.7	14	18	290	0.78	0.55	87
5	Menorrhagia	F	36	31	0.49	23	25	390	0.92	0.66	105
6	Diaphragmatic Hernia	M	31	28	0.89	20	25.5	366	0.78	0.59	94
7	Menorrhagia	F	26	28		3	15	352	0.18	0.15	23
	Mean values and Range			32 28-38	0.70 0.7-1.0	17 3-22	19 15-25.5	377 290-460	0.86 0.18-1.29	0.61 0.15-0.85	95 23-134

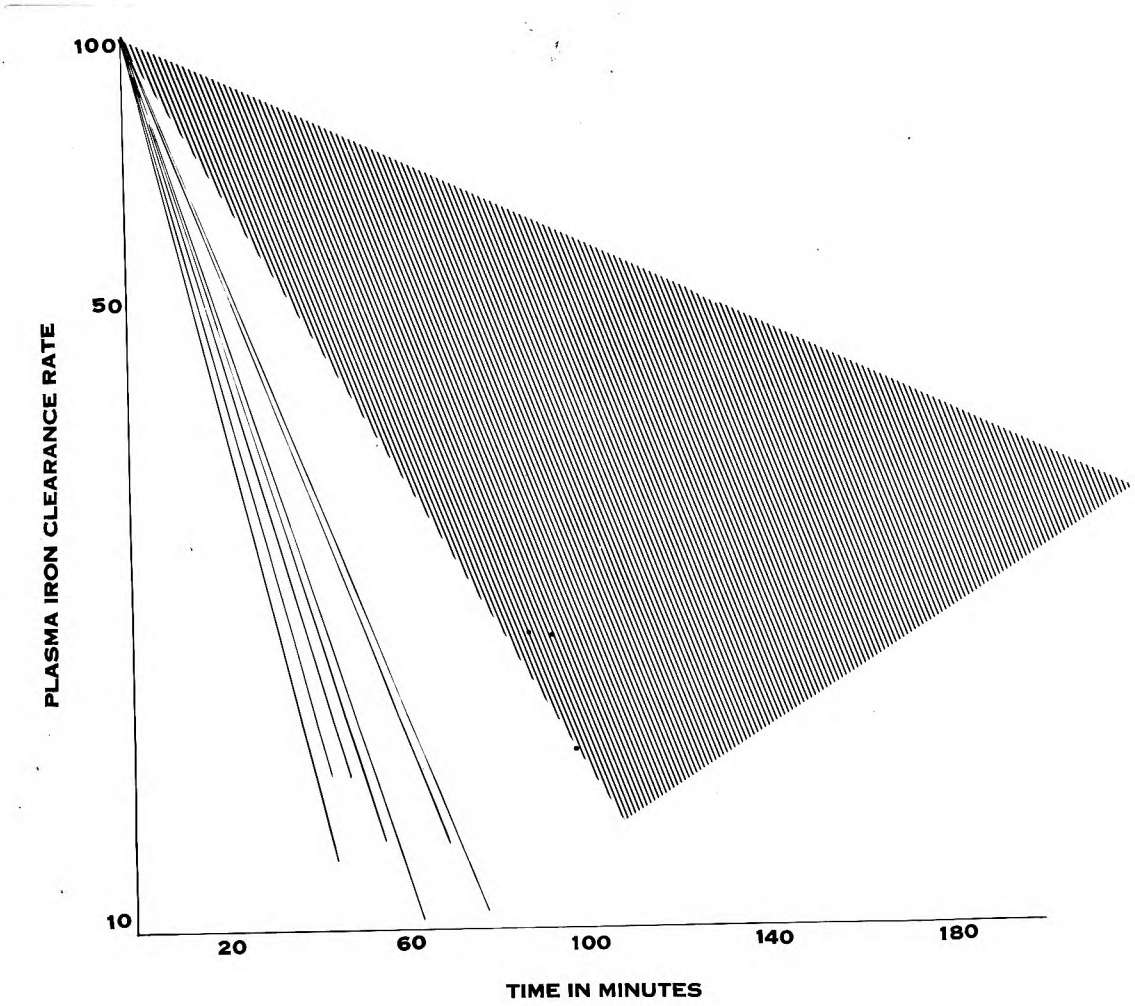


Figure 22 - Plasma Iron Clearance Rates in Seven Patients with Iron Deficiency Anaemia.

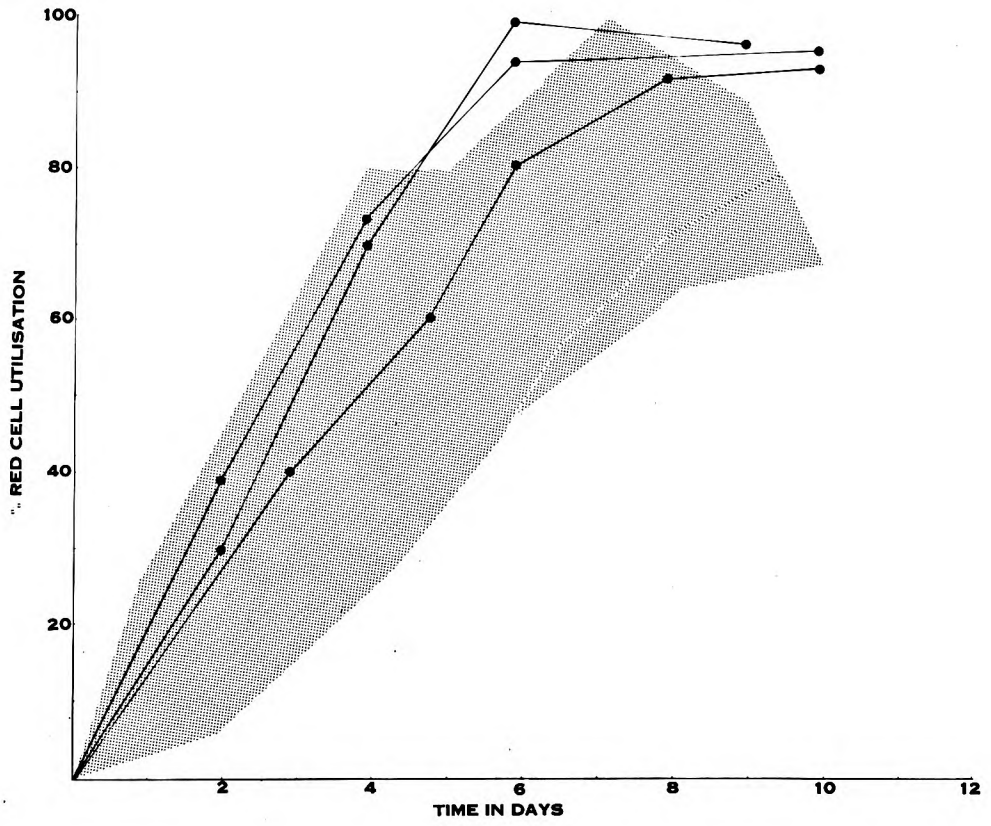


Figure 23 - Percentage Red Cell Utilisation Curves in Three Patients with Iron Deficiency Anaemia.

have all shown a decreased survival of iron-deficient cells. However, Bush et al (1956) could not show a shortened survival of microcytic hypochromic cells in an iron-deficient swine.

Case No. 7 (Table XII, p. 87) is unusual. The serum iron was 3 micrograms per cent, yet the rate of clearance was not greater than in the other cases of iron deficiency anaemia (half-time 17 minutes). It has been suggested that the rate of clearance of iron from the plasma is limited by the time required for the passage of the plasma pool through the marrow (15 minutes) (Loeffler, 1956), hence faster plasma clearance rates are not seen.

REFRACTORY ANAEMIAS

This term is applied to patients with anaemia of unknown aetiology which fail to respond to any treatment other than blood transfusions (Wintrobe, 1956). The group is not homogenous and includes cases with hypocellular (hypoplastic anaemia), normal and hypercellular bone marrows (Bomford and Rhoads, 1941). The range of the plasma iron turnover in refractory anaemias, like the marrow histology, is wide. The range in the series described by Huff (1950) was 0.7 to 11 times normal. The percentage red cell utilisation has been variable and has not necessarily corresponded with the plasma iron turnovers. Najean (1959) found three different types of ferrokinetic patterns in patients with refractory anaemia and hypercellular marrows :-

a. increased plasma iron turnovers and adequate red cell utilisation.

b. increased plasma iron turnovers and depressed red cell utilisation.

c. normal plasma iron turnovers in the presence of severe anaemia.

In group (a) the pattern is that of haemolysis with suboptimal marrow response. In group (b) the pattern was that of ineffective erythropoiesis. The patients in group (c) are functionally similar to those described under decreased marrow function.

MISCELLANEOUS DISORDERS

A plasma iron turnover was done in one patient with chronic blood loss (Case No.1, Table XIII, p. 92). Sideropenic dysphagia was found in 1957. This followed excessive menstrual blood loss over many years. She was given iron intramuscularly, but in September 1959 her serum iron was again low and a very rapid rate of clearance of radioiron gave a plasma iron turnover 3.5 times normal. One patient had an acute idiopathic haemolytic anaemia (Case No.2, Table XIII, p. 92). He was admitted in November 1959 because of haematuria. Three days after admission he had an episode of severe chest pain with haemoptysis. On admission his haemoglobin was 9.6G per cent. Four weeks later, although he had had no further haematuria and he had fully recovered from the pulmonary embolus, his haemoglobin was only 6.6G per cent. The reticulocyte count was 8 per cent. Blood bilirubin 2mg per cent; and excess urobilinogen was present

TABLE XIII

Case No.	Age	Sex	PCV	% Reticulocytes	Time taken for radioiron to reach half concentration (minutes)	Plasma Iron ug/100ml	UIBC	Plasma Iron Turnover mg/day/100ml plasma	Plasma Iron Turnover mg/day/100ml whole blood	Erythropoietic Index	Comments
1	33	F	33		13	41		3.15	2.2	349	Sideropenic dysphagia. Menses were excessive. No occult blood in stools. Serum iron 19ug% MCHC 28%.
2	43	M	31	5.2	38.5	58	186	1.5	1.08	172	Pulm.embolism Nov.1959 and haemorrhagic cystitis. Hb.9.6G%. MCHC 30%. WBC 4,400/cmm. Platelets adequate. Polymorphs 54%. Normal osmotic fragility. Marrow-erythroid hyperplasia. Myeloid & platelet genesis n.a.d.
3	30	M	19		360	250		0.693	0.57	90	Marrow-hypoplasia of erythroid and myeloid series. Hb.8.9G%. WBC 3,500/c.mm. Polymorphs 12% Lymphocytes 8.2%. O.E.- Temp.101. Metacorten 80mg/day. Otitis media, boils and petechia.
4	26	F	33	0.8	122	88	239	0.72	0.50	79	Hb.10.1G%. WBC 2,300/c.mm. Polymorphs 22%. Lymphocytes 73% History of chloramphenicol. O.E.- fundal petechiae. Platelets scanty.
5	43	F	39		53	58	202	1.09	0.71	113	Gross myxoedema. PBI 1.5 Cholesterol 290mg%.
6	42	F	28.5	0.8	36	50	183	1.39	0.90	143	Anaplastic ca. of R. breast. BP 190/125. Gr.III retinitis. ?infection R upper lobe. Urea 44mg%.
7	29	M	20	2.6	46.5	57	228	1.22	1.00	159	R.nephrectomy 1957 followed by perinephric abscess. Urea 110, rose to 268, fell to 98mg%. O.E. 1959 - 2 finger splenomegaly. Urea 55mg%. WBC 16,800. Alb.4.4 Glob.1.61. Bl. transfusions. Marrow - erythroid hyperplasia.

TABLE XIII(cont.)

Case No.	Age	Sex	PCV	% Reticulocytes	Time taken for radioiron to reach half concentration (minutes)	Plasma Iron ug/100ml	UICB	Plasma Iron Turnover mg/day/100ml plasma	Plasma Iron Turnover mg/day/100ml whole blood	Erythropoietic Index	Comments
8	36	M	26	4.7	69	155		2.24	1.70	356	Cirrhosis of liver. Porphyria cutanea tarda and a haemolytic anaemia
9	47	M	28	0.6	55	163		2.96	2.22	470	Persistent vomiting, Partial gastrectomy, complicated by tetany. Responded to small and frequent feeds.

in his stools. The serum iron level was 58µg per cent. The rate of clearance of the radioiron from the plasma was rapid and the plasma iron turnover was 1.08mg/100ml whole blood/day. (Erythropoietic Index 1/2). No cause was found for the haematuria and the anaemia remitted spontaneously. Two patients had marked marrow hypoplasia (Case No. 3 and 4) and severe infection. The plasma iron turnover was normal in each patient. This is not surprising since a normal plasma iron turnover is found in the anaemia of infection as well as marrow hypoplasia. The high serum iron and slow rate of clearance of radioiron in Case No. 3 indicated that infection was of little significance in this case.

A normal plasma iron turnover was found in one patient with gross myxoedema in whom the haematocrit was slightly reduced. Reduced plasma iron turnovers have been noted in experimentally induced myxoedema (Austoni et al, 1958) and these observations support the hypothesis of Bomford (1938) that in hypothyroidism there is a reduction in the total mass of red cells and their precursors resulting from reduced oxygen consumption in the tissues.

Patients No.6,7 and 8 demonstrated further the limitations of the plasma iron turnover test. Mrs.G., aged 62 years (Case No.6, Table XIII, p.92) had severe hypertension for many years and in January 1959 she had a right radical mastectomy for an anaplastic carcinoma of the breast. Fibrosis of the right upper lobe followed deep x-ray therapy. Several months later the haematocrit fell. The blood urea was 74mg per cent. It was not possible to decide whether the fall in haemoglobin was due to infection in the fibrosed right upper lobe, or dissemination

of the carcinoma. A plasma iron turnover of 1½ times normal was compatible with either possibility. A subsequent rise in the haemoglobin suggested that infection had been the cause of the anaemia.

Mr.B (Case No.7, Table XIII, p.92) had been an alcoholic for many years. In 1957 a right nephrectomy was complicated by a perinephric abscess and an acute episode of renal insufficiency. The blood urea rose to 268mg per cent, but fell on recovery to 98mg per cent. When examined in October 1959 he was found to be anaemic. The spleen was enlarged 2cms below the left costal margin. The blood urea was 55mg per cent. Pyrexia (101°C) and a leucocytosis of 16,800/c.mm. suggested recrudescence of the perirenal infection. The plasma iron turnover was 1½ times normal, an increase found in anaemia whether due to cirrhosis of the liver, infection or possibly renal insufficiency.

Mr.V. (Case No.8, Table XIII, p.93) was a patient with cirrhosis of the liver and a haemolytic anaemia. His past history revealed that between 1936 and 1948 he had recurrent boils. In 1948 he was told that he had pernicious anaemia and was given a course of vitamin B_{12} (Cytamen, 1000$\mu\lambda$ x 12). Weakness, tiredness and pallor persisted. In April 1957 he developed a severe mastoiditis on the left side and he was treated with intensive antibiotic therapy. From April 1958 he required four pints of blood (2,000ccs) at monthly intervals

and he became progressively darker in complexion. The only other relevant features in the history were that he had been a heavy drinker for twenty years and that the skin of the dorsum of the hands abraded easily.

The patient was first seen in August 1959. A two-finger hepatomegaly and generalised increased pigmentation were the only findings on clinical examination. There was no evidence of infection.

Investigations: Haemoglobin 9.9G per cent
White blood count 4,000/c.mm.
Neutrophilic leucocytes 59.5%
Monocytic " 5%
Lymphocytic " 35%
Eosinophilic " 0.5%
Reticulocytes 3.5%
Platelets 250,000/c.mm.

The serum bilirubin was not raised. Urine - urobilinogen ++ and excess uroporphyrins (1,160 micrograms per 24 hours; normal ± 125). The latter finding established the diagnosis of porphyria cutanea tarda. Occult blood and excess urobilinogen were present in the stools. Coombs' and Schumm's tests were negative. Ham's and Donath Landsteiner tests were also negative. The screening test for red cell osmotic fragility was negative.

Marrow - the report on a bone marrow smear was as follows:

> "This marrow is of normal cellularity. Myelopoiesis is normal apart from curious vacuolation of the myelocytes the significance of which is not clear. Erythropoiesis is normoblastic and appears to be of normal activity. An interesting feature here is the marked polyploidy of the normoblasts to which no significance can be attached."

Red cell survival - ^{51}Cr labelled red cells had a half life of

seven days (normal 27-29 days). ^{51}Cr was not recovered in his stools. The past history of alcoholism, disturbed liver function tests (Thymol turbidity ++, Takata Ara +++, serum albumin 36%) and excess uroporphyrins suggested cirrhosis of the liver. The serum iron was 155, but this could be partly explained by the fact that he had received over 60 pints of blood. The plasma iron turnover was 1.7mg/100ml whole blood/day (2.4 times normal). In this patient with cirrhosis of the liver there was suboptimal erythropoietic response to a severe haemolytic anaemia. Since the serum iron level was raised part of the increase in the plasma iron turnover may have been due to redistribution of iron stores.

Case No. 9 (Table XIII, p. 93), Mrs. D., aged 50 years, was admitted in July 1959 because of persistent and severe recurrence of symptoms from a duodenal ulcer. A partial gastrectomy was done and she made an uneventful recovery. Shortly after discharge, however, she started vomiting, several times a day. There was marked loss of weight and progressive weakness. On admission in October 1959 the patient was emaciated and dehydrated and the persistent vomiting had resulted in tetany (hypochloraemic hypopotassaemic alkalosis). The haemoglobin was 12G per cent. The disturbed electrolytic state was corrected; a barium meal showed no obstruction, but the vomiting persisted and a progressive anaemia and leucopenia developed.

11th November, 1959 - haemoglobin 9.0G per cent

white cell count 3,000/c.mm.
neutrophilic leucocytes 30%
lymphocytic " 60%
reticulocytes 1%

Excess urobilinogen was present in urine and stools. The blood bilirubin level, however, was not raised. There was free gastric acid in the stomach remnant and no occult blood in the stools. There was no evidence of steatorrhoea. The bone marrow smear was reported as showing hypoplasia of all elements. She was given various haematinics, including vitamin B12, liver extract and iron, but there was no response. The serum iron was 163µg per cent. A rapid plasma iron clearance rate gave a plasma iron turnover of 2.22mg per cent (normal 0.62). The increased plasma iron turnover with a low reticulocyte count suggested a large element of ineffective erythropoiesis. Although folic acid deficiency may have been present repeated bone marrow examinations did not show any megaloblastic erythropoiesis. When she was given frequent small feeds the vomiting subsided and there was a rise in the haemoglobin level (9 - 11G per cent). The patient left hospital eight days after the plasma iron turnover. No definite diagnosis was made.

The following case is presented in detail to show how the plasma iron turnover may help in the elucidation of an anaemia.

Mr. L., aged 60 years.

Date of first admission - 7.6.59 : Date of discharge - 26.7.59.

Present history - for several weeks prior to admission the patient had experienced abdominal discomfort particularly after meals and had noticed over the same period progressive distension of the abdomen and swelling of the feet. There was marked loss

of appetite and loss of weight.

Systematic history - This was essentially negative but for dyspnoea and tiredness on exertion.

Past history - In 1941 while in the army there had been an episode of abdominal pain and jaundice, which lasted three weeks. He made an uneventful recovery.

Habits - The patient had for many years drunk a half to one bottle of brandy a day.

Examination revealed a spare, elderly European male. There was no clubbing or cyanosis, but pallor and an icteric tinge of the conjunctiva were noted. In the mouth there was a purpuric spot on the left fauces. The abdomen was distended with fluid and there was oedema of the feet. Neither palmar erythema nor spider naevi were present.

Investigations - Ascitic fluid - straw coloured with a low specific gravity (1004). After a large volume of fluid had been drained a four finger spleen was palpable.

11.6.59 -	Haemoglobin	9.4G per cent
	Red blood cells	2.89 million per cu.mm.
	Haematocrit	27 per cent
	M.C.H.C.	34.8 per cent
	Leucocytes	4,500/c.mm.
	Neutrophilic leucocytes	69 per cent
	Monocytic "	8.0 per cent
	Lymphocytic "	18.5 per cent
	Eosinophilic "	3.5 per cent
	Basophilic "	1.0 per cent
	E.S.R. (Westergren)	65mm in 1 hour
	Reticulocytes	1.4 per cent

The platelets were normal in appearance and number.

Urine - Albumin present. Specific gravity 1004; occasional white blood cells and an occasional red cell were seen in a high power field.

Blood urea 86mg per cent. The electrolytes were otherwise normal.

Liver Battery (9.6.59)

Thymol turbidity test	5 units (normal 2 units)
Thymol flocculation test	+++
Colloidal red test	+++
Cephalin cholesterol flocculation test	+++
Takata Ara	+++
Zinc sulphate turbidity	35.8
Total lipid	511
Alkaline phosphatase	17.5
Bilirubin direct	0.6mg per cent
Total	1.2mg per cent

Cholinesterase 41 per cent of average normal activity.

Total protein	5.9G per cent
Albumin	1.61G per cent
Alpha 1 Globulin	0.39G per cent
Alpha 2 "	0.57G per cent
Beta "	0.68G per cent
Gamma "	2.65G per cent

Coombs' and Schumms tests negative.

The reticulocyte count varied between 2.8 and 4.8 per cent.

In several blood counts there was a true eosinophilia.

Occult Blood - Orthotolidine reaction +
Amidopyrine reaction +

X-ray chest - n.a.d.

Barium meal - oesophageal varices demonstrated.

