A STUDY OF IRON KINETICS IN NORMAL AND ABNORMAL HUMAN SUBJECTS

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My thoughts, at the time of writing this chapter, go back to the time when, fresh from housemanship duties in June 1968, I confronted the world of 'SCIENCE'. This confrontation was initiated more by accident than by design, by an invitation from Professor E. Dowdle, at that time head of the Radio Isotope Diagnostic Services of Groote Schuur Hospital, to come and 'learn to think like a scientist'. Whether or not I have acquired this faculty is debatable, but by accepting his invitation, I have seen a world of excitement and reason, mixed with very hard work and a search for knowledge. In this world I have made friends, acquired certain skills, and developed an understanding of the limits of my knowledge, as well as the extremes of knowledge available.

The choice of a project upon which I could work was directed by a happy coincidence. I had an interest in haematology and the Department had no other individual with a particular interest in isotopic haematological investigations. The possibility of describing the pathophysiology of obscure anaemias, and of quantitating erythropoiesis seemed to me very exciting, and committed me firmly to this line of investigation.

Having "fallen" for this project, I needed to develop the skills necessary for carrying out the work. This period of my life is associated with many happy memories. New friends were made and skills acquired. Particularly helpful during this period were

Lyn Petersen, Heather Weir, Val Connell, Egbert Herring, Keith Blake and Dennis Fenner. Their friendship and help saw me through the initial period of awe and mistrust, when all was strange and the laboratory seemed dominated by complicated machines. During this period I learned to use a pipette, to know when to use 'hot' and 'cold' glassware, to manipulate counters, rate meters and scanners, to understand the vocabulary of radio-isotope work, and to acquire an understanding of what isotope decay is and how it is measured. Each day was a mixture of excitement and despair, elation and depression, anxiety and relief, as I moulded into the laboratory situation and pondered over my own ignorance.

Once the basic skills had been acquired, the time came for setting up an experimental protocol and working with patients. I shall never forget my delight when our first experiment worked and meaningful data was obtained, nor the need to share this experience with anybody with an open ear. During this period I was extremely fortunate to have the technical assistance of Miss Heather Weir. Her sense of proportion and perspective, technical ability and coolness under pressure are well known to her friends. Her friendship and help were invaluable.

With the experimental work running smoothly, the time came to elaborate on the basic work, and to question the meaning of my results. This period was spent browsing through the library reading papers, learning mathematics and statistics, and realising each day how little I really knew. At this stage I sought the

help of mathematicians, and eventually became associated with Mr. Paul Haddingham, a Chemical Engineer doing post-graduate work at the University of Cape Town. His criticisms of my experimental protocol helped me to repair shortcomings in my data, and his expertise produced the mathematical model detailed in this thesis.

Finally, the time came when it was necessary to write up the experimental work, and to interpret the data obtained. In some ways this was the most interesting period of work, as theories were developed, tested and accepted or rejected on their merits. However, it is doubtful that I would ever have seriously started to write this thesis without the constant help and encouragement that I received from my wife. The debt that I owe to my family is immense. They have been neglected, have seen me carry my papers away on weekends and vacations, and suffered through my period of writing up this thesis. Throughout they have been loving, helpful, reassuring and encouraging.

At the end of this period I was left with an untidy bundle of unedited notes. The sorting out and presentation of these notes in an acceptable form is due solely to Mrs. Angela Phillips, who has battled away at my writing through a rough draft and finally through the final copy of this work. This she has managed to do with a smile always ready to appear, and for this alone, I doubt I will ever forget her.

A constant source of encouragement and help has been Dr. Ann Orren, who started working in the Department of Isotope Diagnosis at

much the same time as I did. She has never been too busy to discuss a problem or to help in its solution. The expertise of her husband, Dr. Michael Orren, has likewise been particularly helpful and appreciated.

In conclusion to this chapter, I would like to reaffirm my gratitude to Professor E. Dowdle, who initially made this work possible and who has guided me constantly through rough patches, and has at all times been available for constructive criticism and help. His own high standard of scientific enquiry and capability is well known, and serves as a standard towards which all, who work with him, reach. My association with him has been totally stimulating and enjoyable.

CHAPTER I. INTRODUCTION

I-(1) The work to be presented in this thesis took place while I was working as a registrar in the department of radio-isotope diagnosi at Groote Schuur Hospital. During this period, I became interested in the ferrokinetic technique for attempting to quantitate normal and abnormal erythropoietis, and the possible development of these techniques so as to allow more accurate quantitation of the data. At the time the study started, the department was offering standard ferrokinetic investigations to aid in the diagnosis of abnormal haematological states. These studies did not, I felt, provide adequate information for the referring clinician, particularly in regard to ineffective erythropoietic activity.

I-(2) The idea that the findings of the research laboratory, in terms of bone marrow function and ineffective erythropoiesis, could be applied to the clinical patient, appealed to me. Furthermore, existing procedures, while providing for the measurement of the rate at which ^{59}Fe label left the plasma and appeared in the circulating blood, did not define what happened to this iron between these two processes. It seemed to me that, by appropriate experimental design and analysis, one could analyse this intermediary function, and this I have attempted to do.

I-(3) My thesis is written in five distinct chapters. Chapters two and three attempt to review the vast literature available on iron metabolism and erythropoiesis, both effective and ineffective. Chapter four attempts to define normal ferrokinetic parameters. I have found that body iron stores and serum iron levels profoundly affect ferrokinetic performances

in otherwise normal patients, and I have attempted to quantitate this variation with the aim of applying these modified parameters to disease states.

I-(4) The fifth part of my work is really a combination of work done by both myself and Mr. Paul Haddingham, who is a chemical engineer attached to the University of Cape Town. In this section, a model of bone marrow function has been developed which attempts to relate input into the bone marrow and output from it in a mathematical manner based upon our experimental findings and the research findings of other workers. The results obtained using this method of analysis are also presented.

I-(5) The final section of my thesis details my results in the study of normals and a number of disease states. This section attempts to determine the usefulness of the ferrokinetic technique I have developed, as well as to characterise the various disease states. In this section a study has been made of

(a) normal patients.

(b) haemolytic anaemias

(c) hypoplastic anaemias

(d) iron deficiency anaemias

(e) polycythaemias

(f) myelofibrosis and reticuloses

(g) neoplasms

(h) infections and inflammations

(i) uraemia

(j) cirrhosis

(k) B_{12} and folate responsive anaemias

(l) symptomatic porphyria.

This section is completed by a combined assessment of the results in these groups.

I-(6) Three appendices are added to my thesis. The first of these details the methods I have used in all the patients studied. The second appendix contains the mathematical proof and development of our model describing bone marrow function, and the third appendix details the bibliography referred to throughout the text.

CHAPTER II. REVIEW OF THE LITERATURE ON IRON METABOLISM.

II(a) Historical developments.

II-a-1 The presence of iron in body tissues was first described in 1713 by Lemery and Geoffrey.[507] Liebig[518] published his theory of respiration in 1843, in which he postulated that combustion in the animal body was related to the presence of iron and took place in the blood where haemoglobin (Hb) iron oxidised biological substrates. In 1867, Hoppe-Syeler[400] crystallised Hb, and McMunn subsequently showed that all cells contained haem.[575]

II-a-2 Non haem bound iron in the body was first described by Fontes and Thivolle in 1925.[267] They described plasma iron and demonstrated that it was present in a form different from Hb iron. In 1927, Barkan[42] demonstrated that plasma iron was protein bound and showed that it was non dialysable at physiological pH. The iron binding properties of plasma were discovered by Holmberg and Laurell[396] as recently as 1945. In 1949 transferrin was isolated by Surgenov et al,[820] and in 1966 was characterised by Roberts et al[723] as a β_1 glycoprotein with a molecular weight of 74000. Surgenov et al[820] had shown transferrin to be a specific iron transporting protein in the plasma. Since this, approximately sixteen genetic variants of transferrin have been identified, but all have been found to handle iron in quantitatively the same manner.[841]

II-a-3 The advent of radioactive iron saw the development of a new technique in exploring iron metabolism. Hahn, in 1939, published a series of articles[337][338][339][342][584] and showed that iron injected intravenously became distributed in the body, predominantly within haemoglobin, but also into body iron stores.

This work was soon amplified by a vast number of authors. Dubach[211] demonstrated the variability of distribution of intravenously injected iron in different disease states.

II-a-4 Up to this stage experimental techniques had been relatively crude, and had certainly not been of a tracer nature, i.e. amounts of 10 mgs. of iron having been used. "Counting" techniques were also laborious, and subject to considerable experimental error. The development of well type scintillation counters with activated crystals and the development of radioactive iron of high specific activity, coupled with increased understanding of the physiology of transferrin led, in 1950, to the development of quantitative ferrokinetics by Huff et al.[404][405][406] With various modifications, Huff's original techniques have since been employed by many workers.

II-a-5 The early realisation that Huff's mathematical formulae tended to overestimate the amount of iron daily being utilised for haemoglobi synthesis led to the development of more complex 'models' or mathematical analyses of the kinetic data.[164][399][686][864] To a considerable extent, these models have relied upon increasing the accuracy of the data obtained by improvement in counting technique. The problems posed by the complexity of these models have mitigated against their use in routine laboratories, and Huff's original methods, slightly modified, are still used by most routine isotope services. These models have however, added considerably to our understanding of internal iron kinetics.

II(b) <u>Development of a basic 'model' of iron kinetics</u>.

II-b-1 As detailed in a review article by Hahn in 1937,[336] the

understanding of iron kinetics prior to the advent of radioactive iron was bedevilled by shortcomings in analytical techniques. It is evident that the incorporation of iron into Hb, and the regulation of body iron balance by iron absorption was understood, as was the fact that, normally, iron loss from the body was minimal.(569) The advent of radioactive iron led rapidly to a more complete understanding of iron kinetics.

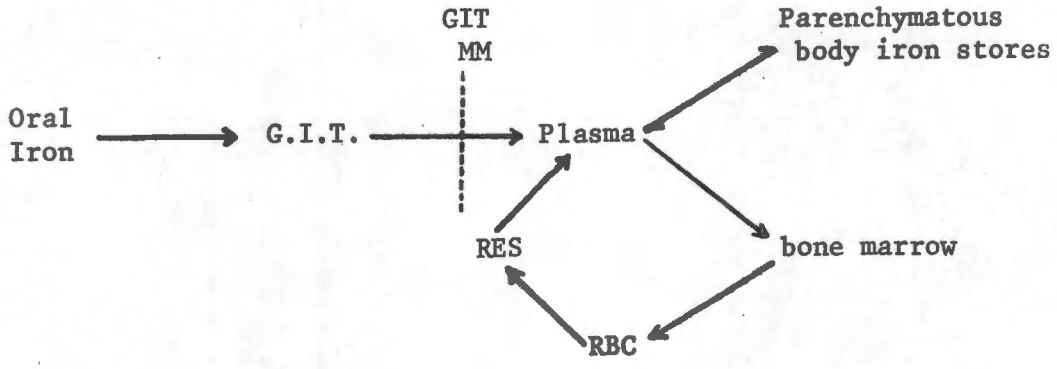
II-b-2 Hahn(337) showed in dogs that labelled iron, given orally, was absorbed into the body and incorporated into Hb. Any iron absorbed and not incorporated into Hb was incorporated into body iron stores. Balfour et al(38) showed that the amount of the oral dose absorbed depended to a considerable extent upon the iron status of his experimental subjects, being increased by iron deficiency. After absorption he was able to show detectable radioactivity in the plasma and Hahn(337) showed how this concentration in the plasma decreased as a funtion of time. In subsequent work, Hahn showed that the percentage of iron absorbed from an oral dose decreased as the size of the oral dose increased.(33 He also studied the distribution of intravenously administered iron(338). However, non tracer amounts were given, i.e. more than 100 mg., and this obviously vastly exceeded the transferrin binding capacity of the plasma. As a result, he found some early urinary loss of Fe, but also found a continuous small faecal loss of intravenously administered radioactive iron. In addition he described biliary iron loss from the body, and showed how this was increased in phenylhydrazine induced haemolytic anaemia in dogs.

II-b-3 Subsequent work by Miller et al[584] confirmed that the circulating radio-iron, following absorption or intravenous injection, was in fact within the red cells, and incorporated into the haemoglobin. Hahn demonstrated[340][341] that this iron, once in the cell, was not released from the red cell until it was destroyed, confirming earlier work by Shiefley[775] and Hawkins.[364]

II-b-4 Granick characterised ferritin as storage iron[309][310][311][312] and, working with Hahn[342] showed that intravenously injected ^{59}Fe labelled ferric ammonium citrate or haemoglobin resulted in labelling of ferritin. In addition these authors showed that colloidal and organic iron given intravenously accumulated in the reticulo-endothelial system(RES) whereas inorganic iron tended to accumulate predominantly in parenchymous liver cells.

II-b-5 Cruz et al[176] showed, in 1941, that haemoglobin iron released from catabolised Hb was re-utilised for Hb formation. At this stage then, a fairly composite picture of internal iron kinetics (see Figure II-1) emerges which has been largely confirmed by later workers with more refined techniques. On the basis of the pioneering work mentioned, extensive study of individual parts of the kinetic pathway of iron have been carried out by a number of workers, and this work will now be reviewed.

Figure II-1.



Schematic representation of internal iron kinetics as postulated by early workers. (see text (II-b-5)

G.I.T. = Gastro-intestinal tract.

RBC = Circulating red cells

RES = Reticulo-endothelial system

GIT.MM = Gastro-intestinal mucous membrane.

II-c Iron Absorption.