Bone marrow examination - this marrow was of normal cellularity; erythropoiesis, myelopoiesis and platelet genesis were normal.

Therapy - In the ward he had an epistaxis and was given an intensive course of all haematinics (ferrous gluconate, parentrovite,

vitamin B forte, vitamin K and vitamin C.) There was no haematological improvement and on 17.7.59 he was given four pints of packed cells and was discharged shortly afterwards. The patient was readmitted in August as there had been a progressive fall in his haemoglobin. The ascites had not reaccumulated. The anaemia could have been due to :-

a) cirrhosis of the liver
b) blood loss
c) hypersplenism
d) renal insufficiency as manifested by a raised blood urea.

Erythropoiesis is markedly increased in anaemia due to blood loss or haemolysis. In the anaemia of renal disease and cirrhosis of the liver there is failure of the marrow to increase its activity in response to a varying degree of haemolysis. The serum iron was 123 micrograms per 100ml of blood and the rate of clearance 180 minutes. The plasma iron turnover was 80 per cent of normal. It was apparent that the major component to the anaemia was marrow hypoplasia, due either to chronic nitrogen retention, or cirrhosis of the liver.

AN EVALUATION OF THE PLASMA IRON TURNOVER TEST

The plasma iron turnover and the percentage red cell utilisation of radioiron have been extensively used over a decade to study erythropoiesis. The plasma iron turnover test is a sensitive index for measuring increased erythropoiesis. In response to haemorrhage or acute haemolysis, there is a three-fold increase in erythropoiesis. In chronic haemolytic

anaemias, as a result of the increase in size of red cell mass, erythropoiesis increases 6-8 times normal. With marrow hypoplasia the plasma iron turnover test is either normal or moderately decreased. While this test is of little value as a quantitative measure of marrow hypofunction, qualitatively it is of great value. A normal plasma iron turnover in any anaemia other than iron deficiency is indicative of marrow hypofunction.

There is a large group of anaemias (the anaemia of infection, rheumatoid arthritis, renal insufficiency and some patients with carcinoma) in whom the plasma iron turnover is normal or moderately increased. Their response when compared with maximal response as found in haemolytic anaemias is suboptimal. The anaemia in these patients is in part due to relative marrow hypofunction.

The plasma iron turnover test has helped to define effective from ineffective erythropoiesis. In pernicious anaemia and in Cooley's anaemia the plasma iron turnover test is considerably increased. This is associated with erythroid hyperplasia and an elevated urobilinogen excretion. The magnitude of this increase is out of proportion to the rate of blood formation as judged by the reticulocyte count and the percentage red cell utilisation of radioiron. Such findings indicate that in these anaemias a large fraction of erythropoiesis is ineffective in so far as the delivery of viable red cells to the circulation is concerned.

Since the plasma iron turnover measures total erythropoiesis, and the percentage red cell utilisation distinguishes effective from ineffective erythropoiesis, it is possible to classify anaemias on the basis of these two tests. Such a classification is a valuable adjunct to the morphological classification in the elucidation of blood dyscrasias.

Diagnosis	Plasma Iron Turnover	Percentage Red Cell Utilisation
Normal	0.63mg/100ml whole blood per day	70 - 90 %
Marrow hypoplasia Lymphatic leukaemia Some patients with Refractory anaemia	Normal or Reduced	Markedly Reduced
Iron Deficiency Anaemia	Normal	100 %
Chronic haemolytic Anaemia (congenital spherocytosis)	Six Times Normal	Less than 70 %
Acute Blood loss Acute Haemolytic Anaemia Some patients with neoplasm	Three Times Normal	70 - 100%
Pernicious anaemia Cooley's Anaemia Some patients with Refractory Anaemia	Three Times Normal	Markedly Reduced
Anaemia of Infection Renal Insuffieiency Cirrhosis of Liver Rheumatoid Arthritis Reticulosis and Carcinomas, i.e. Haemolysis with relative marrow aplasia.	Less than Three Times Normal	70 - 90 %

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C H A P T E R IV

FERROKINETIC STUDIES IN PORPHYRIA

RADIOIRON STUDIES IN PORPHYRIA IN EUROPEAN AND BANTU SUBJECTS

Biochemical Aspects

Haemoglobin is a compound which is made up of a protein molecule globin, on the surface of which there are four small prosthetic groups of haem. Haem consists of an iron atom in the centre of a porphyrin structure, protoporphyrin which is built up from glycine (Muir and Neuberger, 1950) and acetate (Shemin and Wittenberg, 1951). These two substances probably by condensation and decarboxylation yield delta amino laevulic acid. Two molecules of delta amino laevulic acid combine to form porphobilinogen; (Fig.1, p.111); a reaction which is enzymatically controlled (Dresel and Falk, 1953). Delta amino laevulic acid and porphobilinogen (pyrrole structure) have been shown to be intermediates in the synthesis of haem (Shemin, 1955; Shemin and Russell, 1953; Neuberger and Scott, 1953).

Relatively little is known of the manner in which porphobilinogen is converted to protoporphyrin. Porphobilinogen can serve as a precursor of uroporphyrinogen and it is almost certain that all normally occurring porphyrins are derived from uroporphyrinogen (Gray, 1955) (Fig.2, p. 112). These porphyrins are tetra-pyrollic pigments in which the four pyrrole rings are united by methene groups to form one large ring structure. Four acetic groups and four proprionic groups occupy the beta positions in the pyrrole rings of the uroporphyrinogens. Four arrangements of these groups are possible, but only two of these four arrangements in their corresponding porphyrin form have so far

SUMMARY OF STEPS IN THE BIOSYNTHESIS FROM GLYCINE + SUCCINATE (FROM THE TRICARBOXYLIC ACID CYCLE) OF PORPHOBILINOGEN, THE SIMPLEST PYRROLIC SUBSTANCE KNOWN TO BE A PRECURSOR OF HAEM AND PORPHYRINS

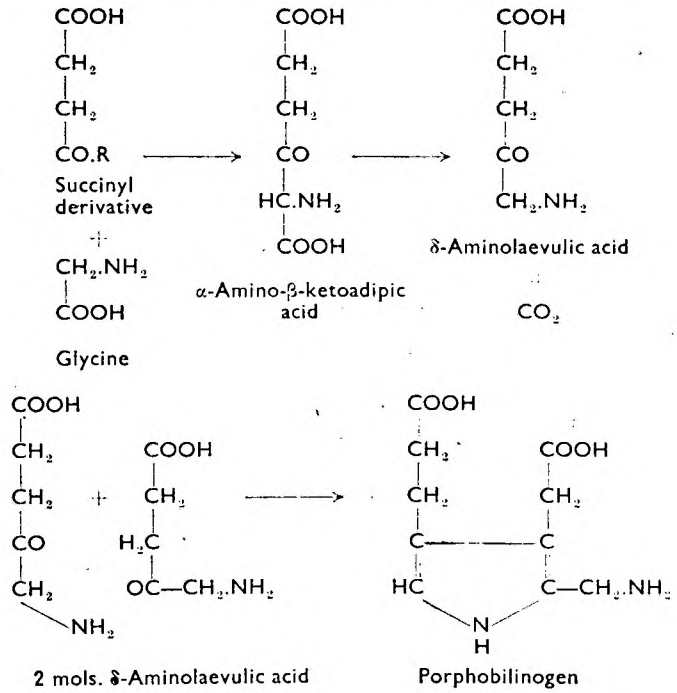
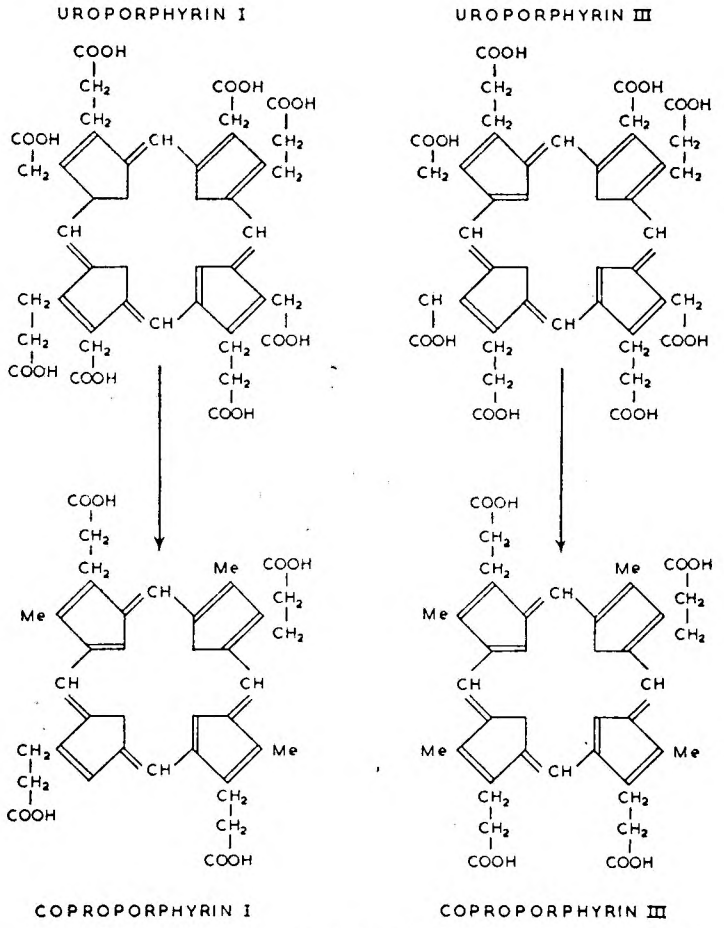


Figure 1 - The steps in the Synthesis of Porphobilinogen from Glycine and Acetate.



Structure of uroporphyrins and coproporphyrins I and III.

Figure 2 - Structure of Uroporphyrins and Coproporphyrins I and III. Type III Isomer is concerned with Haem Synthesis.

been found in nature, uroporphyrins I and III (Fig.2, p.112). In uroporphyrinogen I the acetic and proprionic acid groups are arranged alternately round the ring, but in uroporphyrinogen III the groups attached to one pyrrole ring have been interchanged. The type III isomer is concerned with haem synthesis.

There are several theories on the synthesis of protoporphyrin from uroporphyrinogen III (Shall, 1959):

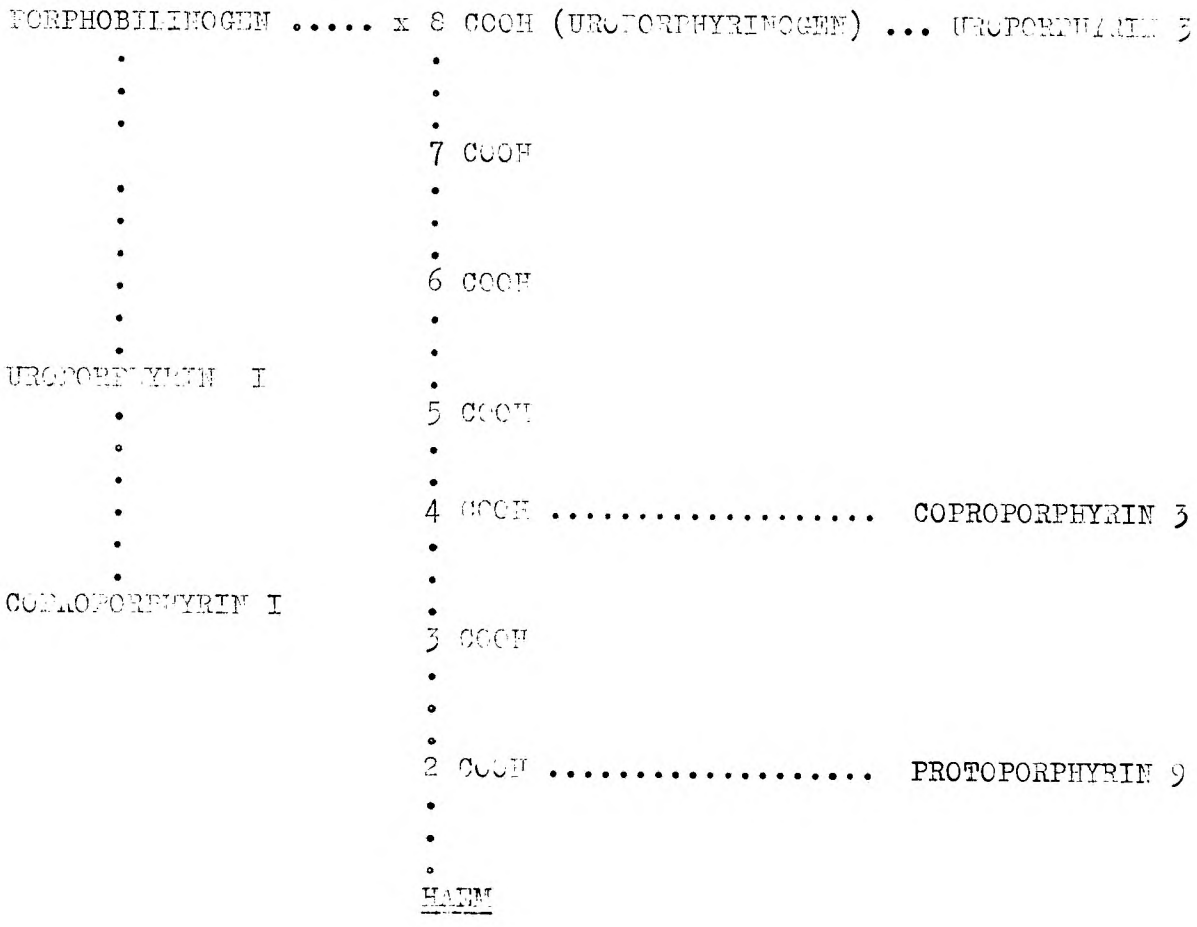
1. Uroporphyrinogen III, with the decarboxylation of acetic acid side chains to methyl groups can be converted to coproporphyrinogen III. Further dehydrogenation and decarboxylation will give protoporphyrin (vinyl methyl and proprionic acid side groups) for which there are theoretically fifteen possible isomers. The protoporphyrin of haemoglobin is classified as protoporphyrin IX type III.

2. Haem may be synthesised as shown on page 114 :-
The X denotes a substance similar to the porphyrins and the number indicates the number of side chains possessing carboxyl groups (Dresel, 1955).

Finally iron is incorporated into protoporphyrin IX to form haem, the prosthetic group of such proteins as haemoglobin, myoglobin, cytochrome C, peroxidases and catalase. This process is accomplished enzymatically (Goldberg et al, 1956) and is potentiated by reducing substances (Goldberg, 1959). Since iron is incorporated into the tetra pyrrole ring at a late stage in the biosynthesis of haem, abnormal porphyrin metabolism could theoretically lead to disturbances in iron kinetics.

Classification of the Porphyrias

Small quantities of coproporphyrins are excreted normally in the urine and stool. A moderate and temporary increase of up to a few milligrams a day is referred to as porphyrinuria and



A theory on the synthesis of haem from porphobilinogen. The x denotes side groups similar to the porphyrins and the number indicates the number of side chains containing carbonyl groups.

is found in several conditions, e.g. infection, haemolytic and pernicious anaemia, gout and acute rheumatic fever. Several hundred milligrams of porphyrins or their precursors are excreted daily in urine or stool in a variety of clinical entities collectively referred to as The Porphyrias. Since the biochemical disturbances underlying the porphyrias are not well understood, various classifications have been proposed based mainly on the clinical picture. A congenital, or photosensitive type and an acute intermittent variety have been recognised since the earliest workers (Gunther, 1922). Waldenstrom (1937) described a late cutaneous form of porphyria and more recently (1957) defined two types --

a) porphyria cutanea tarda symptomatica (frequently seen in cirrhosis)

b) porphyria cutanea tarda hereditaria or protocoproporphyria.

In this latter entity there is photosensitivity with acute episodes which are similar to those which occur in the acute intermittent type. Rimington et al (1949, 1952, 1953) demonstrated reciprocity in the excretion of porphyrins in this group; in remission excess copro- and proto-porphyrins are found in the faeces and in an active phase the excretion of urinary porphyrins is considerably increased and there is a corresponding fall in the faecal excretion. This group corresponds with mixed porphyria as described by Watson (1954).

PATHOPHYSIOLOGY OF THE PORPHYRIAS

In 1945 Prunty found porphobilinogen in the liver of a patient in an acute phase of intermittent porphyria. This finding was confirmed by Gray (1950). In 1954 Schmid et al carried out tissue porphyrin assays and fluorescent microscopy on the liver and marrow of 31 patients with porphyria. These included two of the congenital photosensitive type, twelve acute, thirteen cutanea tarda and three of the mixed variety. Two had latent porphyria. They designated the two patients with congenital porphyria, erythropoietic porphyria on the basis of abnormal porphyrin formation demonstrable in some normoblasts in the marrow. In the acute intermittent form, cutanea tarda and mixed types, the porphyrin content in the marrow was normal, but in the liver porphyrin or porphyrin precursor was markedly increased and these porphyrias were collectively referred to as hepatic porphyrias. The findings in one patient of the mixed type was of particular interest. During a period of photosensitivity without abdominal pain or nervous symptoms, the liver contained preformed porphyrins. Later, without evident photosensitivity, but when abdominal pain was prominent, the liver contained very little preformed porphyrin, but considerable amounts of precursor. Goldberg (1959) by chemical analysis on post mortem tissue, confirmed the hepatic distribution of porphyrins and their precursors in the acute intermittent type.

PORPHYRIA AS SEEN IN SOUTH AFRICA

The congenital form of porphyria is extremely rare and in South Africa only one case in a Bantu subject has been described (Findlay and Barnes, 1950). A recent review of the literature found only 34 cases of true congenital porphyria (Schmid et al, 1955). This condition is inherited as Mendelian recessive and is due to a disturbance in pigment metabolism giving a disproportionate or disorderly type of synthesis in favour of the type I isomers (Dobriner and Rhoads, 1940) which are not utilised in the synthesis of haemoglobin and therefore excessive amounts of uro and copro-porphyrins of type I are excreted in the urine. These fluorescent porphyrins account for the red urine, the erythrodontia, the pigmentation of bone and probably the extreme skin photosensitivity; the last causing bullous or erythematous lesions of the skin (hydroa aestivale) which may result in mutilation of the hands and face. Some erythroblasts in the bone marrow fluoresce, due to the presence of uroporphyrins and this finding has led to the condition being called erythropoietic porphyria. The erythropoietic function is markedly affected. Anaemia may develop and was originally thought to be due to insufficient formation of protoporphyrin from uroporphyrinogen. Clinically however, haemolysis is a characteristic feature (Wintrobe, 1956; Rosenthal et al, 1959).

The observation that splenectomy was followed not only by marked clinical improvement, but also by a decrease in the

concentration of uro- and copro-porphyrins in the erythrocytes, marrow and urine (Aldrich et al, 1951), suggested that the increased blood destruction in these cases resulted in increased erythropoietic activity and excessive formation and excretion of porphyrins.

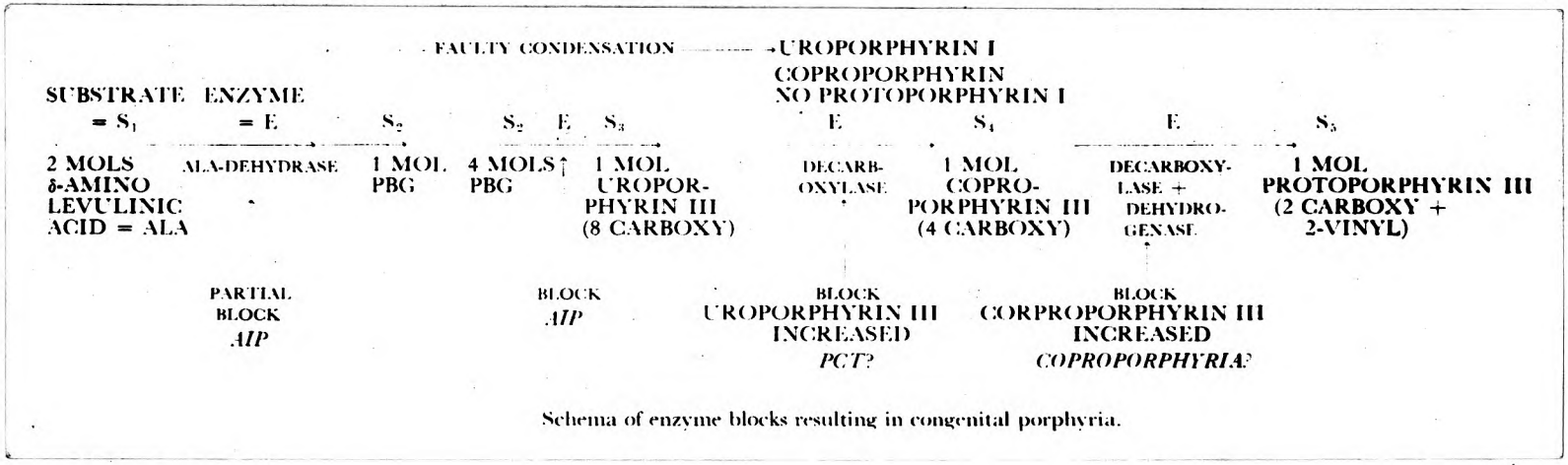
There are no radioiron studies in congenital porphyria and permission to study the one South African subject was refused. However, in view of the erythroid hyperplasia found in congenital porphyria it can be anticipated that the plasma iron turnover will be increased. In the presence of anaemia the increase will not be in proportion to the rate of red cell destruction. The high reticulocyte count in congenital porphyria suggests that radioiron will be incorporated into red cells which will then be released into the circulation, i.e. erythropoiesis will be effective.

In white South Africans a form of acute intermittent porphyria is seen (Porphyria Variegata). It is a common entity; the incidence in the white population of the Eastern Province is about 1 per cent (Dean and Barnes, 1958). It is transmitted as a Mendelian dominant (Dean and Barnes, 1955). The attacks are identical to those seen in acute intermittent porphyria. In the acute phase there are episodes of abdominal colic, vomiting, tachycardia and constipation. Complications involving the nervous system are common. These include paraesthesiae, irritability, delirium, coma and paralysis of limbs, trunk and cranial nerves. Death may result from respiratory failure.

It differs clinically from acute intermittent porphyria in that cutaneous manifestations occur in the same family group and are regarded as different manifestations of the same inherited disorder. Furthermore, excess porphobilinogen in the urine is only found in the acute phase, whereas in the acute intermittent group, porphobilinogen in the urine is frequently found in the phase of remission. Latent porphyria can be detected in white South Africans by the excess amounts of copro- and proto-porphyrin in the stool (Barnes, 1958). Excess porphyrins or their precursors have been found in the liver in patients who have died in an acute phase (Barnes, 1959). Porphyria as seen in the white South African is therefore indistinguishable clinically, biochemically and genetically from the mixed cases described by Watson (1954) and hereditary proto- copro- porphyria as described by Waldenstrom (1957).

There are two possible mechanisms by which disturbances in iron kinetics might arise during the acute phase of hepatic porphyria -- 1. the finding of porphyrins or their precursors in the liver in an acute episode of porphyria suggests that this organ is the site of the biochemical disturbance, and disturbances in liver function can result in a high level of serum iron: 2. iron is incorporated into the porphyrin nucleus at a late stage in the synthesis of haem and a block in the earlier stages of porphyrin synthesis (Fig.3, p.120) would lead to impaired incorporation of iron into haem complexes.

For these reasons iron metabolism was studied during the acute phase of porphyria variegata.



Schema of enzyme blocks resulting in congenital porphyria.

Figure 3 - Schema of possible blocks in porphyrin metabolism to explain the various porphyrias.

Methods and Material

Twelve patients with porphyria variegata were studied in an acute attack (Table 1, p. 121; Fig. 4, p. 122). The disease is hereditary and the trait was transmitted as a Mendelian dominant in each case. The history and clinical findings are briefly outlined in Table II (p. 133). Five cases had a history of skin sensitivity. Abdominal pain and tenderness were present in every patient. All three patients with paresis died. Met-haemoglobinaemia and sulph-haemoglobinaemia were present in one patient. The diagnosis in each was established by examining stools for coproporphyrin and protoporphyrin by the method of Holti and co-workers (1958) and by examining the urine for delta amino laevulic acid and porphobilinogen by the method of Mauzerall and Granick (1956) (Table III, p. 123). Serum iron estimations and radioiron techniques have been described in Chapter II.

High serum iron levels were found in ten patients either on admission or during observation (Fig. 5, p. 124).

The remaining two patients with normal serum iron levels died in respiratory failure, two and six days respectively after observation, and their serum iron estimations were done only on admission.

Discussion

The finding of a high serum iron level in the acute phase of porphyria variegata was initially regarded as a manifestation of hepatic dysfunction. Disturbances in hepatic function and structure can give rise to a raised serum iron level, e.g.

TABLE I

Case No.	Sex	Age	PCV	% Reticulocytes	Time taken for radioiron to reach half concentration (minutes)	Plasma Iron ug/100ml	Plasma Iron Turnover mg/day/100ml plasma	Plasma Iron Turnover mg/day/100ml whole blood	Erythropoietic Index
1	F	19	37	1.6	100	108	1.08	0.73	116
2	M	27	61	0.1	296	255	0.86	0.39	62
3	F	34	37	1.6	68	87	1.28	0.86	137
			34	2.2	100	81	0.81	0.56	89
4	F	27	49	0.4	100	150	1.5	0.83	132
5	F	25	50	0.1	48	89	1.85	1.02	162
6	F	30	46	1.0	90	88	0.97	0.57	90
7	F	30	49		127.5	228	1.8	1.01	160
8	F	28	48		117	155	1.33	0.76	121
9	F	34	43	0.2	285	248	0.87	0.53	84
			43		72	110	1.53	0.93	148
10	M	37	42	0.5	220	210	1.05	0.65	103
			42	0.2	60	72	1.20	0.86	137
11	M	32	32	1.4	161	207	1.29	0.92	146
12	F	40	39	1.4	91	96	1.06	0.69	110
Mean values and Range			45	0.77	147	166	1.26	0.75	119
			32-61	0.1-1.6	48-296	87-255	0.86-1.85	0.39-1.02	62-162

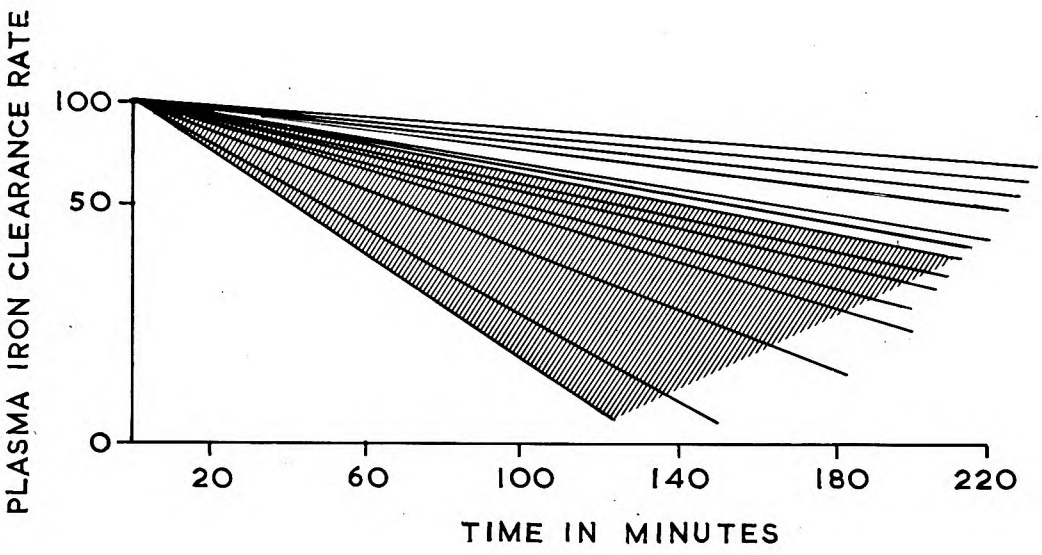


Figure 4 - The plasma iron clearance rates in Twelve patients with porphyria variegata studied in the acute phase.

TABLE III

Stool and Urine Analyses for Porphyrin

Case No.	URINE	FAECES		
	Amino Laevulic Acid	Porphobilinogen	Coproporphyrin	Protoporphyrin
2	247	126	-	-
3	278	123	-	-
4	111	146	178	105
5	127	29	-	-
6	41	10	625	793
7	138	81	625	805
8	31	30	105	590
9	40	41	667	750
	44	36	-	-
10	-	Moderate excess	428	307
11	80	63	-	-
Normal Values	4mg/litre	1mg/litre	15mg/litre	45mg/litre

In Cases No. 1 and No. 12 quantitative analyses were not carried out, but screening tests on the urine showed large amounts of porphyrin and porphobilinogen.

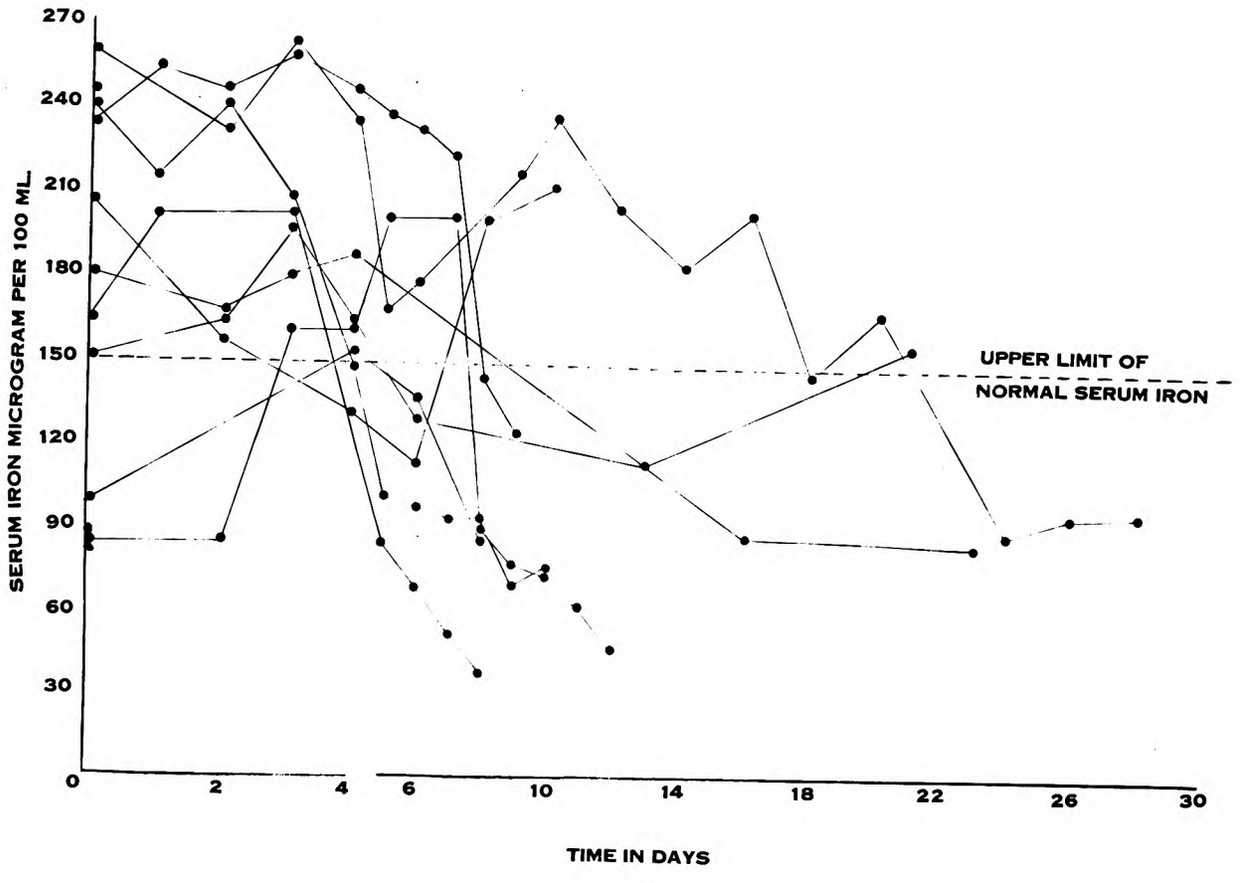


Fig. 5 - Serum iron estimations in twelve patients with Porphyria Variegata, studied in the acute phase.

infective hepatitis (1952), and disturbed liver functions in the white South African type of porphyria have been noted by Dean (1956) and were found in three of six cases studied in this series (Table IV, p.126). The high serum iron level in infective hepatitis is said to arise from necrosis of liver cells with release of iron from the storage depots (Stone et al, 1955; Weissman et al, 1954). However, in the present study glutamic oxalo acetic transaminase and glutamic pyruvic transaminase levels, which are regarded as good indices of hepatocellular necrosis, were only slightly raised (Table IV, p.126). Furthermore, a high serum iron level has been noted during the acute phase of intermittent porphyria (Vannotti, 1955) while minimal histological changes are found in the liver in those patients dying during acute attacks (Schmid et al, 1954; Goldberg, 1959). It would appear unlikely therefore that the raised serum iron levels in the acute phase of porphyria variegata and intermittent porphyria are due to liver cell necrosis.

In experimentally induced porphyria the most striking early change observed is a rapid decrease in the activity of hepatic catalase (Schwartz, 1955). It is however, extremely unlikely that iron not utilised for haem enzyme formation will give a sustained rise of the serum iron level. However, a fall in haem enzymes in the liver, e.g. catalase or cytochrome, will impair aerobic cellular metabolism. Mazur et al (1955) have shown that in anaerobic liver cellular metabolism iron is released from ferritin. Such a mechanism could explain a high serum iron level in the absence of cellular necrosis.

TABLE IV

Correlation Between Liver Function Tests and Plasma Iron Levels

Case No.	Plasma Iron Level	Transaminase		Thymol Turbidity	Thymol Flocculation	Alkaline Phosphatase	% Cholin-esterase	Total Bilirubin	Serum Proteins	
		Glutamic-Oxaloacetic	Glutamic-Pyruvic						Alb.	Glob.
1	112	158	230	4.5	3	5.9	100	1.0	3.0	3.9
2	255	230		1.0	Neg.	5.2	100	0.6	4.0	3.9
3	162	133		1.0	Neg.	9.8	100	1.6	4.4	3.4
	68	65		1.0	Neg.	7.9	64	0.6	2.5	2.3
4	150	218		5.5	3	8.8	69	0.6	2.8	4.0
10	212	150	210	1.0	Neg.	9.1	97	1.2	4.3	3.4
	72	110	210							
11	207	80	65	2.5	Neg.	8.8	77	0.6	2.9	2.5
12	152	115	180							
Mean Values and Range	70-150 G/100ml	35-100 units	10-95 units	0-2 units	Neg.	4-13 units	100%	0-1.2 mg/100ml	4.0-5.7 G/100 ml.	1.3-3.0 G/100ml

The serum iron level bore a close relationship to the clinical state. In every patient except one the serum iron level fell when the clinical condition of the patient improved. This close relationship was particularly obvious in one patient in whom the serum iron level fell with clinical improvement only to rise again with the sudden onset of generalised weakness. In one patient the serum iron level fell, but there was a progressive deterioration in the clinical condition. A marked disturbance in electrolytes proved irreversible and the patient died in a pseudo-Addisonian crisis (Kark, 1955) (Case No. 3, Table I and II, p. 121 + 133). The fall in the serum iron level, however, was accompanied by a marked decrease in urinary excretion of delta amino laevulic acid and porphobilinogen. It was postulated that the disturbance of porphyrin metabolism had subsided, but that it had caused irreversible biochemical changes in the kidney resulting in excessive sodium depletion.

The results of the plasma iron turnovers done in the acute phase are recorded in Table I (p. 121). The mean plasma iron level was raised (166, range 87-255ug per 100ml plasma). However, as the mean time taken for the plasma clearance rate to reach half concentration was prolonged, 147 minutes, the mean plasma iron turnover per 100ml whole blood per day was only slightly increased (.75, range .39-1.02mg per 100ml whole blood). A serum iron greater than 200ug per 100ml of plasma was associated with a normal plasma iron turnover in two patients (Cases No. 9 and 10, Table I, p. 121) and a significantly reduced plasma iron turnover in a third subject (Case No. 2 Table I, p. 121).

A reduced packed cell volume was found in three patients (Cases No. 1, 3 and 11, Table 1, p.121), in each of whom the plasma iron turnover was normal, or only slightly increased. These various findings suggested impaired haemoglobin synthesis since a raised serum iron level in any condition other than hypoplasia of the marrow is associated with an increase in the amount of iron passing through the plasma per day and secondly a normal plasma iron turnover in any anaemia other than iron deficiency anaemia is indicative of marrow hypofunction. Impaired haemoglobin synthesis was further suggested by the finding of a diminished utilisation of the radioiron for red cell production in six of seven subjects (Fig.6, p.129). In one patient recovery was associated with a marked uptake of radioiron into red cells.

The acute episode of South African cases of porphyria and acute intermittent porphyria are similar clinically and biochemically and both are inherited as Mendelian dominants. That the impaired haemoglobin synthesis demonstrated in the present series of cases may be observed in the acute episode of intermittent porphyria is suggested by the significantly reduced plasma iron turnover reported in one patient (Bothwell et al, 1956). A block in haemoglobin synthesis should result in anaemia. This is, however, an uncommon finding of the hepatic form of porphyria. Goldberg (1959), in fifty cases, showed a mean haemoglobin of 14.7 with a range of 11.8 - 17.5 gms%. Furthermore, stimulation of the marrow of a patient in an acute episode by venesection was

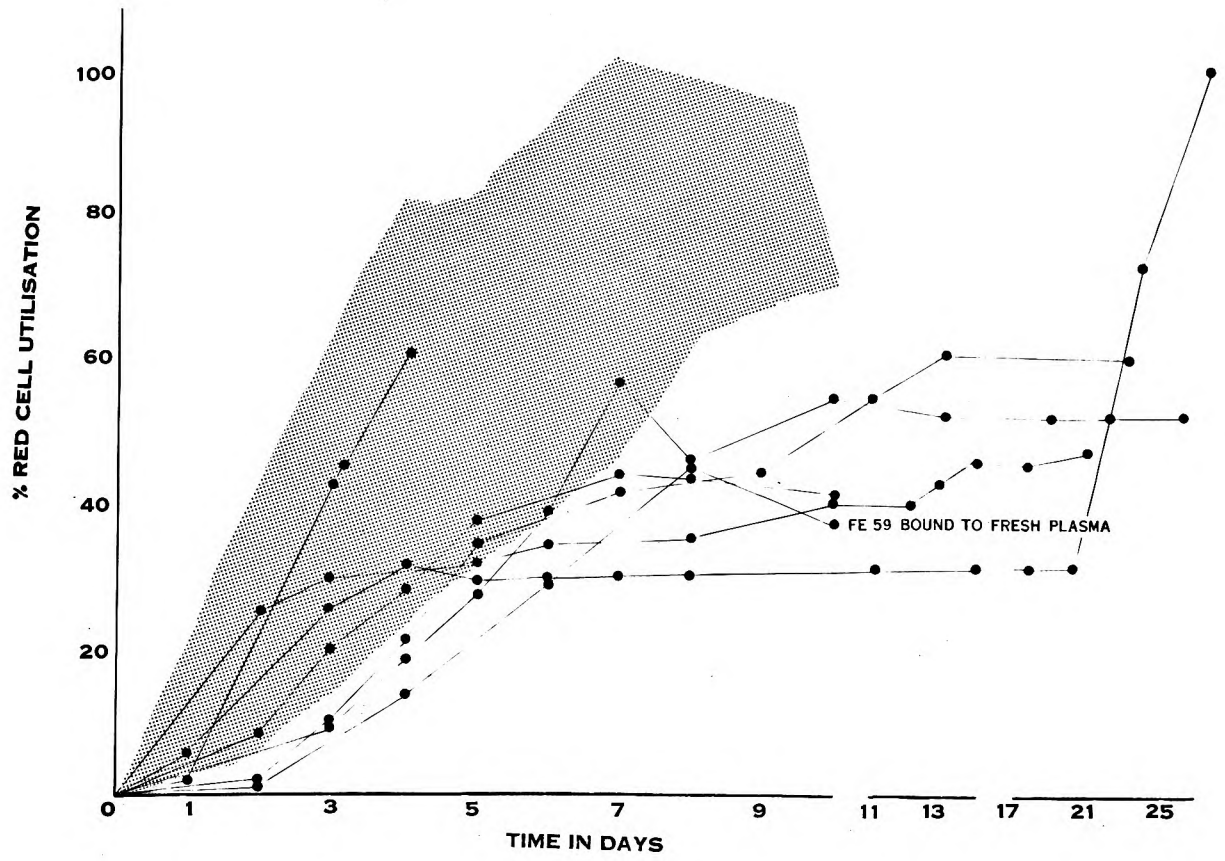


Figure 6 - Percentage Red Cell Utilisation Curves in Seven Patients with Porphyria Variegata in an Acute Phase. A marked increase in the percentage uptake of radioiron was noted in one case after the acute phase had subsided.

followed by a reticulocytosis and a rise in the haemoglobin level, (Iber et al, 1956). Anaemia has, however, been observed in several case reports of porphyria cutanea tarda (Gazet, 1953) and acute intermittent porphyria (Smeed, 1956; Fischer, 1957). Martin and Heck (1956) found that four of eighty-one patients with acute and mixed porphyria had a haemoglobin of less than 10 grams, and the haemoglobin levels were just below the normal limit in three of nine patients studied by Barnes (1951). Since 1.5 grams of haemoglobin per 100ml of blood are normally replaced every twelve days, several weeks must elapse for anaemia to follow on hypoplasia of the marrow. Of the present series of patients four died and six recovered within a period of ten days. One patient was observed for several weeks in an acute episode (Fig.7, p. 131). On admission the high serum iron level and significantly reduced plasma iron turnover suggested impaired haemoglobin synthesis (Table I, Case No.2, p.121). The percentage red cell utilisation was reduced. During the acute episode the haemoglobin fell from 17 to 13 grams. Remission was associated with a fall in serum iron and a reticulocytosis (Fig. 7, p. 131).

The intermittent nature of the disease, fluctuations in an acute episode, haemoconcentration and the fact that impaired haemoglobin synthesis must exist for several weeks before anaemia is evident, could explain the infrequent finding of anaemia in the acute episodes of porphyria.