II-c-1 Perhaps no one aspect of iron metabolism has received as much attention as has iron absorption. Prior to the introduction of radioactive iron, absorption was measured by balance studies[336] which were, generally, time consuming, liable to considerable experimental error, and unpleasant to perform. The concept of intestinal regulation of iron balance by variable absorption was developed as a result of studies showing how iron absorption was, to a large extent, inversely proportional to the size of the body iron stores,[38][337] and that iron loss from the body was minimal, and not important in regulating iron balance.[679][158][569][338] Most of the work done on iron absorption since this time has been done in pursuit of an understanding of the mechanism and regulation of intestinal iron absorption.

These may be conveniently subdivided as follows:-

(a) luminal factors.

(b) mucosal factors.

(c) endogenous factors.

II-c-2 Luminal factors regulating intestinal iron absorption.

Food iron occurs mainly in the ferric form where it may be complexed with organic compounds or present in an inorganic (or ionic) form. These two forms (organic and inorganic iron) of iron appear to be absorbed by two different mechanisms, haem iron not being influenced by the factors normally influencing ionic iron absorption.

(a) Inorganic iron absorption.

This occurs in the diet mainly in the ferric form and may or may not be complexed to other chemical groups. A number of studies have shown that inorganic iron is absorbed into the mucosal cell in the divalent state[426][33][40] and in order for this to occur, complexed iron must be released and then reduced to the ferrous state. This process occurs primarily in the stomach, because of its acid secretions, and in the upper small bowel[41][99][160] and lumenal factors promoting or inhibiting both the release of complexed iron and its reduction to the ferrous state will obviously influence the amount of iron presented to the mucosal cell for absorption, and (as will be detailed later) this will influence the amount of iron absorbed. Figure II-2 details the important factors promoting or inhibiting the presentation of ferrous salt to the mucosal cell.

Table II-1 Factors effecting the presentation of ferrous iron to the intestinal mucosal surface.

Factors Inhibiting the presentation of Ferrous Iron to the mucosal cell surface.	Factors promoting the presentation of ferrous iron to the mucosal cell surface.
Phytates (225)	Ascorbic Acid (394) (154) (120) (172) (601) (603) (678)
Phosphates (225) (25)	Gastric Acidity (120) (792) (426) (182) (422) (594) (423) (424) (420) (105) (314) (366) (163)
Carbonates (225)	Succinic Acid (394) (120) (346)
Achlorhydria (750) (299) (421) (118) (389) (798) (843) (467)	Cysteine (555) (346)
Desferrioxamine (33) (41) (481b)	Methionine (555)
Cobalt (749) (57)	Fructose (138) (188) (681)
Food (394)	Alcohol (140)
Excess food iron (394) (395) (555)	Decreased Pancreatic Secretions (185) (66) (186) (837) (870) (745)
	Iron deficient diets (453) (456) (616) (34)
	Cirrhosis (171) (578) (157)

All these factors act by promoting or inhibiting the release of iron from complexed compounds and the reduction of ferric to ferrous iron.

(b) Organic Iron Absorption.

Evidence that the absorption of organic iron compounds, notably haem, by a different process to that for inorganic iron absorption abounds. Haem iron absorption is, generally, not influenced by lumenal factors acting upon inorganic iron absorption[33][41], and the reading of plasma radioactivity following ^{59}Fe labelled haem ingestion occurs later and persists for longer than that following oral inorganic iron loading.[40][160] It is believed that haem iron is absorbed intact into the mucosal cell, the iron being split off the parent organic compound within the mucosal cell.[189][617] A small amount of haem iron appears to be absorbed intact into the plasma[117] but the majority appears to be split up into the porphyrin ring and free iron by an enzyme whose activity can be inhibited by the presence of excessive iron locally.[875] This mechanism may be a regulatory process, determining not only the amount of iron released but also the eventual amount absorbed. Xanthine oxidase appears to be important in the release of iron from haem[189] as it generates peroxidases that release the iron from haem. Once the iron is separated from its parent organic compound, it appears to be absorbed in the same way as inorganic iron.

II-c-3. Mucosal factors regulating inorganic iron absorption.

Ferrous iron, once presented to the intestinal mucosal cell, is transported across the cell by an active transfer process[205][426][540][541][542][543] capable of moving iron against both concentration and potential gradients. This system is dependent upon oxidative metabolism and generation of phosphate bond energy, and because of this has a limited capacity.

Uptake by the mucosal cell of iron is rapid, occurs in all segments of gut, is non regulated and varies according to the amount of iron presented to the cell.[88][792][99] Serosal transfer of iron is however a slow process, occurs predominantly in proximal gut segments and involves the transfer of divalent iron only. This process is blocked by metabolic inhibitors because of its dependence upon oxidative hexose metabolism.[81] Transfer is stimulated by ascorbic acid and low pH. Increased transfer activity occurs in conditions associated with increased iron absorption whereas transfer activity is depressed in conditions associated with decreased iron absorption.

Two iron pools are present within the intestinal mucosal cell.

(i) A rapidly turning over Fe^{2+} pool passing through the mucosal cell into the plasma and

(ii) A slowly turning over Fe^{3+} pool, some of this iron being associated with ferritin.

The activity of these iron pools is variable and decreased activity can be induced by inhibition of protein synthesis in the proximal small bowel.[327]

Granick has shown[313][314][316] that the presence of iron in the intestinal epithelial cells is associated with the formation of ferritin. Apoferritin, the non iron containing protein precursor of ferritin, has been shown to be actively synthesised and degraded within this epithelial cell, becoming stabilised only when combined with iron. Synthesis of apoferritin at this

site is considerably augmented by the presence of iron or where there are increased body iron stores.(791) Crosby, in a beautiful series of experiments has shown how, by mucosal desquamation, ferritin is continuously being lost from the intestinal mucosa, and on the basis of this work postulated a regulatory mechanism for iron absorption.(172)(174)(173)(175)(158)(359)(674)(874)(881)(882)(883) His work has now been confirmed by many workers, and postulates that a portion of the iron taken into the intact mucosal cell is rapidly transferred across the cell into the plasma, the size of this portion being determined by a number of endogenous factors which I shall detail at a later stage.(II-c-4) The characteristics of this iron during transfer are not known.(544)(668)(881) The remainder of the iron taken into the cell becomes complexed within the cell, the majority being complexed with apoferritin, to form ferritin.(139)(141) This is subsequently lost from the body by desquamation (158)(75).

It is postulated that the determination of how much iron will be transported across the cell into the plasma is "built into" the mucosal cell at its conception and is dependent upon the amount of iron delivered to it by transferrin at this stage. This messenger iron stimulates the formation of apoferritin which will complex absorbed iron in amounts proportional to its own concentration. By varying the amount of iron complexed in this way, iron absorption can be regulated. The amount of 'messenger iron' delivered to the developing mucosal cell appears to depend upon the body's iron requirements(881)(882)(883) and the amount

of endogenous iron available for storage. Where excessive endogenous iron is available for storage increased amounts of 'messenger iron' will be delivered which will, by increasing apoferritin synthesis also increase mucosal iron sequestration and diminish iron absorption. The reverse occurs where body iron requirements are high.(543)(881)(883). Transferrin preferentially transports iron to erythroid tissue in the presence of diminished body iron stores, and because of this, messenger iron deposition in the mucosal cell will decrease in iron deficiency or in conditions associated with increased erythropoietic activity (at given serum iron levels). The reverse will apply where erythropoietic activity is decreased.