There are two explanations for the reduction in haemoglobin synthesis in the acute episode of porphyria-- either

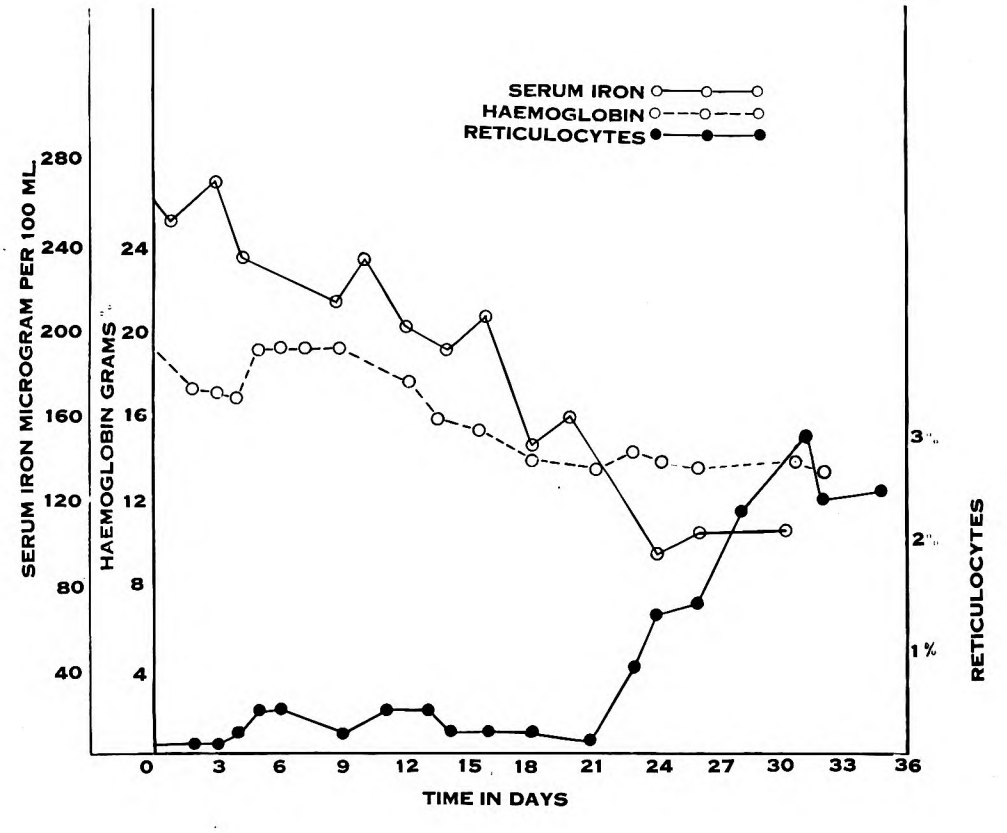


Figure 7 - Serum Iron, Haemoglobin and Reticulocyte Estimations in a Patient with Porphyria Variegata who remained in an Acute Phase for Three Weeks.

a block in the synthesis of protoporphyrin or some less specific depression in overall erythropoietic activity (marrow hypofunction). A block in pyrolle pigment metabolism would lead to a lack of available protoporphyrin for combination with iron. However, porphyrin precursors have not been demonstrated in the marrow of subjects with mixed porphyria (Schmid et al, 1954) and there is strong experimental evidence to show that porphobilinogen found in the liver in acute porphyria is formed in situ and not transported from an extrahepatic site (Goldberg, 1955). Furthermore, a block in pyrolle pigment metabolism will not affect the uptake of iron by the cells and may in fact lead to an increase in the plasma iron turnover (Bothwell, 1960). Finally, the observation that the reticulocyte count was reduced in a subject during an acute attack and rose with recovery, is more in keeping with a temporary depression in overall erythropoietic activity. It would appear, therefore, that the pattern of iron kinetics demonstrated in the present study is most likely the result of hypofunction of the marrow due to some non-specific manifestation of the metabolic upset in the acute phase rather than to any direct block in porphyrin synthesis in the marrow.

TABLE II

CASE HISTORIES

Miss V.Z.

This patient was admitted because of severe abdominal pain, vomiting and progressive weakness. These symptoms had been present for three weeks.

On examination generalised paresis and hypoaesthesia were found. All reflexes were absent. Pulse 130/minute. Blood pressure 120/90mm.Hg. The patient died in respiratory failure 48 hours after admission.

Mr. V.R.

This patient had a past history of recurrent bullae on the dorsum of the hands and during each episode he noted that his urine was red. He had had several episodes of acute abdominal pain and had been submitted twice to laparotomy. He was admitted to the Johannesburg General Hospital because of severe abdominal pain and constipation. Both these symptoms had been present for three weeks.

Pulse 130/minute. Blood pressure 130/95mm.Hg. The eschars of previous bullae were noted on the dorsum of both hands. The symptoms and signs subsided slowly over a period of six weeks.

Mrs. A.O.

Bullae had been recurring on the dorsum of her hands for two months and abdominal pain had been present for two weeks before the diagnosis of porphyria variegata was made.

On admission the patient was confused and there was generalised muscle weakness and hypoaesthesia. Pulse 130/minute. Blood pressure 100/60mm.Hg. The scars of a recent bullous eruption were present on the dorsum of her hands. Her blood electrolytes were grossly abnormal (Sodium 110m.eq/litre; Potassium 5.4m.eq/litre; Chloride 90m.eq/litre; Urea 60mg%). The patient was given 3% saline intravenously, D.O.C.A., hydrocortisone and 9 alpha fluoro hydrocortisone, but in spite of this intensive therapy she died in a pseudo-Addisonian crisis.

Mrs. L.G.

This woman was given phenobarbitone gr.½ b.d. because she complained of nervousness and tension. This was followed by abdominal discomfort, nausea, constipation, and pain in both thighs. She also noted that her urine was red.

On examination her pulse was 100/minute. Blood pressure 130/100mm.Hg. There was generalised abdominal tenderness.

All symptoms and signs subsided over ten days.

Mrs. L.S.

This patient was a known case of porphyria variegata. She was admitted because of severe and continuous abdominal pain which had been present for three weeks.

Pulse 100/minute. Blood pressure 120/80mm.Hg. There was generalised abdominal tenderness.

Her symptoms and signs diminished in severity over the next five days, but on the sixth day there was a sudden recurrence of severe abdominal pain. A rapid ascending quadriplegia was followed by respiratory failure and death.

Mrs. A.N.

This patient had a past history of three attacks of jaundice. During the last episode she had passed a red urine and had developed blisters on the dorsum of her hands. When this patient was studied she was recovering from an episode of severe abdominal pain and tenderness associated with vomiting and constipation.

Mrs. L.P.

Nervousness and anxiousness had been present for several months but abdominal pain had been present only for four days prior to admission.

On examination minimal hirsutism was noted on the side of her face. Pulse 110/minute. Blood pressure 120/85. There was generalised abdominal tenderness. All symptoms and signs subsided within ten days.

Mr. E.

This patient was admitted with a history of abdominal discomfort, constipation and weakness of the legs. She also had noted that her urine was dark in colour. All these symptoms had been present for five weeks.

On examination old eschars of previous bullae were present on the dorsum of her hands and there was generalised abdominal tenderness. Pulse 100/minute. Blood pressure 130/90mm.Hg.

There was a rapid diminution of all symptoms and signs.

Mrs. E.

This patient was admitted 14 days after the onset of severe abdominal pain and constipation.

On examination the pulse was 130 per minute. Blood pressure 130/100mm.Hg. There was generalised abdominal tenderness.

She made a rapid and uneventful recovery.

Mr. V.W.

History of abdominal pain for three weeks. The patient was also constipated.

On examination the pulse rate was 120/minute. Blood pressure 130/90mm.Hg. There was generalised abdominal tenderness.

He made a rapid and uneventful recovery.

Mr. V. de W.

This patient had a six weeks' history of severe abdominal pain. During this period his urine was dark in colour. These symptoms followed a general anaesthetic for repair of a right indirect inguinal hernia. (Pentothal had been administered).

On examination the pulse was 120/minute. Blood pressure 130/105mm.Hg. There was generalised abdominal tenderness and weakness of the legs.

The paresis progressed and the patient died two weeks later in respiratory failure.

Mrs. C.B.

This patient was admitted with a three week history of severe

abdominal pain.

On examination a slaty-grey hue was noted and there was generalised abdominal tenderness. Methaemoglobinaemia and sulph-haemoglobinaemia were both present. The patient had previously taken a mixture containing phenobarbitone and phenacetin.

Progress was uneventful.

Many hundreds of cases of porphyria have been seen in the Bantu (Barnes, 1955). It is characterised by the formation of blisters on the exposed parts. The bullae may vary in size from a millimeter or two, to a few centimeters. They often rupture to leave depigmented scars with an annulus of hyperpigmentation. There is frequently generalised hyperpigmentation of the face and hands. Temporal and pre-auricular hypertrichosis is common and the patient may comment on the passage of a red urine. Neither episodes of abdominal pain and constipation, nor neurological manifestations, which are common in acute porphyria in white South Africans, occur in symptomatic porphyria in the Bantu. The differences are not only clinical. Whereas in porphyria in European patients excess copro- and proto-porphyrins are found in the stools in remission and delta amino laevulic acid and porphobilinogen in the urine in an acute episode, an excess amount of uroporphyrins and to a lesser degree copro-porphyrins are passed in the urine in Bantu porphyria (Barnes, 1959). In the classification of late cutaneous porphyria Waldenstrom (1957) distinguished between hereditary proto-copro-porphyria and symptomatic porphyria. Bantu porphyria is grouped with the symptomatic porphyrias. The clinical picture and biochemical abnormalities are the same as in the subjects described by Brunsting et al (1951).

The exact mechanism of production of porphyria in the Bantu is not known. The imbibing of adulterated liquor, the high incidence of malnutrition and toxins have all been suggested as

possible aetiological factors. Damage of liver parenchyma could lead to hepatic dysfunction and abnormal porphyrin metabolism. In porphyria cutanea tarda symptomatica, excess porphyrins are found in the liver. It is thus a form of hypatic porphyria.

Clinical Material

Plasma iron turnovers were done in thirteen cases in whom spectroscopic examination of the urine had confirmed the clinical diagnosis (Table V, p. 139). No significant amount of porphyrin precursors was found in the urine (Table VI, p.140). The percentage red cell utilisation was estimated in five cases (Fig. 8, p. 141). Since there is a high incidence of iron overload and liver disease in hospital Bantu patients liver biopsies were done on eight cases and examined histologically after staining with haematoxylin and eosin. The eight liver biopsies and marrow smears in three further patients were stained with Perl's stain to assess body iron stores (Rath and Finch, 1949).

Results

The haematocrit was normal in eleven and slightly reduced in two patients (Table V, Cases No. 6 and 12, p. 139). These two patients had cirrhosis of the liver. In both of these patients the plasma iron turnover was slightly increased ($1\frac{1}{2}$ times normal) and the percentage red cell utilisation was normal in the one patient studied (Case No.12, Fig. 8, p. 141). These findings are similar to those reported in the anaemia associated with cirrhosis of the liver (Loeffler, 1956).

In Case No.10 a high plasma iron level was found with little iron

TABLE V

Bantu Porphyria.

Case No.	Sex	Age	PCV	Plasma Iron ug/100ml	UIBC	% Reticulocytes	Time taken for radioiron to reach half concentration (minutes)	Fraction of iron cleared/hr.	Plasma Iron Turnover mg/day/100ml plasma	Plasma Iron Turnover mg/day/100ml whole blood	Marrow and Liver
1	F	43	70	175	270		70	0.594	2.5	0.92	Cirrhosis of liver Haemosiderin +++
2	F	31	43	182			74	0.562	2.46	1.50	Marrow haemosiderin +++
3	M	25	47	79	182		54	0.77	1.46	0.85	Marrow haemosiderin +
4	F	22	42	78	161		74	0.562	1.05	0.67	
5	F	27	70	85			52	0.5996	1.64	0.62	Marrow haemosiderin +
6+	F	38	40	206			138	0.301	1.49	0.95	Frank cirrhosis Haemosiderin +++
7	M	40	52	220	13		233	0.462	2.44	1.29	Liver haemosiderin +++
8	F	33	51	150	94	0.8	128	0.33	1.19	0.64	Normal liver histology Haemosiderin +
9	F	41	48	198	12	2.2	122	0.341	1.62	0.92	Heavy deposits of iron in parenchyma, kupffer cells and portal tracts.
10	F	24	45	187	20	2.0	130	0.32	1.44	0.85	Liver haemosiderin +
11	F	38	50	45	147	1.0	45	0.924	1.00	0.55	Liver haemosiderin in portal tracts +++
12	F	37	40	94	50	1.8	64	0.65	1.47	0.94	Cirrhosis of Liver Liver haemosiderin +++
13	F	28	50	178		0.4	110	0.378	1.62	0.89	

+This patient in addition was in congestive cardiac failure from chronic rheumatic carditis.

TABLE VI

URINE

Case No.	ALA Normal less than 4mg/litre	PBG Normal less than 1mg/litre
1	8	less than 1mg PBG
2	18	less than 1mg PBG
3	negative	negative
4	6	less than 1mg PBG (1.7)(Repeat)
5	6	1 (0.8)(Repeat)
6	4	less than 1mg PBG
7	negative	less than 1mg PBG
8	4	1
9	3.8	less than 1mg PBG (0.6)(Repeat)
10	5	less than 1mg PBG
11	negative	negative
12	negative	negative
13	negative	negative

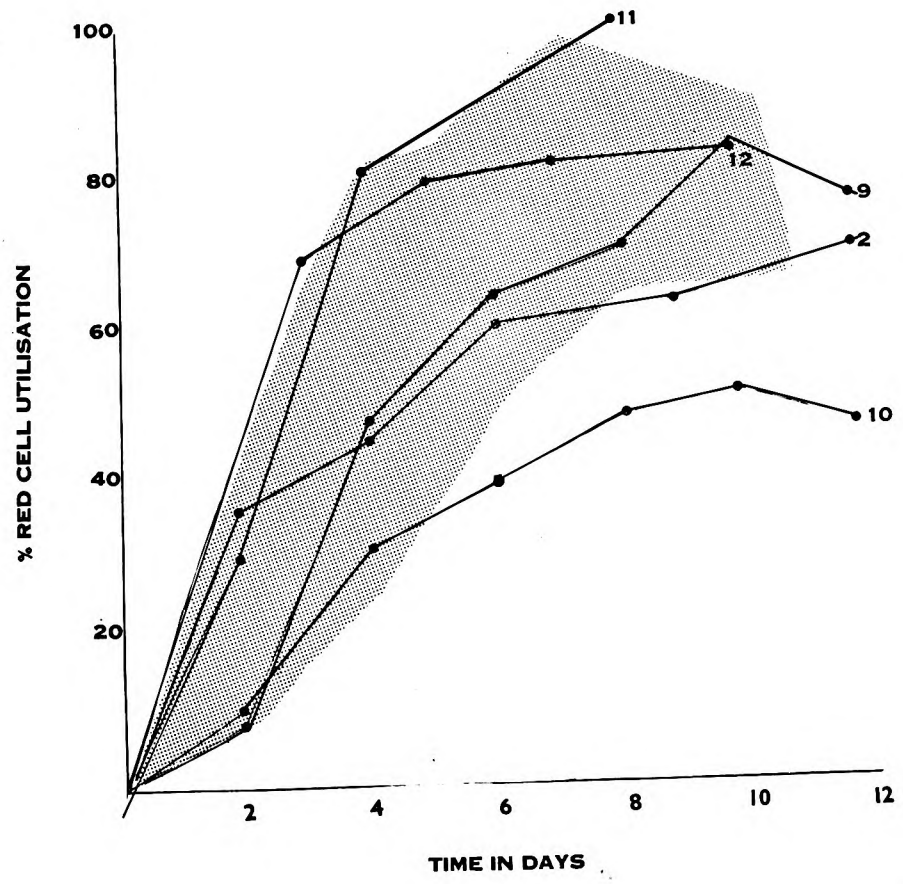


Figure 8 - Percentage Red Cell Utilisation Curves in Five Bantu Patients with Symptomatic Porphyria.

in the liver. There was no apparent explanation for this discrepancy. It is unlikely that it was technical as the serum iron was done in duplicate and in keeping with the high serum iron level the percentage red cell utilisation was reduced. Conversely, low serum iron levels and normal percentage red cell utilisation curves were found in two patients (Cases No. 11 and 12, Table V, p. 139, Fig. 8, p. 141) in whom heavy deposits of iron were found in the liver. Sub-clinical infection could have reduced the serum iron level.

In all other patients the plasma iron turnover was directly related to the serum iron level. The plasma iron turnover in one patient (Table V, Case No. 2, p. 139) was done five days after recovery from a right upper lobe pneumonia. The serum iron was raised, the plasma iron turnover was 2½ times normal and the percentage red cell utilisation was normal. Cytosiderosis and recovery from marrow depression secondary to pulmonary infection could explain this increase in the plasma iron turnover. This increase in the plasma iron turnover could not have occurred if there had been a significant block in porphyrin synthesis in the marrow, or a non-specific impairment in haemoglobin synthesis. Thus, in contrast to porphyria variegata, there is no evidence direct or indirect, of impaired haemoglobin synthesis in symptomatic porphyria.

SUMMARY

Iron kinetic studies were done during the acute phase in 12 patients with porphyria variegata.

1. A high serum iron was found in 10 of 12 patients either on admission or during observation. However, as the mean time taken for the plasma clearance rate to reach half concentration was prolonged, the mean plasma iron turnover in these patients was only slightly increased.

2. In 3 patients a reduced packed cell volume was associated with a normal plasma iron turnover.

3. The percentage red cell utilisation of iron was reduced in 6 of 7 subjects.

4. In one patient remission was associated with a reticulocytosis.

These various findings suggested that in the acute phase of porphyria variegata there was a non-specific depression of erythropoiesis. In contrast to porphyria variegata erythropoiesis was unimpaired in secondary porphyria as found in the Bantu.

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C H A P T E R V

FERROKINETIC STUDIES IN SCURVY

STUDIES IN SCORBUTIC PATIENTS TO ELUCIDATE THE RELATIONSHIP OF ASCORBIC ACID TO IRON.

Introduction

Iron occurs either in the oxidised (ferric) form or in the reduced (ferrous) form. Ferrous iron is more readily absorbed than the trivalent form (Moore et al, 1944). In the classical concept of the absorption of iron (Granick, 1955), ferrous iron enters the mucosal cell and is immediately oxidised to ferric iron, in which form it diffuses to that part of the cell nearest the capillary. Here there is a reducing mechanism which changes the ferric iron back to the ferrous form. The ferrous iron passes through the cell membrane and is chelated on to beta-1-globulin (siderophilin).

In the plasma iron is transported in the ferric form. Most of the iron passing through the plasma is used in the synthesis of haemoglobin and appears to be directly transferred from beta-1-globulin to receptors on the reticulocyte membrane (Jandl et al, 1959) and then through the cytoplasm for the synthesis of haemoglobin (Allen and Jandl, 1960). A reducing mechanism must be present in red cell precursors as ferrous iron is incorporated into protoporphyrin in the formation of haem.

When large amounts of iron enter the mucosal cell some of the ferric iron as ferric hydroxide is complexed with a protein apoferritin to give ferritin (Granick, 1955). Ferritin and haemosiderin (granules of iron and ferritin) are also the storage forms of iron (Laurell, 1947). Electron microscopy

has shown that the erythroblast normally contains ferritin (Bessis and Breton Gorius, 1959). Since the iron in ferritin is in the ferric form it must presumably be reduced prior to delivery to the plasma or incorporated into haem.

Several mechanisms have been proposed for the reduction of the ferric iron to the ferrous form. Mazur et al (1955) have shown that anoxia brings about the release of ferrous iron from ferric hydroxide in ferritin and Heilmeyer (1958) postulated that in the intestinal mucosal cell reduction might be brought about by the powerful reducing substance ascorbic acid. There is some evidence to show that ascorbic acid plays a part in the lumen of the gastro-intestinal tract facilitating the absorption of iron. Large amounts of ascorbic acid when fed with ferric iron reduced the trivalent iron and caused it to be more completely absorbed from the intestinal tract (Moore et al, 1941). Several authors have reported that the absorption of iron in the diet was augmented by the addition of ascorbic acid (Moore, 1955a; Steinkamp et al, 1955; Moore, 1955b).

That reducing substances are necessary for the incorporation of iron to protoporphyrin in the biosynthesis of haem is strongly suggested by the in vitro studies of Goldberg (1959), who showed that reducing substances such as ascorbic acid, glutathione and ergothioneine in concentrations which are normally present in red cells increase enzymic proto-haem formation in a system to which the iron has been added in the ferric form.

In view of the possible relationship of iron metabolism to ascorbic acid, deficiency of this vitamin could theoretically lead to: (a) impaired absorption, (b) failure to release iron from the reticulo-endothelial system and (c) defective incorporation of iron into haem. It was therefore decided to investigate these various aspects of iron metabolism in scorbutic Bantu subjects. Because of the difficulty in interpretation of the results of radioiron absorption studies in the Bantu, additional radioiron studies were done on scorbutic guinea pigs. Finally plasma iron turnover and percentage red cell utilisation studies in scorbutic Bantu patients were correlated with various haematological indices in an attempt to explain the anaemia of scurvy.

Material

All patients studied were adult Bantu subjects. The diagnosis of scurvy was made clinically. All were anaemic and manifested two or more features of scurvy -- haemorrhagic gums, perifollicular haemorrhages, discolouration of skin and haematomas into muscles. In one patient there was a haemorrhagic pleural effusion (Rozner and Lloyd, 1958). A not infrequent finding was scleroderma of the feet, evidence of old scurvy (Grusin and Kincaid-Smith, 1954). In all patients response to ascorbic acid and ward diet alone was dramatic and complete. There was no clinical evidence of any other vitamin deficiency, and infection was not present in any patient. Plasma iron estimations and

radioiron studies were commenced immediately on admission before the taking of a normal ward diet.

A. THE ROLE OF VITAMIN C IN THE ABSORPTION OF IRON

The Measurement of Iron Absorption in Scurvy with Fe^{59}

Methodology

There are only a limited number of methods for studying iron absorption. In the earlier attempts to assess the retention of iron by mouth, chemical balance studies were done. The method is accurate (Joseph, 1958), but technical difficulties are great and the procedure laborious. Plasma iron plus plasma radioiron curves have also been used to measure iron absorption. These methods, however, are of limited value and cannot give any quantitative assessment of absorption (Joseph, 1958; Bothwell et al, 1955).

In the earlier studies with radioactive isotopes of iron, the proportion of an oral dose of labelled iron appearing subsequently in the red cells was taken as a measure of iron absorption (Hahn et al, 1939). However, when Dubach et al (1948) measured not only radioiron recovered in newly formed red cells, but the unabsorbed radioiron in the faeces, it became evident that in normal subjects and in diseased states not all the absorbed iron was incorporated into red cells; some of the radioiron was stored. In chronic haemolytic anaemias, while all the absorbed iron may be incorporated into red cells, labelled red cells are removed from the circulation at a rapid rate and the percentage incorporation of iron will be less than 100 per

cent (Bothwell et al, 1956). Under these conditions the proportion of radioiron in the red cells after an oral dose could only be a close approximation of the percentage of iron absorbed. In iron deficient subjects, however, the proportion of the oral dose appearing in the blood is indeed a good measure of absorption (Dubach et al, 1948). This would be expected as radioactive studies have shown that iron travelling through the plasma of iron deficient subjects is almost completely utilised for red cell formation (Bothwell et al, 1956).

In a pilot study plasma iron turnovers were done on five scorbutic patients. These patients were then given 500mg of vitamin C intravenously. The incorporation of radioiron into red cells was measured at two day intervals. Nearly all the radioactive iron was incorporated into the red cell mass at eight days (Fig. 1, p. 152). Hence, as in iron deficient subjects, the percentage of an oral dose in red cells eight days after therapy was considered to be a satisfactory measure of iron absorption.

Method and Results

Four patients with scurvy were given an oral dose of 5mg of iron as ferrous sulphate labelled with 15μc of radioactive ferric chloride. The patients were fasted for at least four hours before and after the dose and 12-24 hours later 500mg of vitamin C were given intravenously to ensure maximum iron utilisation (Table I, p. 154). In a further four patients the

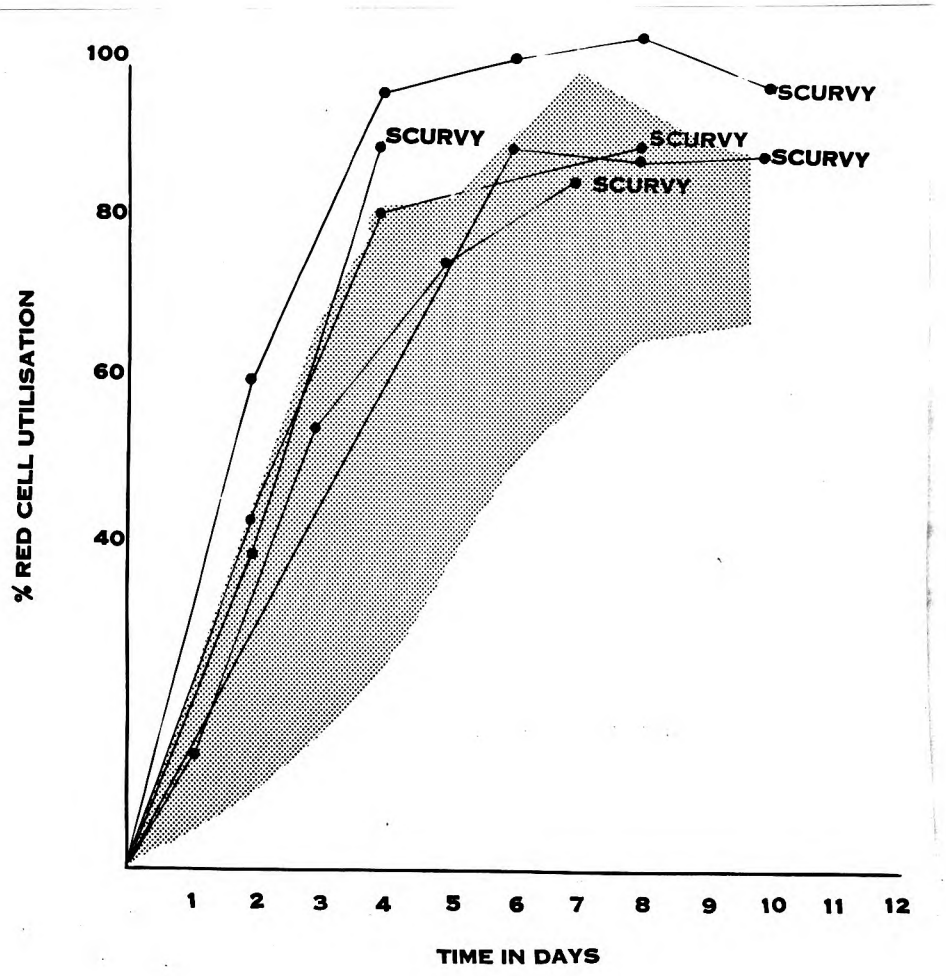


Figure 1 - The Percentage Red Cell Utilisation Curves in Five Cases of Scurvy after Plasma Iron Turnovers had been followed by 500mgm of vitamin C intravenously.

test dose was given after the patients had been treated for varying intervals with ascorbic acid (Table IB, p. 155). The patients were fasted for ten hours before and a further four hours after the oral dose. Vitamin C was recommenced 12-24 hours later. Percentage incorporation was calculated as follows:

$$\frac{\text{CPM/ml of blood} \times \text{BV (as cal. from body Wt.)}}{\text{Total number of CPM administered}}$$

An aliquot of radioiron was kept and measured at eight days to allow for decay of radioiron.

The number of cases studied was small (Table I, p.154+5), the range of absorption was wide and there was a marked overlap between the two groups, hence no valid conclusion could be drawn. Iron absorption was apparently not completely depressed in scurvy, nor was there any obvious increase after therapy.

Discussion

The state of the iron stores and the rate of erythropoiesis are of major importance in the control of iron absorption (Bothwell et al, 1958). Stimulation of erythropoiesis increases the absorption of iron whereas increase in iron stores diminishes iron absorption. In scurvy, as will be shown, there is increased erythropoiesis. However, iron overload (cytosiderosis with heavy deposits of iron in the portal tracts) was found in 11 of 12 liver biopsies, done after the patients had fully recovered (Fig.2, p. 156). The effect of opposing factors cancel out each other. This has been shown experimentally (Bothwell et al, 1958) and confirmed clinically (Callender, 1959)

TABLE I A

Percentage Oral Dose of Radioiron absorbed in Four Untreated Patients with Scurvy.

Case No.	Sex	Packed Cell Volume	Haemoglobin	% Reticulocytes	Serum Iron ug/100ml	% Oral Dose Absorbed
1	M	33	11.7	2	40	3
2	F	20.5	6.7	5	71	4
3	M	30	9.1	4	45	1.5
4	M	30	10.0	1.2	35	8
Mean Values and Range		28.4	9.2	3.05	48.75	4.1
		20.5-33	6.7-11.7	1.2-5	35-71	1.5-8

TABLE I B

Percentage Oral Dose of Radioiron Absorbed in Four Treated Cases of Scorvy

Case No.	Haemoglobin G.%.	P.C.V.	% Reticulocytes	Serum Iron	Oral Dose Absorbed	Comment
1	10.6	34	4.2	165	16.1%	Prior to study given 100mg Vit.C I.V. and 1000mg orally over six days.
2	11.6	37	3.4	180	4.0%	Prior to study given 1000mg I.V. and 750mg orally, Vit.C over four days.
3	12.3	39	0.8	215	2.0%	Prior to study given 500mg Vit.C. I.V. and 600mg Vit.C. orally over six days.
4	10.1	32	17.2	100	11.0%	Studied 72 hours after admission. Patient in early clinical remission.
Mean Values and Range		35.5 32-39	6.4 0.8-17.2	165 100-215	8.0% 2-16.1%	

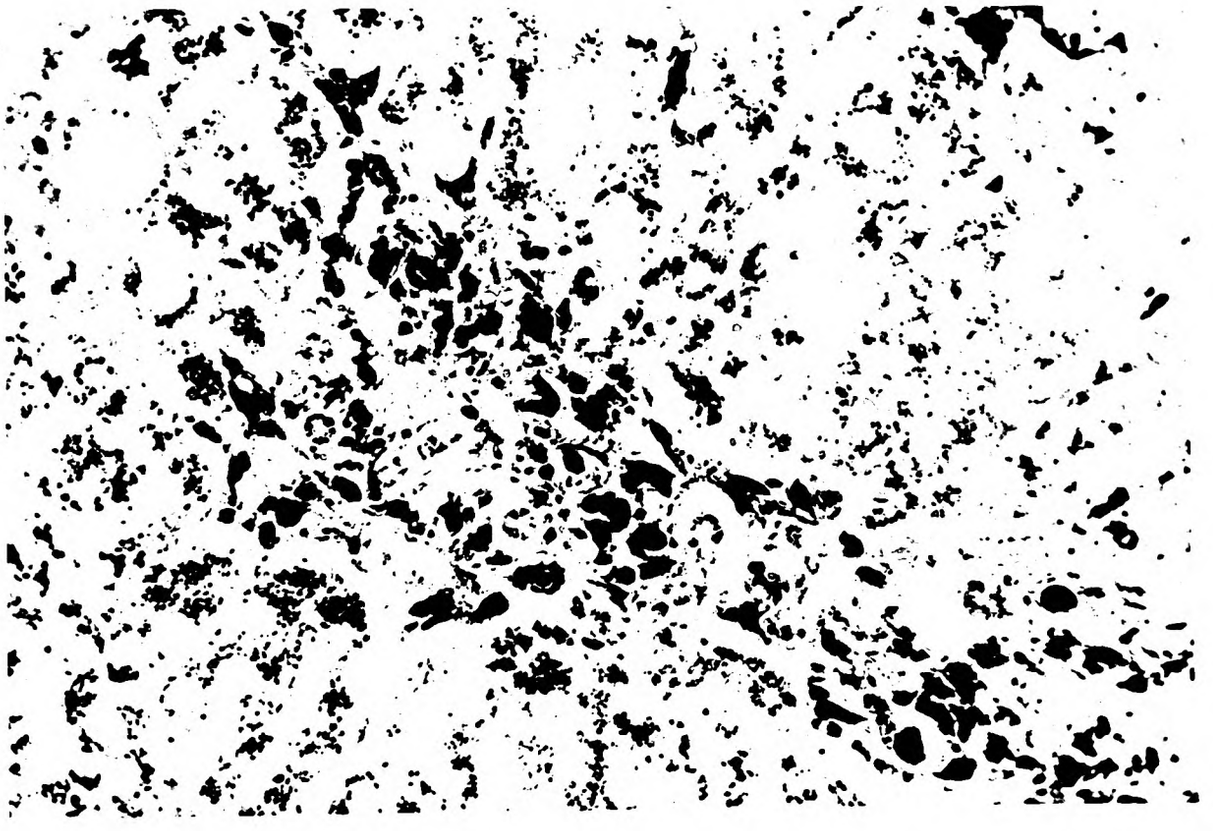


Figure 2 - Heavy deposits of Iron in Portal Tracts, and to a lesser degree in liver cells in a treated case of Leucy.

and probably accounted for the normal range of iron absorption (1.5 — 16.1 per cent) in untreated scurvy in the Bantu (Smith and Pannacciulli, 1958).

Because of the marked degree of iron overload in scorbutic Bantu patients depressing iron absorption, it was difficult to determine whether avitaminosis C was associated with decreased absorption. It was therefore necessary to study experimentally the effect of a scorbutic state on iron absorption.

Experimental Approach

Method

Adult guinea pigs were made scorbutic on a diet of bran (45%), oats (30%) and dried skimmed milk (25%). The latter was heated to 110° for two hours to ensure destruction of all vitamin C. Twice a week each guinea pig was given 1cc of cod liver oil. This diet will produce scurvy in 2-3 weeks .

Twenty milligrams of vitamin C per guinea pig were added to the drinking water of half the colony each day. That the diet was effective in the other half of the colony was shown by the finding of periarticular haemorrhages in five of those guinea pigs who died 3-4 weeks later. After 22 days five scorbutic guinea pigs and eight control animals were each given 50µg of iron as ferrous sulphate labelled with 2µc of Fe^{59} as ferric chloride. This amount of radioiron is equivalent on a weight basis to the amount given to the scorbutic Bantu patients. The guinea pigs were fasted the night before and for four hours after the experiment. Seven hours after the experiment all guinea

pigs were given 10mg vitamin C intramuscularly and 24-hours after the experiment all the guinea pigs were allowed a normal diet. The haemoglobin was estimated in each animal 48-hours after the experiment when the condition of the scorbutic guinea pigs had improved (Table II p.159).

Ten days after the oral dose of radioactive iron the animals were weighed and exsanguinated and the livers perfused with saline. The amount of radioactivity was measured in 2cc of blood, the liver and the spleen of each animal. There was minimal radioactivity in the liver and spleen in all animals hence only the findings in blood have been considered. The total blood volume was calculated from the body weight. Since a known amount of radioactivity had been given and a standard of that had been kept to allow for decay, the percentage absorbed was calculated as follows:

$$\text{Percentage absorbed} = \frac{\text{CPM/ml of blood} \times \text{BV}}{\text{Total number of CPM administered}} \times 100$$

Results

The range of absorption in four scorbutic guinea pigs was 4.1 per cent to 31.1 per cent - mean 20.1 per cent - while the range in eight control animals was 0.6 per cent to 4.5 per cent - mean 1.6 per cent. The percentage of an oral dose of iron absorbed was much greater in the scorbutic guinea pigs than in the controls.

Discussion and Conclusion

The increase in iron absorption in scorbutic guinea pigs is probably the result of increased erythropoiesis. The marrow

TABLE II

Control Animals			Scorbutic Animals		
No.	Haemoglobin (48hrs. after the oral dose of radio-iron)	Percentage Radioiron Absorbed	No.	Haemoglobin (48hrs. after the oral dose of radio-iron)	Percentage Radioiron Absorbed.
1	14.9	0.7%	1	12.2	31.1%
2	15.4	1.4%	2	13.3	4.1%
3	15.7	4.5%	3	12.4	30.2%
4	13.1	0.6%	4	14.6	18.0%
5	15.4	2.1%			
6	14.2	2.0%			
7	14.9	1.6%			
8	14.7	1.7%			
Mean Values	14.8	1.6%		13.1	20.1%
and Range	13.1-15.7	0.6-4.5%		12.2-14.6	4.1-31.1%

of scorbutic patients is hyperplastic and the plasma iron turnover is increased two to three times normal (see next section). Increased erythropoiesis is known to increase iron absorption. Since increased amounts of iron can be absorbed in the absence of vitamin C, ascorbic acid is not essential for the absorption of iron.

THE RELATIONSHIP OF VITAMIN C TO BODY IRON STORES

The effectiveness of ascorbic acid to release iron from the reticulo-endothelial system was assessed qualitatively

1. by comparing in scorbutic patients on admission the serum iron levels with body iron stores and
2. by measuring the serum iron levels at varying intervals after the intravenous administration of vitamin C to scorbutic patients.

While the serum iron level represents a balance between the amount of iron released by the reticulo-endothelial system and that utilised by the marrow, the plasma iron turnover measures quantitatively the amount of iron passing through the plasma. The plasma iron turnover is primarily a measure of erythropoiesis. The main source of iron for erythropoiesis is that released from effete red cells in the reticulo-endothelial system. Hence the plasma iron turnover is also a semi-quantitative measure of the amount of iron released by the reticulo-endothelial system.

Plasma iron turnovers were done in 11 scorbutic patients and repeated in three of them at varying intervals after the intravenous administration of vitamin C to measure - 1. the amount

of iron released by the reticulo-endothelial system of scorbutic patients and 2. to determine whether in these patients intravenous vitamin C was followed by any increase in the amount of iron released by the reticulo-endothelial system. All the methods used in this section have been described in Chapter II.

Results

The serum iron level on admission in 17 scorbutic patients ranged from 20-83µg/100ml - mean 56.3µg/100ml (Table III, p.162). (Normal mean Bantu serum iron level 81.7µg/100ml) This serum iron level was low in comparison with the markedly increased iron stores found in the marrow of 8 patients on admission. There was an immediate and marked rise in the serum iron after the intravenous administration of 500mg of vitamin C (Fig.3, p.163), equivalent to the release of 4mg of iron in 4 hours. This rise in serum iron was not observed in normal subjects (one with mild cytosiderosis), in a patient with scurvy observed for 16 hours on a vitamin C free diet, in treated cases of scurvy, nor in a variety of haematological conditions associated with a low serum iron level and haemosiderin in the marrow, (infection and carcinoma). Heparinised (3u/ml) scorbutic blood was incubated at 37°C for 4 hours with vitamin C (1mg per 10cc blood), i.e. the same amount as would be present in 10cc of blood immediately after 500mg had been given intravenously. There was no rise in the serum iron level as compared with a control to which no vitamin C had been added. Hence the in vivo rise in the serum iron level was not due to the effect of the high concentration of vitamin C on scorbutic red cells. In 12 patients the serum iron estimation

TABLE III

Serum Iron Levels in Scorbutic Subjects before and after treatment

Case No.	Sex	Serum Iron ug/100ml on admission	Marrow Haemosiderin on admission	Serum Iron ug/100ml. Ten days after Treatment
1	M	28	-	-
2	M	66	-	152
3	M	40	-	-
4	F	71	-	-
5	M	55	+++	233
6	M	71	-	215
7	M	43	-	-
8	M	83	+++	-
9	M	75	-	233
10	M	20	+++	75
11	M	59	+++	240
12	M	68	+++	264
13	M	70	-	75
14	M	40	+++	199
15	M	72	-	208
16	M	35	+++	252
Mean Values and Range		56.3 20-83		194 75-264

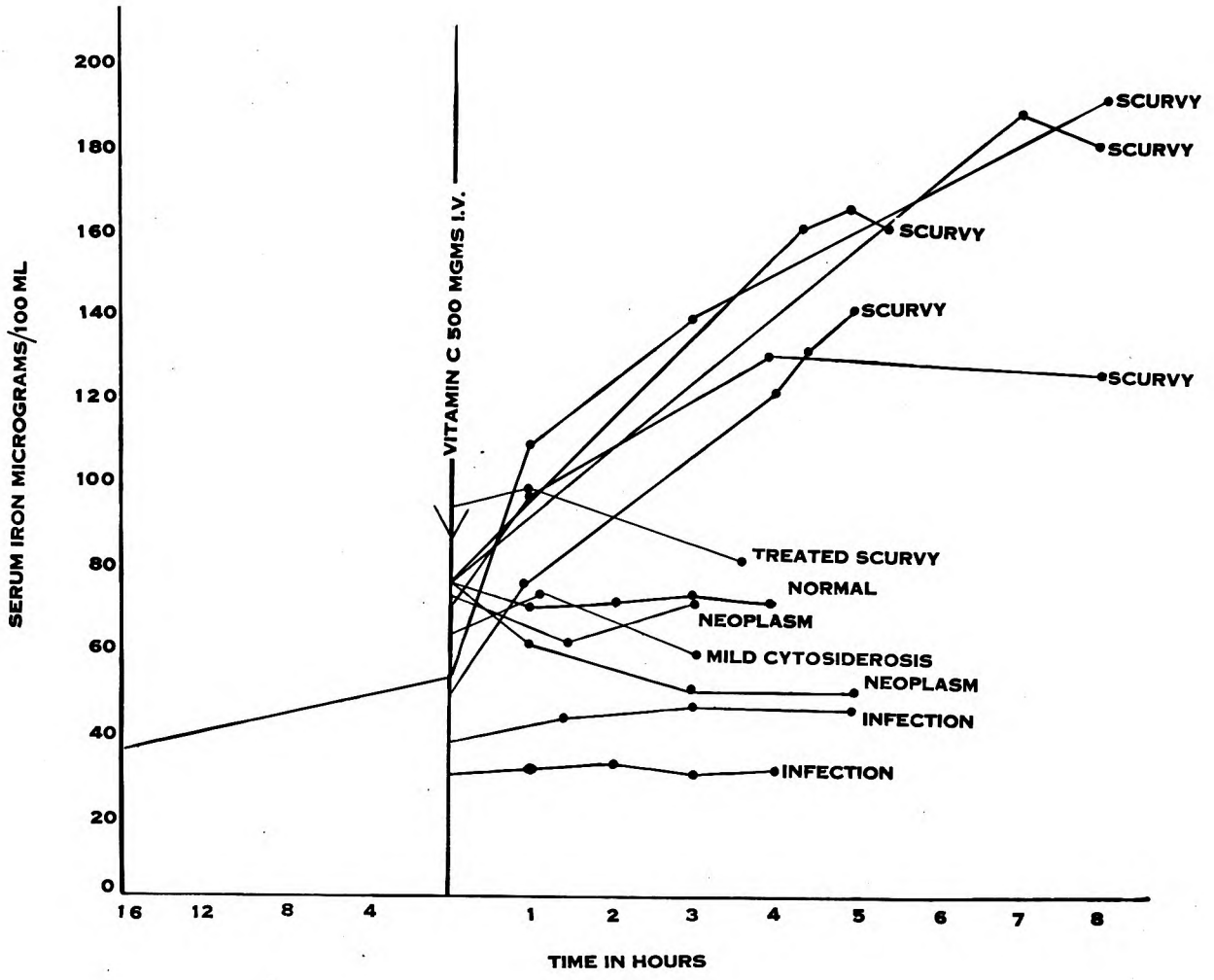


Figure 3 - The marked rise in the Serum Iron of Scorbutic Patients following Intravenous Vitamin C. No rise was noted when vitamin C was given intravenously to a normal subject; treated scorbutic patients and patients with infections or neoplasms who had hypoferremia with haemosiderin in the marrow. No significant rise was noted in a scorbutic patient observed for 16 hours.

was repeated 10 days after treatment (Table III, p. 162). The serum iron ranged from 75-264ug/100ml - mean 202ug/100ml). This was in keeping with the finding of marked cytosiderosis in 11 of 12 liver biopsies done after recovery. Plasma iron turnovers in 11 scorbutic patients ranged from 1.1 to 3.13mg of iron per 100ml of blood per day - mean 1.79, normal mean 0.65) (Fig.IV , p.165). In three patients the plasma iron turnovers were repeated at varying intervals after therapy (Fig. 5, 6, 7, p.166-8). In only one of these patients did the *plasma iron turnover increase after therapy (1.8 to 3 times normal).* (Fig. 7, p. 168).