This postulate has been shown to describe the clinical situation very well(177)(847)(678)(81)(679)(481)(159)(61)(737)(74) in most cases(212)(158)(881)(287)(393)(476)(252)(75)(453)(236)(156)(713)(746)(617) although Bothwell's group have shown that this regulatory mechanism can be overcome by increasing the amount of iron presented to the mucosal surface. They have shown that although the percentage of an oral dose absorbed may decrease as the load increases, the absolute amount absorbed actually increases.(426)(80b) This indicates that the amount of iron absorbed into the mucosal cell does influence the amount of iron actively transported across the serosal surface into the plasma thus over-riding physiological control of iron absorption.(898)(344)(97)(604)(791)(74)(172)(394)(154)(252) Some workers however have been unable to determine any correlation between mucosal iron concentration and iron absorption.(17)(35)(453)(739)

The vast majority of the iron taken into the body appears to be absorbed by a controlled mechanism as described. Other pathways for iron intake into the body have been postulated including lymphatic uptake[235][602] and ingestion of iron by inwardly migrating iron laden macrophages.[904][172][24] The amount taken from the gut by these pathways appears to be negligible. There does not seem to be an exchange of iron between body stores and intralumenal iron apart from the loss of messenger iron in the desquamated mucosal cells[682][791][162][423][424][750][188][681][33][41][481b][105][314][366][163][420][422] although increased body iron loss via mucosal desquamation has been demonstrated in diseases associated with increased mucosal cell turnover.[326][782][171][172][822][28][75][453] The mucosal regulation of iron absorption appears to be deficient or 'set at a lower level' in idiopathic haemochromatosis.[177][172]

II-c-4 Endogenous factors regulating iron absorption.

There appears to be, as previously stated, a good correlation between iron absorption and a number of endogenous factors. Increased iron absorption has been found in conditions associated with decreased body iron stores[82][81][212][347][599][604][679] and increased erythropoietic activity.[81][515][715][743][874][236] Changes in iron absorption, according to these authors, appear to be unrelated to levels of Hb, serum iron, transferrin, Hct or transferrin saturation.

Some studies however, have shown that anaemia alone may influence iron absorption.[577][578][752][906][77][248][751][369]

II-d Plasma Transfer of Iron.

II-d-1 Plasma iron was first demonstrated in 1925[267] and found to be non dialysable and protein bound in 1927.[43] Holmberg and Laurell[396] first recognised the iron binding properties of plasma in 1945, and Surgenov et al[820] isolated transferrin in 1949. Roberts et al characterised it as a β_1 glycoprotein in 1966.[723] To date approximately sixteen genetic variants of transferrin have been demonstrated, all handling iron in the same way.[858][841]

II-d-2 In 1939 Hahn had demonstrated the presence of radioactive iron in the plasma following oral loading with labelled iron, and had showed how its concentration in the plasma diminished as a function of time,[337] and a number of authors have since shown how iron transferred from the intestinal mucosal cell to its serosal surface becomes bound to transferrin.[841][87b][335][500][600][692][702][747][783][864]

II-d-3 Chemically, transferrin is a β_1 globulin (mucoprotein, glycoprotein) with a molecular weight of approximately 74000[723] Genetic variants of transferrin, demonstrated by differences in electrophoretic migration[858] result from variation in a series of co-dominant autosomal alleles at one locus. All are, however, antigenically related and bind, transport and deliver iron in an identical fashion.[841] Transferrin has been found at an early stage in foetal development, the main site of synthesis being the liver[296] although synthesis by circulating lymphocytes

has also been described.[793][372]

II-d-4 Each molecule of transferrin is capable of binding two molecules of ferric iron, and in doing so, changes from a colourless to a salmon pink colour.[501] The bound iron is extremely tightly held with an estimated affinity constant of 10^{31} [1][45] at physiological pH. In vitro, iron can be dissociated from transferrin only at very low pH values.[469][702][501]

II-d-5 The amino acid and carbohydrate composition of transferrin is known although their sequences are not.[328] Two identical branched heterosaccharide chains are present terminating in sialic acid and containing galactose, N-acetyl glucosamine and mannose. These are linked to the protein moiety by an asparaginyl residue.[428] The protein moiety itself consists of a single polypeptide chain.[328] Various carbohydrate groups can be removed from the heterosaccharide chains with no change in biologic function.[474][612]

II-d-6 Two metal binding sites are present on the transferrin molecule which appear to be identical in their capacity to co-ordinate iron.[501] They are separated by a distance of more than nine Å.[1][10] Two histidyl and three tyrosyl groups are involved in binding the iron. Under ordinary circumstances three protons are released for each ferric iron (Fe^{3+}) bound, and one bicarbonate ion (HCO_3) is taken up[10][472][520][894] although binding of Fe^{3+} can be effected in the absence of HCO_3.[11] When this occurs however, heterogeneity exists for the two binding sites at pH 4 - 6 although not at pH 7 - 11. Iron binding capacity is

insensitive to blockage of free amino acid groups and changes in net charge although both of these manoeuvres effect protein binding to iron receptor sites on reticulocytes.[474][114][473]

II-d-7 When iron free transferrin (apotransferrin) binds with iron, a marked change in the physico-chemical properties of the protein occurs due to changes in configuration of the molecule.[475][69] Recent investigations have questioned whether transferrin can exist in two different forms of iron binding i.e. with one or both iron binding sites occupied.[901] It has also been questioned whether the physically and chemically identical iron binding sites are in fact biologically identical[475][263][262][264][265] but equality of delivery from either or both of these iron binding sites has been demonstrated.[494][398]

II-d-8 Transferrin appears to be a true iron carrier. Alone it appears to have no enzymatic function[502][501][658] although it has been demonstrated to inhibit viral, bacterial and fungal growth, a capacity which appears to be related to its ability to bind iron[126][245][551][552] and which decreases as it beccmes progressively more saturated with iron.[126][793]

II-d-9 Another iron binding protein reversibly combining with two ferric ions has recently been demonstrated in body fluids and neutrophils. This has been called lactoferrin and is thought to be important in resistance to infection. It binds Fe^{3+} with a greater affinity than does transferrin and is stable down to a pH of 3.[556]

II-d-10 Transferrin distributes itself approximately equally between intra and extra vascular extra cellular fluids, and has been found in lymph, ascites, cerebrospinal and oedema fluid[27][295][435][450][864] in studies where it has been labelled. Equilibration between these various compartments, following intravenous injection of labelled transferrin, takes four to five days. After this, transferrin disappears exponentially from the plasma with a half time of approximately nine days. No correlation exists between the turnover times of transferrin and iron, supporting the thesis that transferrin is a true iron carrier.[500][502][501] There is some evidence that transferrin becomes transiently fixed to the reticulocyte membrane during delivery of its iron[501][432][434][609][606][227] but is not consumed during this period and readily exchanges with plasma transferrin. Specific transferrin binding sites have been demonstrated on the cell membrane of maturing erythroid cells. These can be destroyed by trypsin and have been shown to be metabolically active, being blocked by metabolic inhibitors.[227][432][434] The amount of transferrin temporarily sequestered by the reticulocyte membrane is small and insignificant when compared to the total transferrin pool.[27][382][610]