Discussion

The initial observations, a low serum iron in association with increased body iron stores and a marked rise in the serum iron after vitamin C suggested that in scorbutic patients there was a block in the release of iron from the reticulo-endothelial system.

While the serum iron represents a balance between the amount of iron released by the reticulo-endothelial system and that utilised by the marrow, the plasma iron turnover measures quantitatively the amount of iron passing through the plasma and is a better index of iron kinetics than serum iron estimation alone.

The most important factor controlling the movement of iron through plasma is erythropoiesis (Bothwell et al, 1957). With erythroid hyperplasia there is an increase in the plasma iron

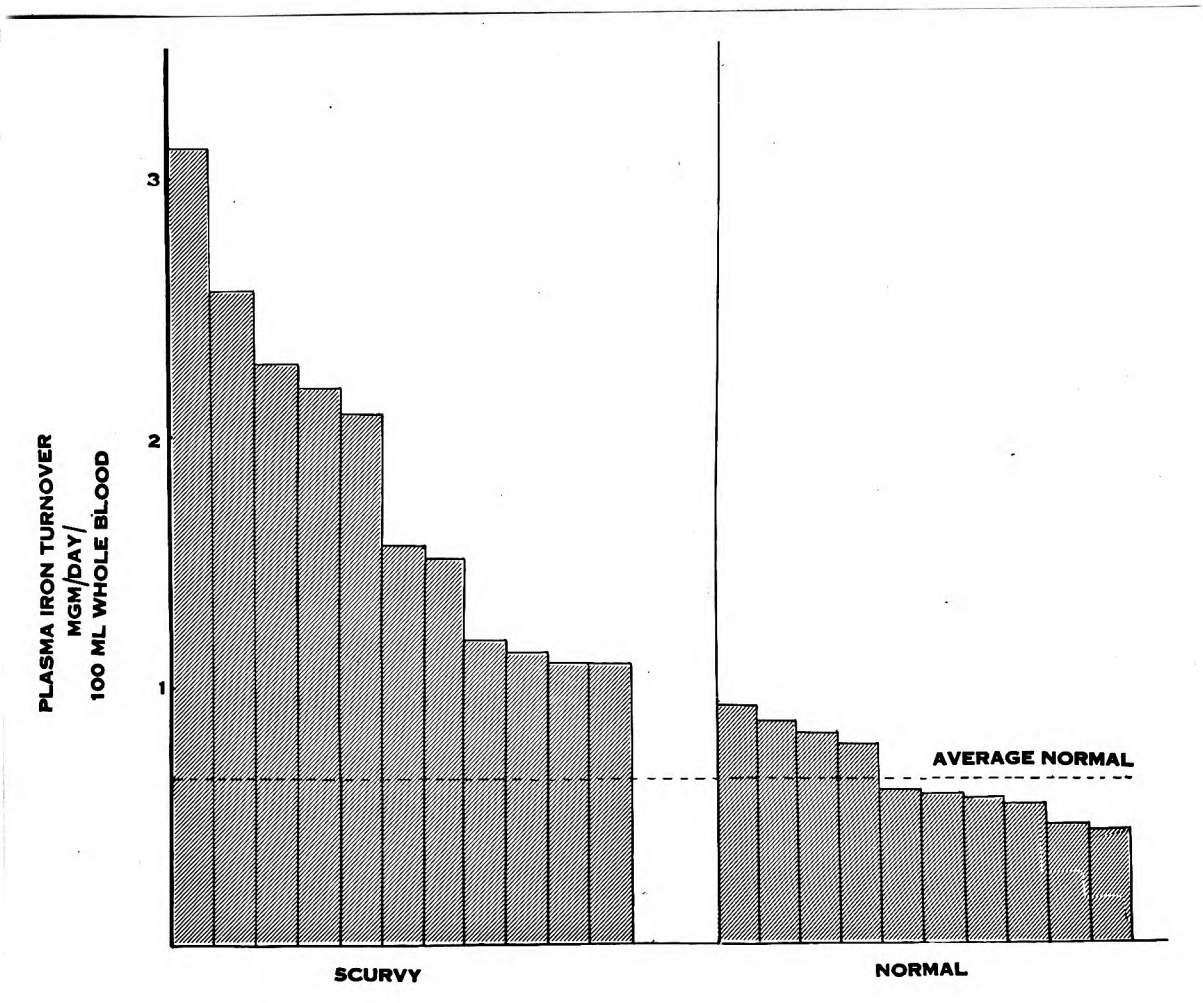


Figure 4 - Plasma Iron Turnovers in Eleven Scorbutic Subjects as compared with Ten Normal Subjects.

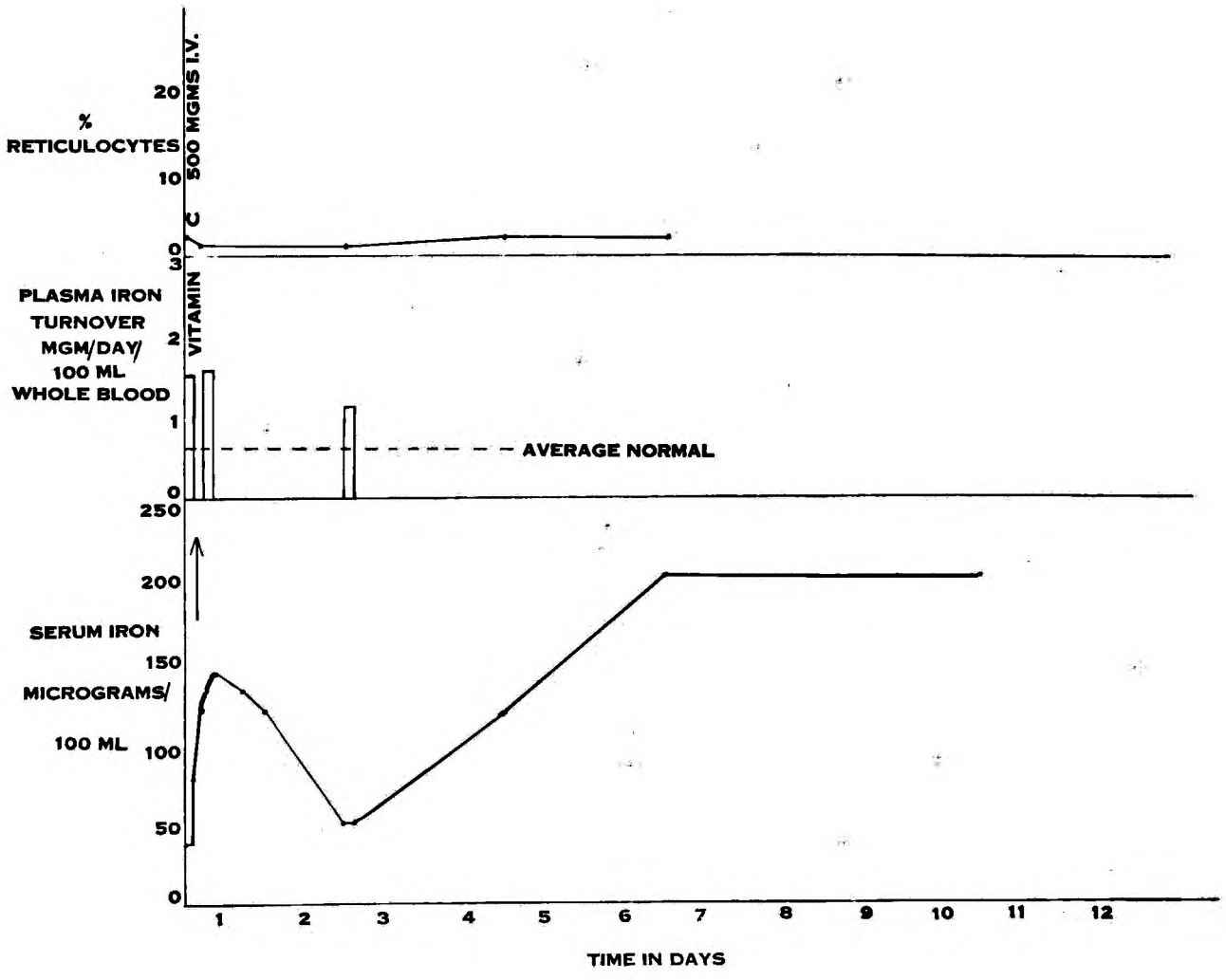


Figure 5 - Serum Iron, Reticulocyte and Plasma Iron Turnover Estimations in a Scorbutic Patient before and after treatment. The arrow indicates the time when 500mgms of Vitamin C was injected intravenously.

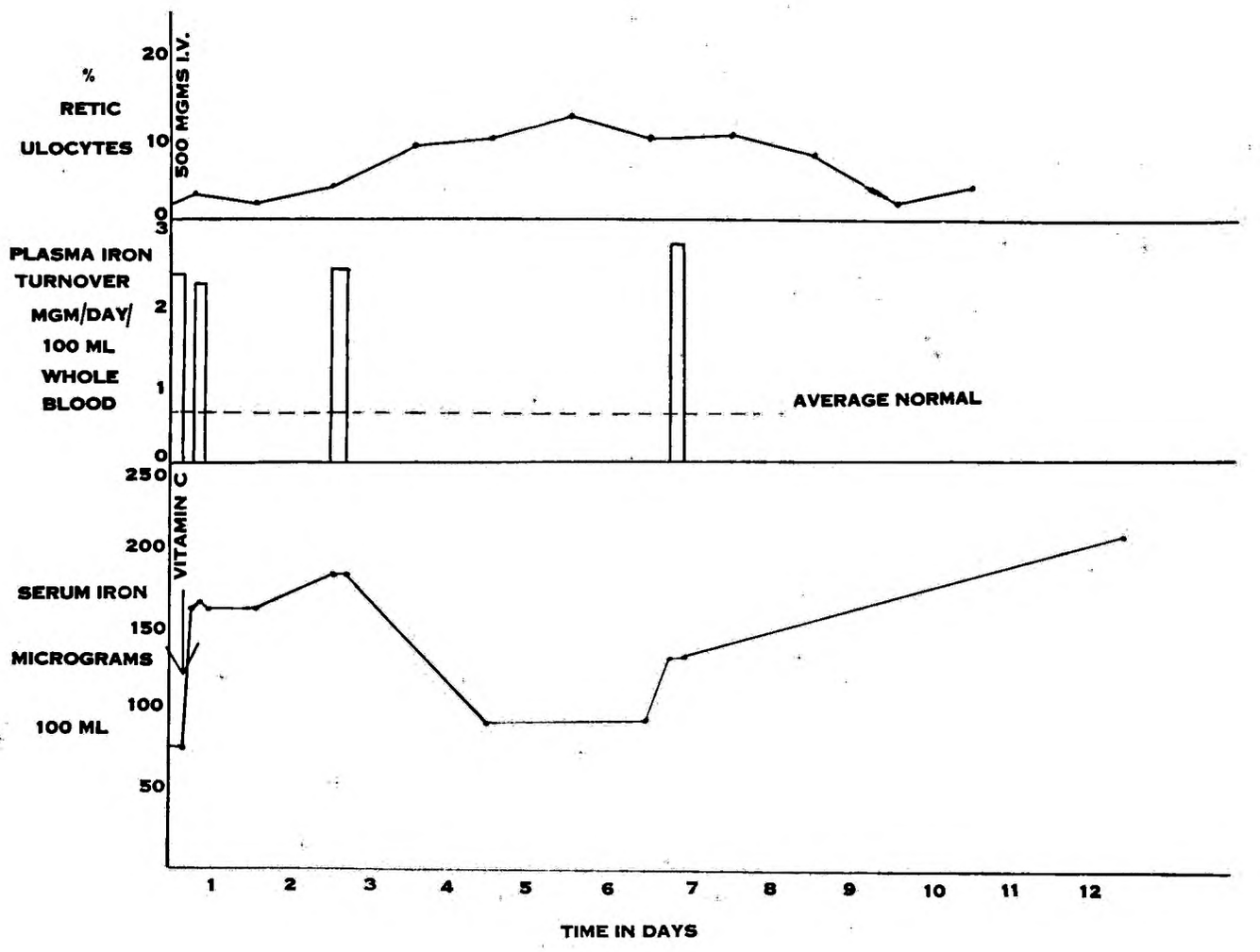


Figure 6 - Serum Iron, Reticulocyte and Plasma Iron Turnover Estimations in a Scorlutic Patient before and after treatment. The arrow indicates the time when 500mgms of Vitamin C was injected intravenously.

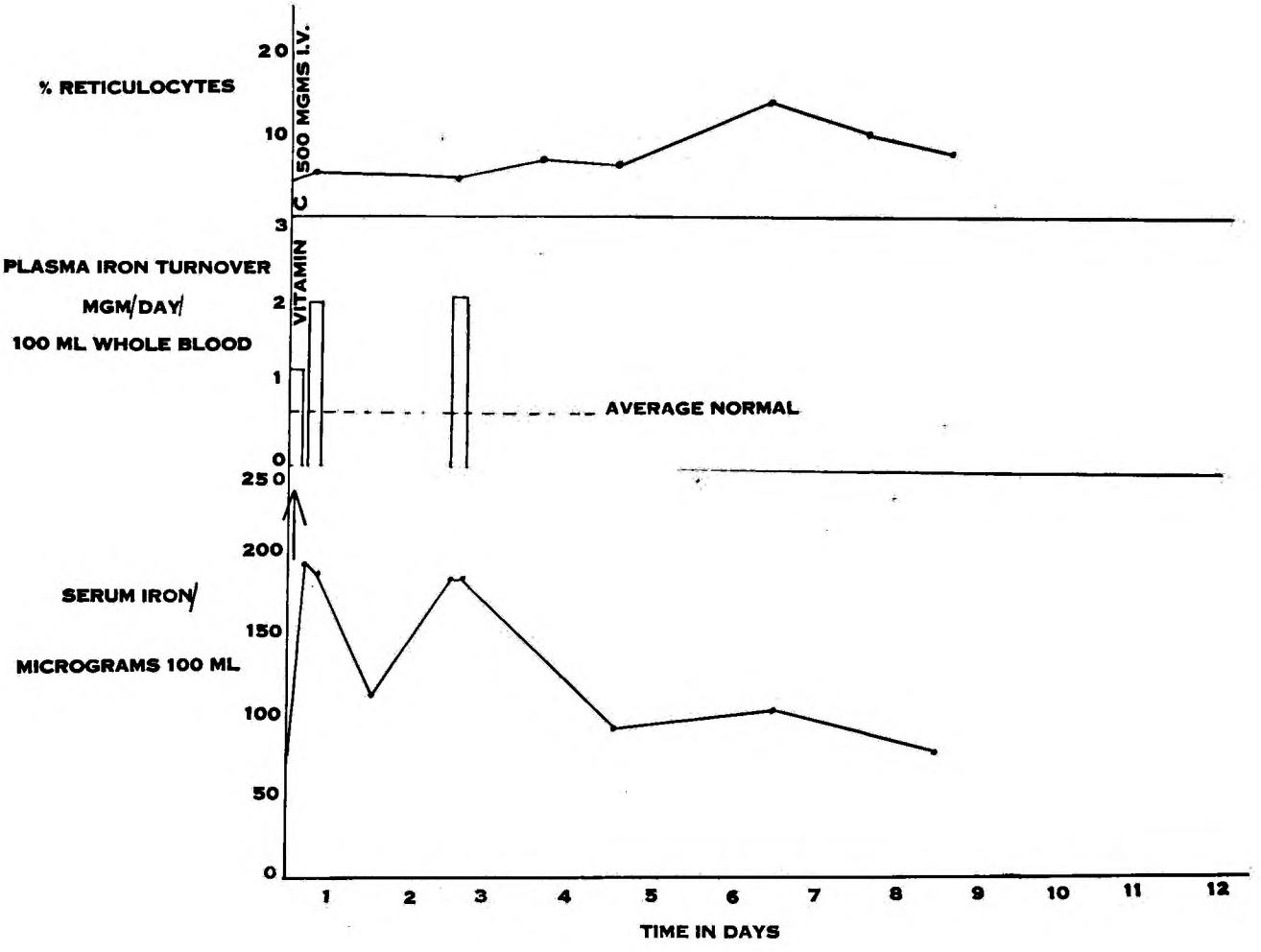


Figure 7 - Serum Iron, Reticulocyte and Plasma Iron Turnover Estimations in a Scorbutic patient before and after treatment. The arrow indicates the time when 500mgms of Vitamin C was injected intravenously.

turnover which must be associated with a corresponding increase in the amount of iron released by the reticulo-endothelial system. A block in the release of iron from the reticulo-endothelial system would result in a fall in the serum iron level and a failure of the plasma iron turnover to increase in response to increased marrow activity. Conversely, the relief of a significant block in the passage of iron from the reticulo-endothelial system to beta-1-globulin will be reflected by a rise in the serum iron level and an increase in the plasma iron turnover. The plasma iron turnover in the 11 scorbutic patients was increased nearly three times normal, an increase which represents maximum marrow response to an acute episode whether haemorrhage or haemolysis. Furthermore, in only one of three patients studied did the plasma iron turnover increase after therapy. Hence in scurvy there is no quantitative impairment in the release of iron from the reticulo-endothelial system. While the initial observations suggested that vitamin C is capable of releasing iron from the reticulo-endothelial system, in its absence other mechanisms and reducing substances, e.g. glutathione, can release increased amounts of iron.

As noted by others, the hypoferremia of infection and neoplastic states, was not associated with a rise in the serum iron after the intravenous administration of 500mg of vitamin C. Hence these low serum iron levels are not due to any quantitative deficiency of vitamin C.

IN VITRO EXPERIMENTS TO DETERMINE WHETHER THE UPTAKE OF RADIOIRON BY SCORBUTIC MARROW IS IMPAIRED

There are two theories on the passage of iron through the erythroblast based on experimental and histological data respectively. Jandl et al (1959) using Fe^{59} showed that iron is transferred from the iron binding protein of plasma to the receptors of the reticulocyte membrane. In its subsequent passage through the cell the radioiron was first associated with particulate fractions (stroma microsomes and mitochondria) and then gradually released to the cytoplasm; where it was incorporated into a "non-haemoglobin" protein and then into haemoglobin (Allen and Jandl, 1960). It was thought possible that in all particulate fractions the Fe^{59} was in the form of a single substance, such as ferritin, having a different solubility in microsomes. Bessis and Breton Gorius (1959) described in immature red cells submicroscopic electron dense particles that resembled ferritin. These particles were regarded as precursors for haemoglobin synthesis. In either hypothesis the ferric iron must be reduced to the ferrous form before it can be incorporated into the protoporphyrin nucleus in the synthesis of haemoglobin. Goldberg (1959) has shown that concentrations of ascorbic acid similar to that which occur in human erythrocytes give optimal uptake of iron by iron incorporating enzyme into protoporphyrin IX. Hence in scorbutic patients there could be impairment in the synthesis of haemoglobin.

A series of experiments was devised to determine if the marrow of a scorbutic subject was capable of synthesising

haemoglobin and if there was any quantitative impairment in the rate of haemoglobin production.

Experiment 1.

An aliquot (2cc) of marrow from a patient with untreated scurvy was incubated at 37° in a silicone-coated tube with 5cc of plasma from the same patient. Ten microcuries of Fe^{59} had previously been bound to the plasma and 0.3mg of penicillin and streptomycin had been added to the silicone-coated tube to prevent bacterial growth. After 16 hours the mixture was washed five times with 0.9% saline, haemolysed by repeated freezing and thawing, and an aliquot run electrophoretically on Whatmans No. 3 filter paper in a veronal buffer (pH 8.6) at volts 120 for 20 hours. Solutions of human haemoglobin from haemolysed red cells and ferritin were run simultaneously. The ferritin had been prepared previously in this laboratory by the method of Granick (1951), from a haemochromatotic liver. The filter papers were then dried and fixed in a corset with a negative x-ray film and left for two weeks. When the film was developed there was a dense black band opposite haemoglobin. It was concluded that some of the radioiron added to the system must have been incorporated into protoporphyrin for the formation of haemoglobin. Haemoglobin can apparently be synthesised from vitamin C free erythroblasts.

Experiment 2.

Three cubic centimeters of plasma from a scorbutic subject were labelled with 15 microcuries of Fe^{59}. To this was added 3cc of the patients marrow. The mixture was then added to 14cc of

Eagles medium (3:7). The tube was mixed thoroughly and divided into two equal parts. To one half ascorbic acid was added in the concentration of 10×10^{-5} (Goldberg, 1959). Aliquots of the two mixtures were then incubated in silicone-coated tubes for 4, 15 and 21 hours respectively, after which time the cells were washed five times in 0.9% saline. The radioactivity of the washed cells was then measured.