II-d-11 Plasma concentrations of transferrin vary between 0.16 - 0.36 gm.[501] One gram of transferrin binds 1.25 mg. of iron. By convention, plasma transferrin concentration is expressed in terms of the plasma iron binding capacity which varies between 200 to 450 µgms/100 ml. depending upon the method used for its assay.[378][702][729]

The mechanisms controlling the transferrin concentration are poorly understood. Transferrin synthesis occurs mainly in the liver[296] and its concentration is dependent upon both anabolic and catabolic functions. Lane has postulated that plasma transferrin levels may be dependent upon the status of body iron stores and the percentage of the circulating transferrin bound with iron in the plasma.[492][493] Low levels of transferrin are present at birth,[500] the levels increasing to normal by adolescence[766] and remaining at this level throughout life.[122][690] Factors influencing serum transferrin concentrations are detailed in Table II-2. No diurnal variation in serum transferrin concentrations has been demonstrated.[122][690][679]

Table II-2

Factors influencing Serum Transferrin Concentrations.

Factors increasing serum transferrin concentrations	Factors decreasing serum transferrin concentrations
1. Hypoxia[122][608][691][809][63]	1. Infection[796][435]
2. Low iron stores[492][493][796][679]	2. Endotoxin[445][446][447]
3. Oestrogens[419][278][607][112][397][547][172][175]	3. Malignant disease[796]
	4. Iron overloaded states[493][755b][63]
	5. Nephrotic Syndrome)
	6. Uraemia) [355]
	7. Protein malnutrition)

II-d-12 Plasma iron levels represent a balance between processes adding to, or removing iron from, the plasma. Normally all the iron present in the plasma is bound to transferrin[26] although, under exceptional circumstances iron may be present, unbound, in the plasma, as ferritin. This usually will only occur following

acute liver damage or occasionally in conditions associated with gross ineffective erythropoietic activity.(248)(883)

II-d-13 Iron in the plasma is in a dynamic state, and its concentration in the plasma varies considerably with time. Marked diurnal variation of plasma iron concentration has been well documented, levels tending to be high in the morning and low in the evening. (78)(87)(351)(503)(662)(663)(794) This diurnal variation seems to disappear in disease states.(663) Serum iron levels tend to increase in the presence of decreased erythropoietic activity, haemolysis, ineffective erythropoiesis, acute liver disease, and where body iron stores are increased.(134)(249)(539)(664)(548) (600)(607)(369)(702)(502)(662)(916) Levels of iron in the serum tend to decrease with infection, malignancy, after surgery, in conditions associated with accelerated erythropoiesis particularly conversion of ineffective to effective erythropoiesis, and in the presence of diminished body iron stores.(702)(783)(122)(690) (273)(548)(30)(50)

II-d-14 The importance of transferrin in internal iron kinetics is well demonstrated in studies performed on patients with congenital atransferrinaemia.(369)(721)(368)(388) In these patients there is severe hypochromic anaemia refractory to therapy, excessive body iron stores, retarded growth and susceptibility to recurrent infections. Injection of iron intravenously into these patients results in extremely rapid indiscriminate clearing of iron from the plasma to iron storage sites with the later appearance of subnormal amounts of the injected iron in the circulating red

cells. Interestingly, iron absorption in this situation is greater than in normal subjects showing that transferrin is not required for iron absorption.

II-d-15 There is no exchange of iron between transferrin and mature red cells,[340][258] nor is there any exchange of iron, in the plasma, between different molecules of transferrin.[258]

II-e Exchange of iron between transferrin and iron receptor sites, and iron storage.

II-e-1 The evidence for the uptake of iron from the intestinal mucosal cell by transferrin, and the deposition, by transferrin, into the developing intestinal mucosal cell, of messenger iron, has previously been reviewed. (Section II-c-3) and will not be repeated.

II-e-2 The interchange of iron between transferrin and parenchymatous iron stores is poorly understood. The evidence for deposition, by transferrin, of iron at these sites is considerable[248][883][564][893] and is derived from

(a) Experiments utilising labelled transferrin and external counting[864][405][149][686][179][258][893][21][846][22][730] or imaging techniques.

(b) Anatomic dissection and organ counting following the intravenous injection of ^{59}Fe labelled transferrin.[258][252]

(c) Observations of increased parenchymal iron stores in conditions associated with raised serum iron levels, and decreased parenchymatous iron stores in conditions associated with decreased serum iron levels[61][387][130][698][815][415][252]

The reticulo-endothelial cell appears to be incapable of taking up iron from transferrin as judged by these studies[258][434][620][609][610][748][908] except under exceptional circumstances.[532][535]

II-e-3 That iron is taken up, by transferrin, from iron stores, whether parenchymatous or reticulo-endothelial, is undisputed. Perhaps the best demonstration of this has been the experimental induction of iron deficiency by repeated phlebotomy which is accompanied by progressive decrease in the histological evidence of iron storage until iron stores are totally depleted.[384][211][360][61][415][252][156] So reproducible is this technique that Finch et al have used this technique to assay body iron stores.[252]

II-e-4 Evidence is available to suggest that recently catabolised haemoglobin iron is more readily available to transferrin than iron previously deposited in iron stores.[211][257][703][361][645][255][328][757] Other authors however, feel that all forms of storage iron are equally available.[360][703][224][252] Iron stores appear to be poorly mobilised to transferrin in the presence of infection,[130][698][61][361][252][916][60][645] neoplasia,[916][60][361] ascorbic acid deficiency[860][859] uraemia,[61][252][916] cirrhosis[61] and chronic inflammatory states.[361][916][60]

II-e-5 Iron is stored intracellularly as ferritin and haemosiderin in approximately equal amounts[776][838][871] in both parenchymal and reticuloendothelial cells of many organs, particularly liver, spleen and bone marrow. Ferritin is a macromolecule with a

spherical core containing iron surrounded by a protein shell made up of approximately twenty identical spherical polypeptide chain subunits[356][659] with a characteristic appearance on electron microscopy. Ferritin is water soluble and contains a variable amount of iron. It stains poorly with Prussian blue.[777] Ferritins derived from different sites in the same animal have been found to have different electrophoretic mobilities due to differences in surface charge, but share antigenic sites.[13][279][280][640][720][819] Ferritin lacking iron is called apoferritin, and synthesis of this has been shown to be accelerated in the liver, in a dose dependent way, following intravenous injection of iron.[147][209][210] The presence of iron in apoferritin appears to stabilise the molecule.

II-e-6 Mazur et al[567] have demonstrated the presence of a number of Fe^{2+} ions on the surface of ferritin stabilised by the presence of surface sulphydryl groups. When these groups are oxidised to disulphide linkages the Fe^{2+} also becomes oxidised to Fe^{3+} and migrates into the central core of the ferritin molecule. Surface Fe^{2+} ions can dissociate from the surface sulphydryl groups in the presence of a suitable iron binding agent, this being facilitated by reducing substances and hypoxia. The mechanism whereby iron is taken up by storage sites from transferrin is porrly understood. Mazur et al[564][565] showed that iron is preferentially taken up by iron poor ferritin molecules. This uptake appears to be dependent upon energy producing oxidation (i.e. ATP synthesis) and the presence of

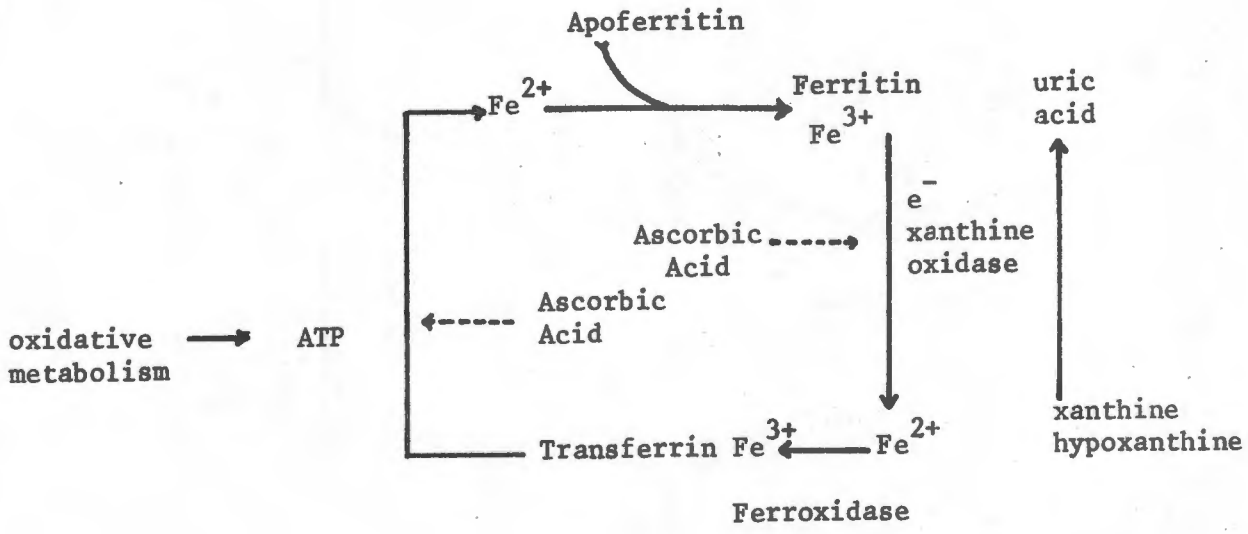
ascorbic acid. The reduction of transferrin Fe^{3+} to Fe^{2+} is stimulated by ATP in the presence of ascorbic acid. The Fe^{2+} dissociates from the transferrin and then binds with surface sulphydryl groups on apoferritin.

II-e-7 Mobilisation of iron from ferritin is similarly poorly understood. Mazur[563][567][568] has postulated that xanthine oxidase is required for this to occur. The reduction of xanthine oxidase during its oxidation of hypoxanthine and xanthine to uric acid is thought to promote reduction of Fe^{3+} to Fe^{2+} in the ferritin molecule (by transfer of an electron), liberating small amounts of Fe^{2+}. This process is stimulated by hypoxia, and administration of xanthine substrates, raising the serum iron level in animals. Against this postulate is the apparent lack of effect upon iron metabolism of chronic allopurinol administration[188][234][88][326] although the reverse has been shown in some animal experiments.[693]

II-e-8 Recent work by Bothwell's group has shown that ascorbic acid is important for the release of iron from iron stores, and that administration of ascorbic acid to iron overloaded Bantu patients increases serum iron levels and desferrioxamine induced urinary iron excretion.[859][860] Normal plasma is capable of oxidising iron at physiological pH and oxygen tensions, this action being based upon the ferroxidase activity of caeuloplasmin.[652] Administration of caeruloplasmin causes prompt release of iron from reticulo-endothelial stores with no effect upon iron absorption.[653][700] These processes are summarised diagrammatically in Figure II-2.

Figure II-2 Diagramatic Representation of Redox System postulated for storage iron uptake and release from transferrin

(See text II-e-6 → II-e-8)



II-e-9 Pape et al[659][660] have proposed an alternate method for the uptake and release of iron by ferritin, not involving redox systems. They propose that the iron micelle in ferritin is initially formed in the cell cytoplasm and subsequently becomes stabilised by the addition of the polypeptide subunits of apoferritin formed in the cell in response to the presence of iron. Removal of iron from ferritin can be carried out by a process involving specific low molecular weight chelating agents. So far this has not been proven in vivo,[210] and apoferritin synthesis has been shown to occur in the absence of an iron core[359] tending to cast some doubt about Pape's theory resolving the problem.

II-e-10 Haemosiderin occurs in granules and stains strongly with Prussian blue. The granules are visible under the ordinary light microscope, and are not water soluble. Haemosiderin appears to be derived from ferritin[557][717][718][719] but can occur in a number of different physical and chemical forms[890] that appear to be aggregates of ferritin[898][878][777] containing a number of additional compounds including porphyrins, lipids and pigments.

II-e-11 There are functional differences between these two forms of storage iron. In iron overload, the proportion of haemosiderin increases. Intravenously administered radioiron is incorporated into ferritin initially, and not into haemosiderin, and following repeated venessection ferritin iron is more readily mobilised than is haemosiderin iron.[778][779]

II-e-12 I have previously mentioned that reticulo-endothelial cells are unable to take up iron from transferrin, except under exceptional circumstances (section II-e-2). However, the reticulo-endothelial system plays a major part in the delivery of iron to transferrin, providing from catabolised haemoglobin nearly all the iron required for erythropoiesis under normal circumstances.[45][645] Iron delivered to the reticulo-endothelial cell from haemoglobin is promptly and almost completely re-utilised for new haemoglobin synthesis, particularly in iron deficiency. Where iron stores accumulate in the reticulo-endothelial cells, they occur as both ferritin and haemosiderin. Under certain conditions it appears as though release of iron from the reticulo-endothelial cells is inhibited i.e. infections, inflammation, neoplasms.[361][460][656] The reticulo-endothelial cell thus appears to have the capacity to finely regulate iron supply to

the plasma. It is important to remember that the majority of iron required for erythropoiesis is derived from this source, and that re-utilisation blocks may lead to significant anaemias. Why the variable avidity of the reticulo-endothelial cells exists, and how it is controlled in these different disease states, does not, at present, appear to be understood.

II-f Body loss of Iron.