Results expressed as absolute counts per minute

Hours after incubation	Control Mixture	Mixture to which vitamin C had been added
4	71,000	67,000
15	132,000	118,000
21	140,000	165,000

The cells were then haemolysed with two volumes of water and centrifuged at 3,000 r.p.m. for 30 minutes. In this way radioiron on the cell membrane (deposit) could be separated from that in haemoglobin and ferritin (supernatant).

Results - percentage radioiron recovered in the supernatants

Hours after incubation	Control Mixture	Mixture to which Vitamin C had been added
4	54%	56%
15	57%	59%
21	63%	54%

The supernatants were heated to 75° over a period of 5 minutes and then cooled to separate ferritin (which presumably would remain in solution) from haemoglobin which would be deposited.

Results expressed as percentage recovered in supernatant

Hours after incubation	Control Mixture	Mixture to which vitamin C had been added
4	33%	36%
15	21%	20%
21	13%	18%

Results

In Experiment No. 2 it is apparent that there was a progressive increase in the uptake of radioiron by scorbutic marrow cells over 20 hours, but at the various time intervals there was apparently no difference in the quantitative uptake of radioiron by scorbutic marrow as compared with a similar mixture to which vitamin C had been added in physiological amounts. Furthermore, the percentage radioiron recovered in cell membrane, ferritin and haemoglobin at the various time intervals was no different in a mixture of scorbutic marrow, plasma, radioiron and Eagles medium, as compared with an identical mixture to which vitamin C had been added.

Conclusion

Under the experimental conditions there was no block in the uptake of radioiron by scorbutic marrow and no difference in the rate and degree of transfer of iron from reticulocyte membrane to presumably ferritin and then haemoglobin. It would appear that the reducing property of vitamin C is not essential for the incorporation of iron into protoporphyrin. It is possible that there are still trace amounts of vitamin C in the marrow of a

scorbutic patient or that in its absence another reducing substance, e.g. glutathione, incorporates ferrous iron into protoporphyrin.

General Summary

In a scorbutic state the body is able to absorb increased amounts of iron, release increased quantities from the reticulo-endothelial system and incorporate iron effectively into protoporphyrin for the formation of haem. A physiological function of ascorbic acid may be the reduction of the ferric iron to the ferrous form, but in its absence other reducing substances probably bring about the reduction of the ferric iron.
Liver Biopsy results - Fig. 2 , p.156).

Heavy deposits of iron in the periportal tract, Kupffer cells and, to a lesser degree, in the liver cells, were found in 11 of 12 liver biopsies obtained from treated scorbutic patients. The frequency of this finding suggests that the Bantu diet which contained excess iron was low in vitamin C, or that heavy iron overload may in some way inactivate vitamin C.

Excess iron in the body may increase the rate of oxidation of vitamin C. Moore (1941) showed that a rise in the serum iron was associated with a fall in plasma ascorbic acid. More than 50 per cent of patients suffering from those blood dyscrasias which are associated with high serum iron values (pernicious anaemia, hypoplastic anaemia) were shown to have less than 0.1mg of vitamin C per 100ml of plasma. Cytosiderosis in the Bantu by the specific oxidative effect of the iron overload and high serum iron may precipitate scurvy in a patient on a low vitamin C diet.

THE ANAEMIA OF SCURVY

In children and in experimental animals (May et al, 1950) the anaemia of scurvy is megaloblastic and thought to be due to the impaired reduction of folic acid to folinic acid. Anaemia is not a feature of experimentally induced scurvy in adults (Crandon et al, 1940; Medical Research Council, 1948). In naturally occurring scurvy in adults, however, although a megaloblastic morphology has been observed (Brown, 1955; Bronte-Stewart, 1953), the anaemia is essentially normocytic normochromic (Grusin and Kincaid-Smith, 1954; Bronte-Stewart, 1953; Brown, 1955).

The anaemia associated with naturally occurring scurvy has been variously interpreted. Goldberg (1959) showed experimentally that ascorbic acid potentiated the enzyme incorporating iron into protoporphyrin and postulated that ascorbic acid deficiency could give rise to a hypochromic anaemia. He found some support for this in a careful analyses of the patients studied by Bronte-Stewart in that the mean corpuscular haemoglobin concentration was less than 32 per cent in 12 subjects studied and rose significantly in a further 7 subjects after vitamin C. While mild hypochromic anaemia may not be an infrequent finding in adult scurvy, gross microcytic hypochromic anaemia is rare and found only in severe scurvy (Metz, 1959). Goldberg (1959) felt that an associated diminished production of erythrocytes could mask the development in scurvy of a microcytic hypochromic anaemia.

Kerskey (1953) transfused normal red cells into scorbutic patients who were kept on a vitamin C free diet and by the Ashby technique observed a decrease in the survival of the transfused cells. From careful observation and urobilinogen excretion, he inferred that no further bleeding had occurred. The ultra microscopic observations of Kisch and Martin (1957) that the scorbutic red cell is abnormal suggests that it may well have a shortened survival. Additional support for a haemolytic element in the anaemia of scurvy was the finding by Vilter et al (1946) of an initial reticulocytosis which fell on therapy. However, the reticulocyte pattern as seen by Bronte-Stewart (1953) was different in that there was a reticulocyte response to therapy. In only five subjects in a series of 32 was there an initial reticulocytosis. He ascribed the anaemia of scurvy to blood loss.

It is apparent that several mechanisms have been invoked to explain the production of anaemia in scurvy and the following study was undertaken to elucidate this mechanism.

Material and Methods

All cases studied were diagnosed clinically as outlined in the first part of this Chapter and all methods used have been described in Chapter II. The anaemia of scurvy was studied by examination of peripheral blood and bone marrow smears and by observing the reticulocyte response to therapy. Blood and bone marrow films were also examined for siderocytes and sideroblasts and plasma iron turnovers and red cell

utilisation curves were done to quantitate marrow activity. In addition, the red cells of three scorbutic patients were labelled with Cromium^{51} (as described by Mollison and Veall, 1955) and transfused into three normal subjects to measure the survival of scorbutic red cells in a normal environment.

Results

Peripheral Blood -- the mean corpuscular haemoglobin concentration in 13 scorbutic patients ranged from 27.6 - 38 (mean 33.1)(Table IV, p.178). While a mild hypochromic anaemia was noted in several of the smears examined, the anaemia as reported by others was essentially normochromic normocytic (Grusin and Kincaid-Smith, 1954; Bronte-Stewart, 1953).

Bone Marrow -- mild to marked erythroid hyperplasia was noted in eight marrow smears examined. Erythroid myeloid ratios were not determined as it may be quite misleading in the quantitative estimation of erythropoiesis, (Wintrobe, 1956).

Reticulocyte Pattern - a variable reticulocyte pattern was found in scorbutic patients (Fig.8, p.179; Table V, p.180). Both an initial reticulocytosis and a low reticulocyte count with a rise after therapy were observed.

Plasma Iron Turnovers done on 12 scorbutic patients on admission ranged from 1.1 to 3.13mg% with a mean of 1.78mg% (Table V, p.180) An increase of three times normal is found in response to acute blood loss or haemolysis. The percentage red cell utilisation curves measured in three scorbutic patient were normal (Fig.9,p.183). These patients were kept on a low vitamin C diet during the six day

TABLE IV

Blood Smears

Case No.	Sex	Packed Cell Volume	Haemoglobin	Mean Corpuscular Haemoglobin Concentration	Slide Microscopy
1	M	32.4	10.2	32,4	Normochromic
2	M	27.2	7.5	27.6	Mild hypochromic
3	M	25.0	8.6	34.4	Mild hypochromic
4	M	25.5	7.9	31.0	Mild hypochromic
5	M	26.7	8.1	30.4	Normochromic
6	M	33.0	11.7	35.5	Normochromic Normocytic
7	F	20.5	6.7	32.7	Mild hypochromic
8	F	30.3	10.0	33.3	-
9	M	20.0	6.7	33.4	-
10	M	39.0	13.3	34.1	Normochromic Normocytic
11	M	30.0	10.0	33.3	-
12	M	29.0	11.1	38.0	Normochromic Normocytic
13	M	29.5	10.3	35.0	
Mean values and Range		28.3	9.4	33.1	
		25-39	6.7-13.3	27.6-38	

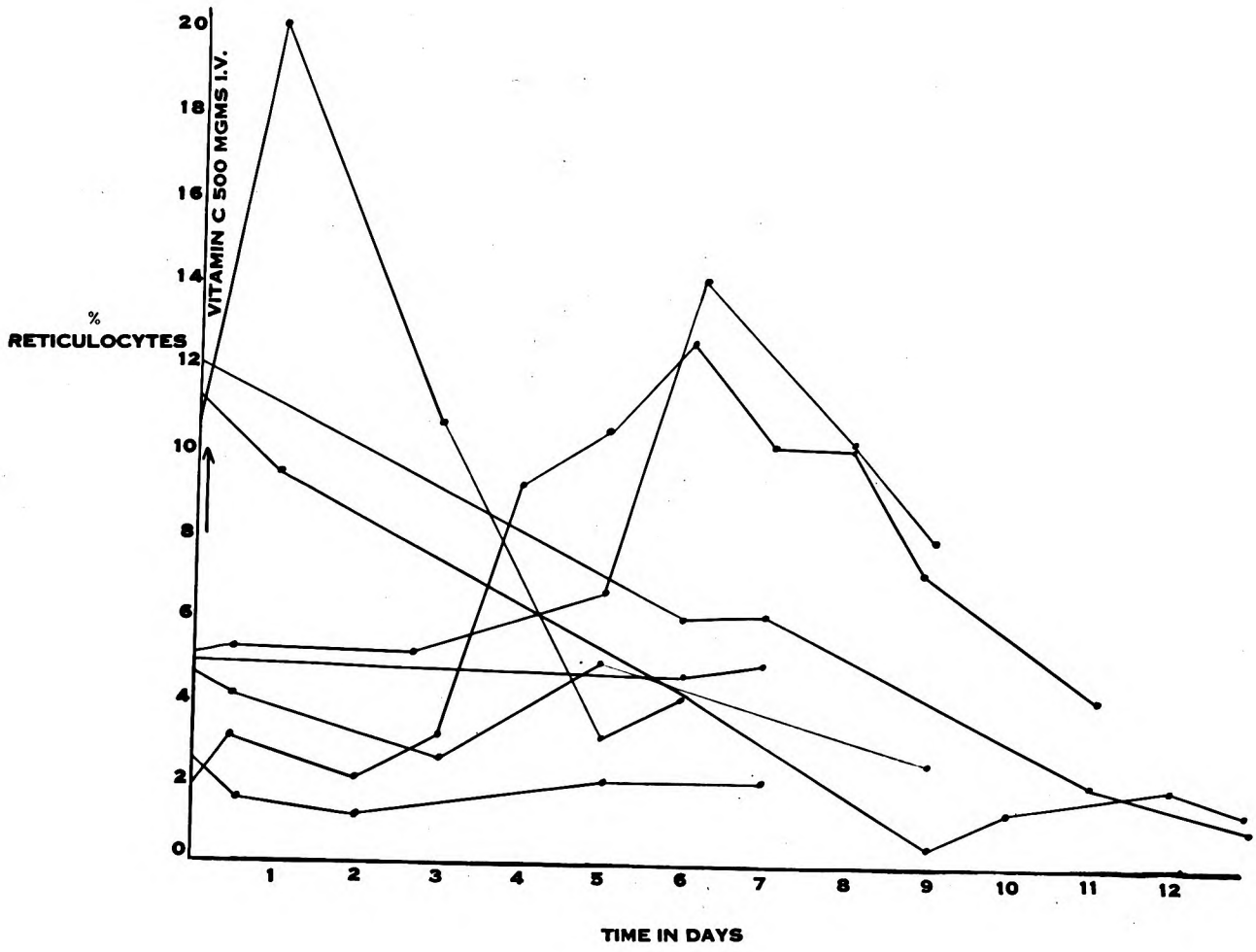


Figure 8 - The Reticulocyte Pattern in Eight Scorbutic Patients after Intravenous Vitamin C. The arrow indicates the time when 500mgms of Vitamin C was injected intravenously.

TABLE V

Scorbutic Patients

Case No.	Sex	PCV	% Reticulocytes	% Corrected Reticulocytes	Plasma Iron ug/100ml	Time taken for radioiron to reach half concentration (minutes)	Plasma Iron Turnover mg/day/100ml Plasma	Plasma Iron Turnover mg/day/100ml Whole blood	Production Index Reticulocytes	Production Index (Plasma Iron Turnover)
1	M	32	4.5	2.9	59	38	1.55	1.10	223	169
2	M	25	11.4	6.2	75	18	4.17	3.13	477	482
3	M	19	12.0	4.5	20	14	1.43	1.19	346	183
4	M	25	5.0	2.5	68	27	3.24	2.52	190	388
5	M	26	4.6	2.3	70	46	1.52	1.17	185	180
6	M	36	2.4	1.6	40	18	2.22	1.51	123	233
7	M	27	3.0	1.6	72	24	3.00	2.28	123	350
8	M	20	10.2	4.1	66	24	2.75	2.26	316	348
9	M	30	2.6	1.6	43	21	2.05	1.50	123	231
10	M	30	0.2	0.12	71	47	1.51	1.10		170
11	M	38	2.2	1.7	112	36	3.11	2.04	130	314
12	M	35	0.2	0.12	96	42	2.29	1.56		220
Mean Values			4.85	2.3	66	29.6	2.40	1.78	(Mean of 9 cases, excluding Nos.2,3 & 8)	
and Range		19.36	0.2-12.0	0.12-6.2	20.-112	14-47	1.43-4.17	1.10-3.13	123	251

period of observation.

The peripheral blood and bone marrow smears of 12 scorbutic patients were stained for iron and counterstained with 1% safranin. Siderocytes were not found in the peripheral blood in any patient and in only one patient was a high percentage of sideroblasts (75-100%) found in the bone marrow when the serum iron was low (35ug%). The marrow storage iron in this patient was markedly increased.

Discussion

An explanation of the anaemia of scurvy must take into account the variable reticulocyte pattern found since various patterns have been described (Bronte-Stewart, 1953, Vilter et al, 1946). In nine of the 12 cases studied, the initial reticulocyte count was low. In several patients there was a reticulocytosis several days after therapy. It is suggested that the low initial reticulocyte count with a rise after therapy is the basic reticulocyte pattern found in scurvy (Bronte-Stewart, 1953). Scorbutic patients are not in haematological equilibrium. In the course of the deficiency there are haemorrhagic episodes. A reticulocyte response to such an acute episode could account for the initial high reticulocyte count observed in three patients. However, a rise in the serum iron and a reticulocytosis in scorbutic subjects at rest on a low vitamin C diet (Fig. 9, p. 183) suggested that small quantities of vitamin C and bed rest could result in haematological remission and this is an alternative explanation

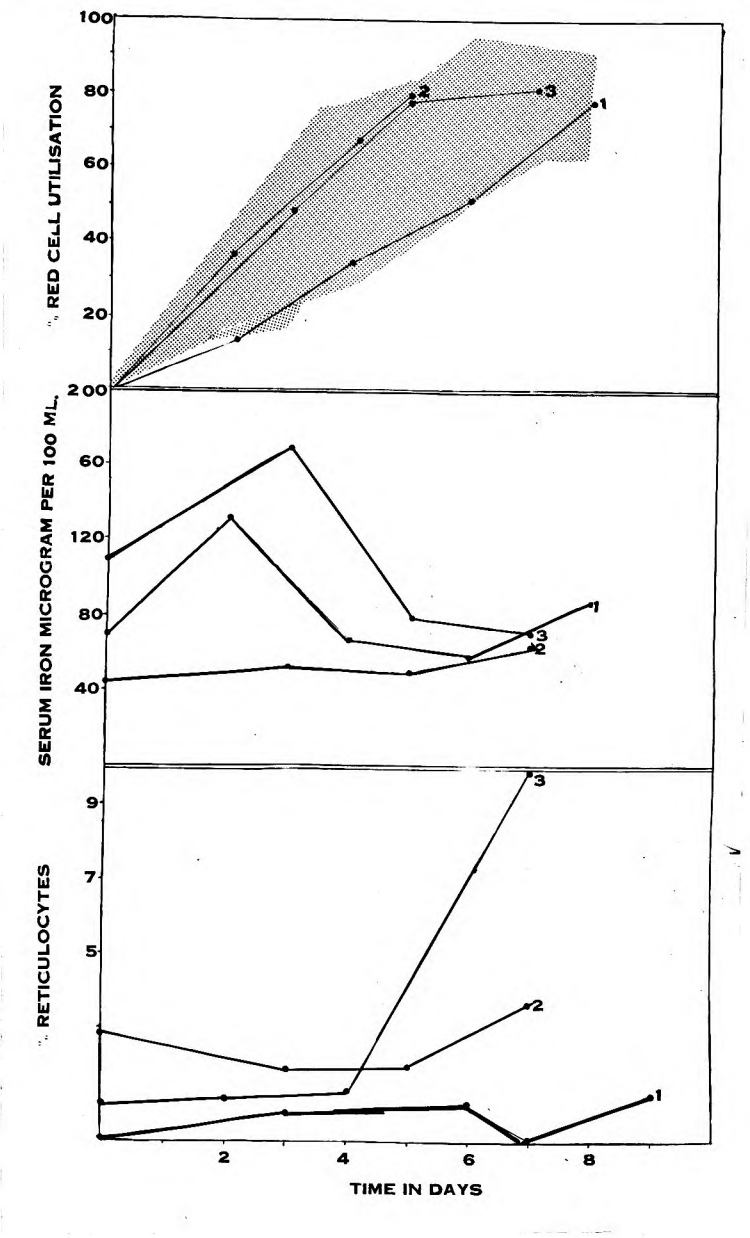


Figure 9 - Percentage Red Cell Utilisation Curves, Serum Iron and Reticulocyte Estimations in Three Scorbutic Patients maintained on a Low Vitamin C Diet for Six Days.

normal. In two of these patients, one of whom in addition had a reticulocytosis of 11%, there was a marked rise in the serum iron level while on the low vitamin C diet. These findings suggested either that there were small, but significant, amounts of vitamin C in the diet, or that haematological remission had occurred with bed rest. The interval between serum iron estimations in the third patient was too great to exclude the possibility of haematological remission.

Recently Crosby and Sheehy (1960) observed a man with hypochromic iron loading anaemia. Iron was entering the erythrocytes and forming ferruginous granules (sideroblasts and siderocytes) in preference to haemoglobin synthesis. The spleen was able to remove these granules from circulating erythrocytes without destroying the cells themselves. Siderocytic iron, like haemoglobin iron, passes from marrow to spleen via red cells and back as plasma iron. The plasma iron turnover was markedly increased, but little injected Fe^{59} was incorporated into haemoglobin.

When the patient was phlebotomised and became moderately iron deficient, the sideroblasts and siderocytes disappeared from the marrow, the plasma iron turnover decreased and the amount utilised for haemoglobin synthesis was 90 per cent. It would appear that the initial increased plasma iron turnover with low red cell utilisation was due to Fe^{59} being cycled through the ferruginous granules of the siderocytes, such a turnover being largely ineffective. In normal marrow the

incidence of normoblasts with iron positive granules ranges from 24 per cent to 81 per cent with a mean value of 48 per cent. These granules only disappear by the reticulocyte stage (Douglas and Dacie, 1953). Iron granules only disappear from the normoblasts in the more severe grades of iron deficiency. On the other hand, in all marrows in which extracorpuscular iron was demonstrable, the normoblasts contained iron. Siderocytes are not normally found in the peripheral blood. Reduction of iron in these granules may be a stage in the synthesis of haemoglobin and in the absence of vitamin C sideroblasts and siderocytes may collect in the marrow and in the peripheral blood, respectively and could account for the ineffective erythropoiesis in scurvy. However, in no patient were siderocytes found in the peripheral blood and in only one of 12 patients examined was there a high percentage of sideroblasts (75-100%) in the marrow with a low serum iron, and in this patient there were heavy deposits of iron in the marrow. The cycling of iron through ferruginous granules in siderocytes does not apparently explain the ineffective erythropoiesis of scurvy.

Labelled red cells (Cr^{51}) from three scorbutic patients were transfused into haematologically normal recipients. The transfused red cells were observed for ten days during which time they survived normally (Fig.10, p.186). Scorbutic red cells may, however, survive normally in normal plasma, but could have a shortened survival in vitamin C free plasma. This is analogous to the anaemia in copper deficient swine. Copper

deficient red cells have a shortened survival only in copper deficient swine (Bush et al, 1956).

To determine the life span of scorbutic red cells it would be necessary to auto-transfuse them and, during the period of observation, maintain the patient on a vitamin C free diet. During this period valid conclusions could only be drawn if there was no evidence of haematological remission, no changes in plasma volume and no haemorrhagic episodes.

Summary

Many routine haematological tests have little value in the understanding of the anaemia of scurvy.

1) In the presence of intramuscular haemorrhages, reticulocyte counts and quantitative estimations of serum bilirubin and stool urobilinogen, are of little value in determining the presence of haemolysis. Because of these haemorrhagic episodes and the ethical and practical difficulty in maintaining a patient in a scorbutic state, the determination of red cell life span from auto-transfused labelled red cells is difficult.

2) The value of the erythroid myeloid ratio is limited in scurvy. This ratio is based on the premise of a normal myeloid series which is inferred if the peripheral white cell count is normal. Leucopenia is, however, a common finding in scurvy. Another disadvantage of the erythroid myeloid ratio is that marrow samples are frequently contaminated by peripheral blood.