II-f-1 Measurement of body iron loss is hampered by a number of factors i.e. contamination of samples by non body iron, faecal loss by excretion as well as mucosal desquamation. The total daily body iron loss appears to be approximately 1 mg/day,[224][213][255][741] the majority of iron being lost via the gastro-intestinal tract as blood,[220] exfoliated mucosal cells and via the bile.[141][213][782] All cells contain iron, and for this reason, the loss of epithelial cells and leukocytes from the body, as well as hair and nails, result in body loss of iron.[782] Green et al[326] calculated that gastro-intestinal iron loss was approximately 0.4 mgs/day, and Crosby has postulated additional loss of endogenous body iron via macrophages into the gastro-intestinal tract,[175] particularly in iron overloaded states. Loss of intravenously injected radioactive iron bound to transferrin in the faeces has been recorded by a number of workers.[782][822][28] The loss of iron in the bile is thought to be approximately 0.26 mgs per day, the source of this iron being uncertain. It may come from transferrin or parenchymatous iron stores. A

large proportion of this iron, lost in the bile, may subsequently be reabsorbed.[782][90][213][326]

II-f-2 Under normal circumstances, loss of iron in the urine is usually less than 0.1 mg/day[181][538] although the loss by this route may be vastly increased under abnormal circumstances such as intravascular haemolysis, particularly in conditions such as paroxysmal nocturnal haemoglobinuria. Iron loss also occurs from the body in sweat, but the amount lost is minute.[255][850][326][605][76][155][48][604][741] Vellar et al[850] have found that loss via this route is not influenced by serum iron levels, although Green et al[326] have shown that iron deposition in the skin increases as serum iron levels increase. Most skin iron appears to be conserved[132] and major loss of iron from the skin is considered unlikely.[326]

II-f-3 Additional loss of iron from the body occurs in menstruating females, approximately 20 mgs. of iron being lost with each period. This increases daily endogenous body iron loss to approximately 2 mgs/day.[345] This amount may be greatly increased in conditions associated with menorrhagia. Iron is also lost with lactation, approximately 1 mg/day being lost by this route.[740]

II-f-4 Abnormal loss of iron will obviously occur in situations where excessive bleeding occurs, as well as in conditions associated with abnormal intravascular haemolysis where iron will be lost via desquamated haemosiderin laden tubular cells in the urine.

An obvious clinical maxim is to look for a source of bleeding in any patient with unexplained iron deficiency anaemia.

CHAPTER III. A REVIEW OF THE LITERATURE OF NORMAL AND ABNORMAL ERYTHROPOIESIS.

III-a Anatomical Considerations.

III-a-1 Blood forming cells are embryologically derived from undifferentiated mesenchymal cells. For the first two months of intra uterine life, blood formation occurs almost entirely in the yolk sac. Subsequently, the liver and spleen become major sites of erythropoietic activity until approximately the seventh month of intra uterine life. Bone marrow erythropoietic activity starts in the third month of intra uterine life and the proportion of blood formed in the bone marrow 'organ' progressively increases until, by the seventh month of intra uterine life, it is the major site of erythropoiesis. By the tenth month, the bone marrow is normally established as the only erythropoietic organ in the body.(193)(319)

III-a-2 Production of red cells occurs extravascularly in the bone marrow stroma, outside the vascular sinusoids. Maturing erythroid cells do have ready access to the nutrients contained in the circulation. Once formed, mature reticulocytes enter the circulation by migration and diapedesis into the vascular sinusoids.(786)(178)(669)(839)(840)(877)(909)

III-b Normal erythropoiesis.

III-b-1 Normal erythropoiesis may be conveniently defined as the generation from primitive undifferentiated cells called stem cells, of mature viable circulating red cells with minimal wastage of precursor material. During the period of differentiation of a stem cell and the time at which mature cells are delivered into the circulation, a number of sequential processes occur in the bone marrow. These include:-

(a) differentiation of the stem cell into morphologically recognisable types by a process we shall call maturation.

(b) erythroblast proliferation, this being a process whereby mitosis increases the yield of a single stem cell so as to produce a number of daughter red cells and

(c) iron uptake; heme formation and globin formation.

III-b-2 To a large extent, the development of our model of bone marrow function has been based upon our interpretation of the literature in regard to these processes, and for this reason it is important to review the relevant literature in some detail.

III-b-3 Differentiation or maturation.

In this process, non morphologically recognisable erythroblast precursors become recognisable erythroblasts and, as they mature, eventually become reticulocytes. Current concepts of erythropoiesis favour the generation of all the elements of the blood from a 'pluri-potential stem cell'. This is called the colony forming unit by some workers and, although it has never been adequately or convincingly defined morphologically, it has been demonstrated convincingly experimentally.(708) (829) (593) (592) (480) (379) (707) (408) (104) (760) (68) (490) (115) (489) (581) (899) (903) (275) (409) (462) (51) (54) (529) (595) (618) (907) (268) (836) (558)

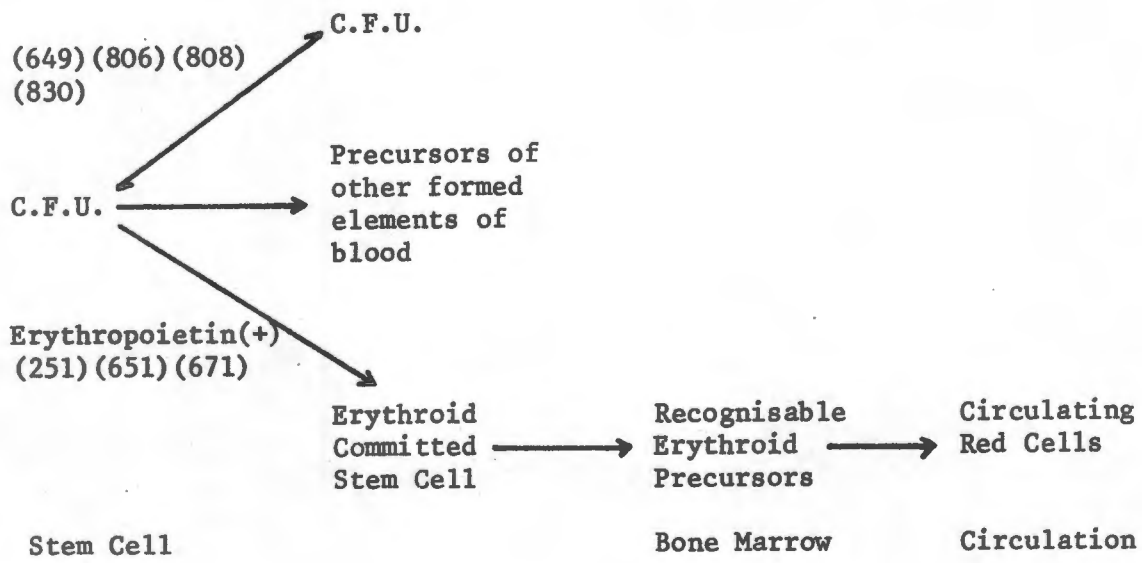
A number of workers have, in addition, postulated, and shown experimentally, a stem cell committed to erythroid development which differentiates from the pluripotential stem cell under the influence of erythropoietin and itself differentiates into the first recognisable erythroblast.(490) (829) (480) (593) (592) This stem cell can also be called the erythroid committed stem

cell, and the size of the "pool" of these erythroid committed stem cells is very sensitive to fluctuations in levels of erythropoietin.(490)(829)(480)(593)(592)(251)(651)(671)

Evidence has recently been presented to show that stimulation of erythropoiesis depletes the stem cell "pool" whereas inhibition of erythropoiesis has the reverse effect.(767)

The stem cell pool is capable of self renewal and of differentiation so that it is self sustaining. Once the erythroid committed stem cell is formed, however, it is processed irreversibly along a non self sustaining pathway terminating either in death or the production of viable red cells. This concept of stem cell kinetics has been detailed diagrammatically in figure III-1.