As a result of recurrent haemorrhagic episodes anaemic scorbutic patients are not seen in haematological equilibrium.

The patients are also in all probability suffering from multiple subclinical vitamin deficiencies. Further, it would appear that haematological remission in scurvy can occur with rest in bed and a low vitamin C diet (Vilter et al, 1946). These factors probably account for the variable reticulocyte pattern reported in scurvy and the difficulty in interpretation of all the data on the anaemia of scurvy. It is suggested that in scurvy there is increased erythroid marrow activity in response to haemorrhage. However, a large fraction of this erythropoiesis is apparently ineffective. Furthermore, red cells released to the peripheral blood are morphologically defective. While these cells apparently survive normally in a haematologically normal patient, there is a decreased survival of normal, as well as autogenous, cells in scorbutic patients (Merskey, 1953; Goldberg, 1961). The pattern of haemolysis and ineffective erythropoiesis is similar to that found in megaloblastic anaemias. Such a mechanism underlying the anaemia of scurvy in children and experimental animals would be expected since their anaemia is megaloblastic. While functionally the anaemia in scorbutic children and adults is the same, morphologically the anaemia of scurvy in children is megaloblastic, while in the adult it is normocytic. This morphological change in the anaemia of scurvy is unexplained.

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Marrow smears and liver sections stained with

potassium ferrocyanide to demonstrate

haemosiderin

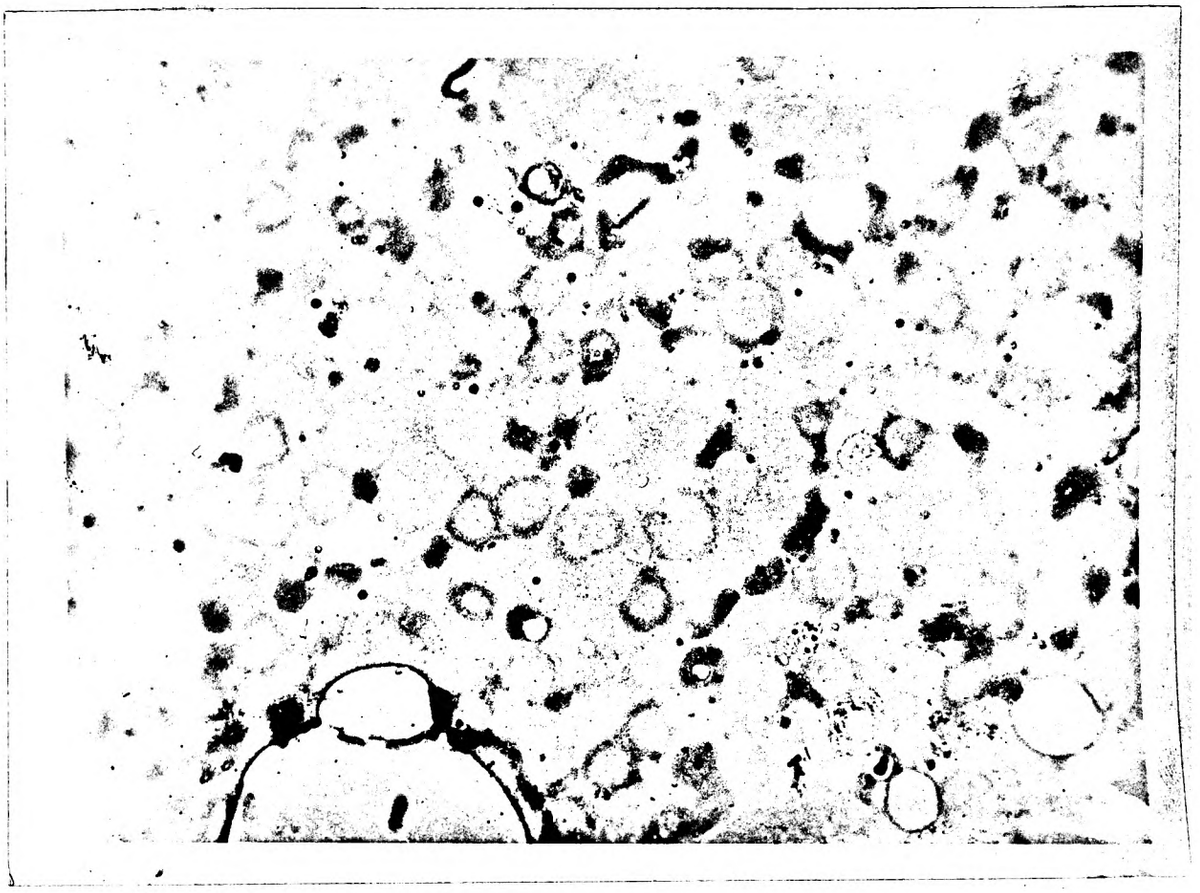


Plate I - Normal Iron Stores in Marrow (+).

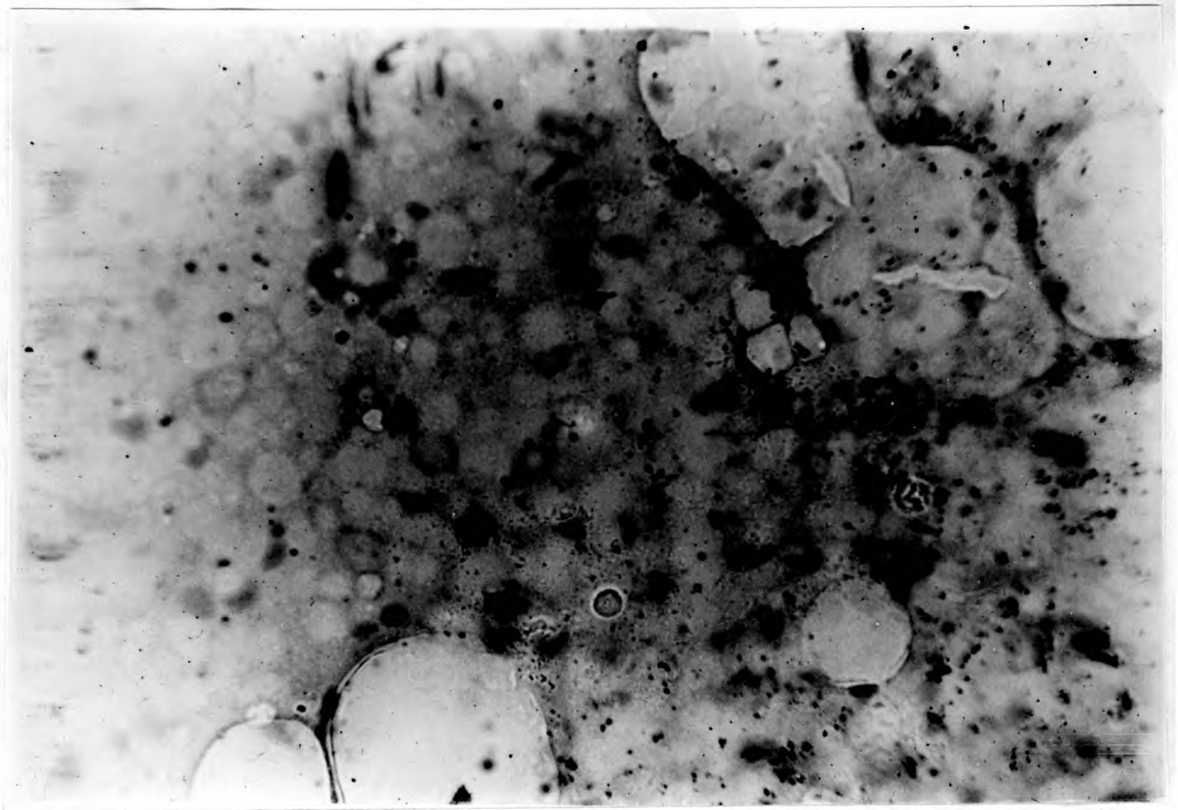


Plate II. - Moderately Increased Iron Stores in Marrow (++). (From a patient with Cytosiderosis).

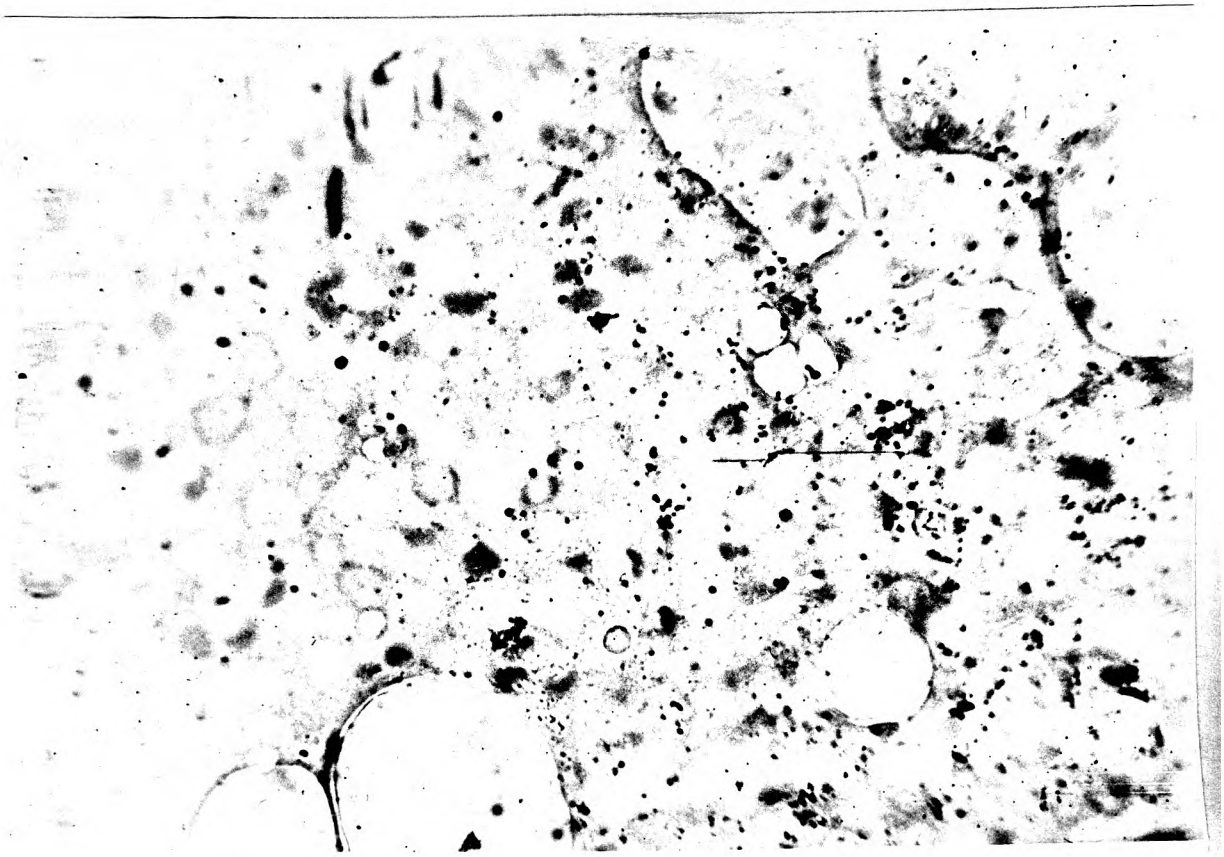


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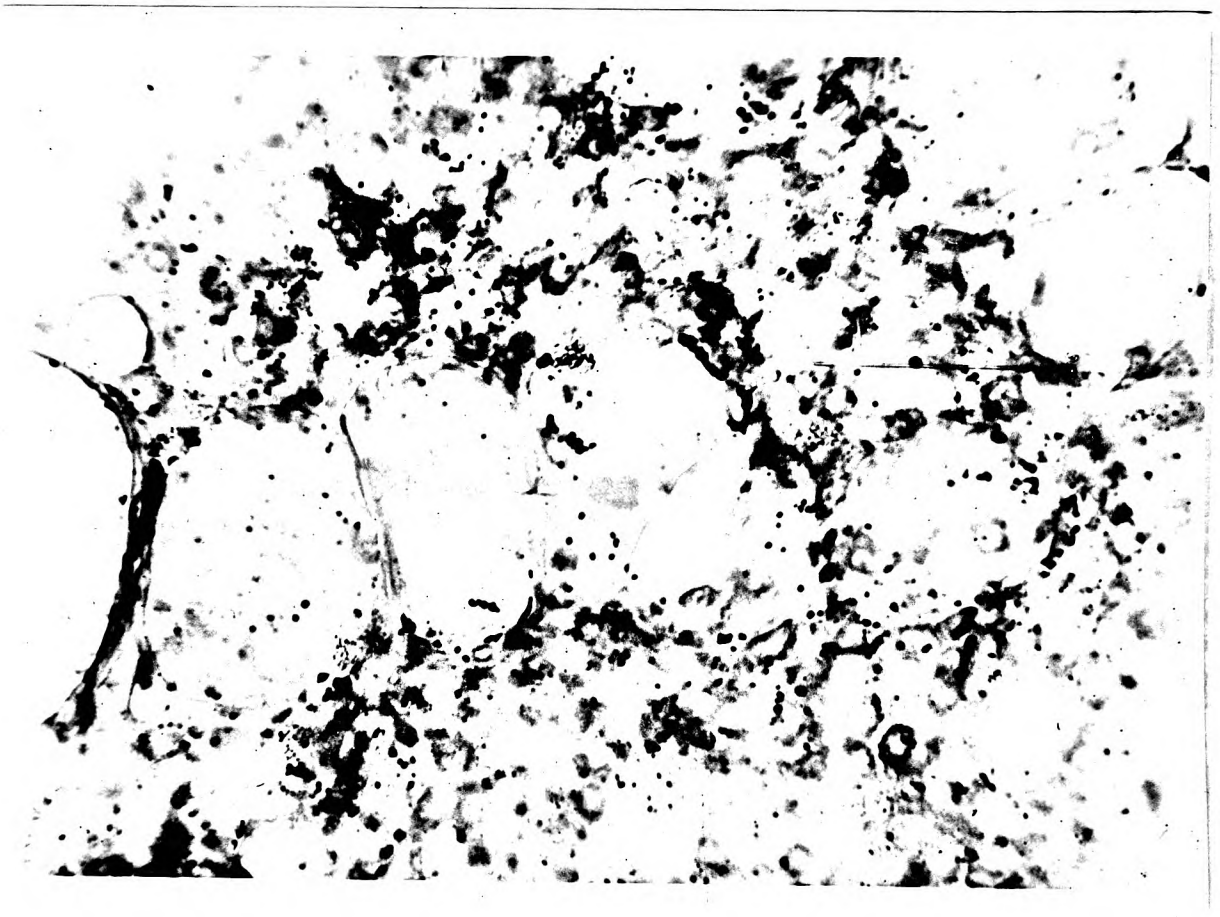


Plate III - Markedly increased iron stores in the marrow (Case No. 1, Table XI, p. 83)

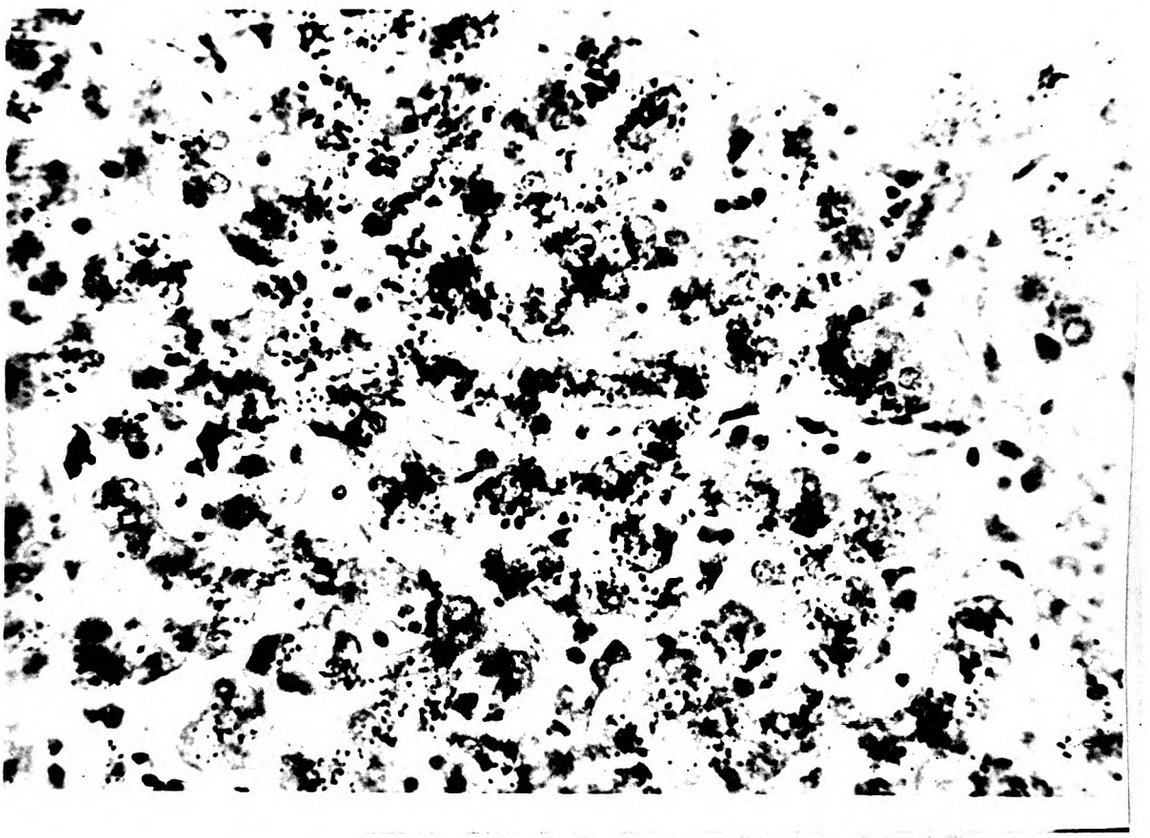


Plate IV - Markedly increased amounts of haemosiderin in hepatic cells (Case No. 9, Table V, p. 139).

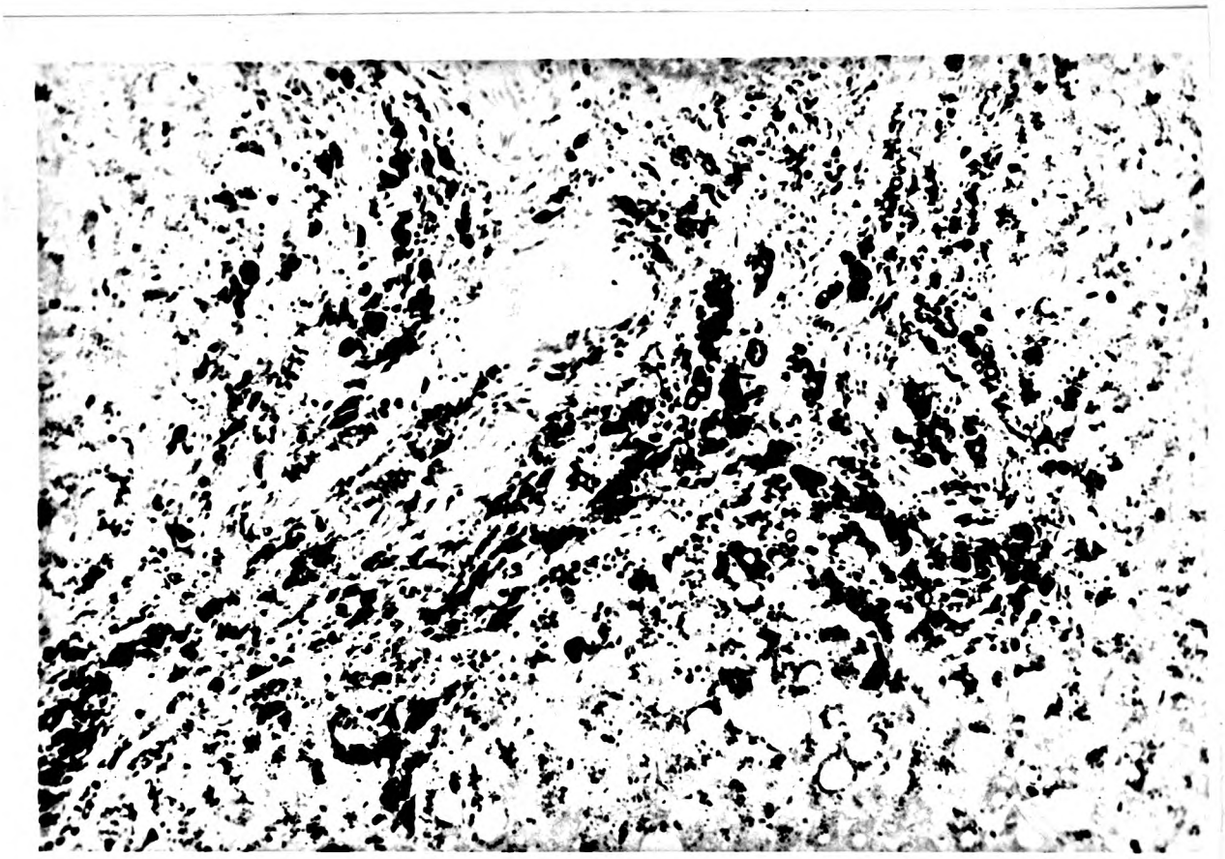


Plate V - Cirrhosis of the Liver with heavy deposits of Iron in portal tracts (Case No. 6, Table V, p. 139).