Figure III-1 Diagrammatic Representation of Stem Cell Kinetics.



(C.F.U = Pluripotential stem cell - see text)

The C.F.U. has been shown to be very sensitive to irradiation and actinomycin[379][707][829][408] whereas the erythroid committed stem cell pool is very sensitive to fluctuation in erythropoietin levels.[829][593][480][592][251][651][671]

Once the erythroid committed stem cell has been formed, it progresses, by differentiation, into the next group of cells in the bone marrow, the recognisable erythrocyte precursors. These have been variously named, either morphologically or on the basis of their position in time in the maturation sequence i.e.:

pronormoblasts
↓
basophilic normoblasts
↓
polychromatic normoblasts
↓
orthochromatic normoblasts
↓
reticulocytes

OR

early erythroblasts
↓
middle erythroblasts
↓
late erythroblasts

Regardless of the nomenclature used, for practical purposes these cells are defined by histological criteria. The process whereby the first recognisable erythroblast proceeds through the various morphological stages to that of a red cell is called maturation. The period required for this to happen can be called the 'maturation period' and during this period the cell

alters its morphology, loses the ability to synthesize DNA and RNA, becomes haemoglobinised and extrudes its nucleus. This process is irreversible terminating only in cell death or the production of a mature red cell.

Most authors seem to agree that the time required for maturation remains constant[379][237][559][713][240][239][243][241][18][19][830][641][354] although recent evidence shows that the whole of this period may not necessarily be spent in the bone marrow. Thus relatively immature cells may be released into the circulation, but these cells still seem to require the same period to reach maturity.[380][381][384][385] Evidence for the constancy of the maturation period is easily obtained clinically while awaiting a reticulocyte response following specific haematinic therapy such as B_{12} in B_{12} deficient anaemia. Other evidence quoted as demonstrating the constancy of this period includes

(a) the constancy of morphologically recognisable erythroblast cell ratios despite varying degrees of erythropoietic activity.[254][201][706]

(b) the recovery response following pretreatment of the bone marrow with inhibitors of stem cell differentiation i.e. hypertransfusion, colchicine or nitrogen mustard.[251][651][671][707][331]

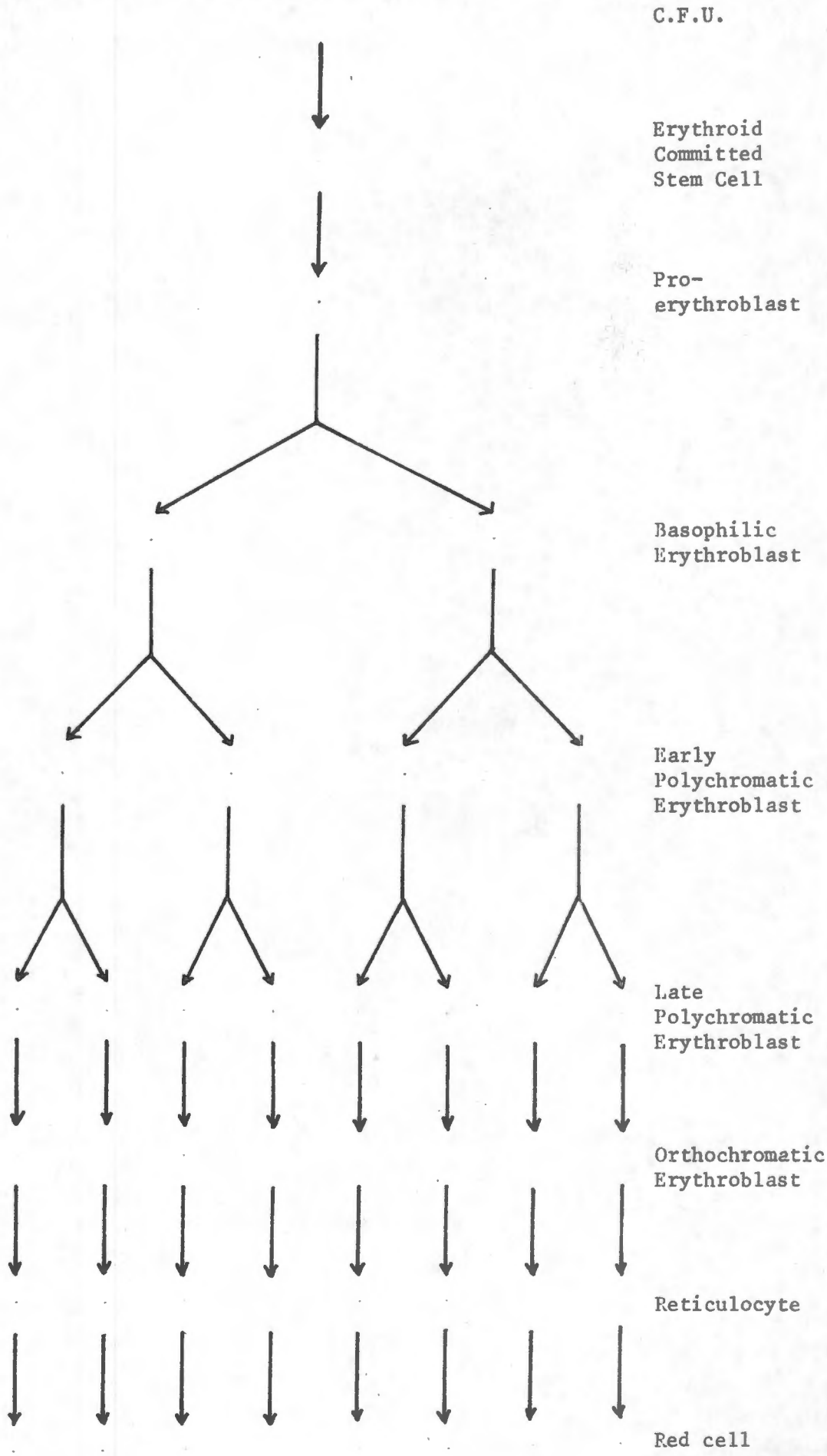
Most authors quote a maturation period of between four and six days.[243] Any change in erythropoietic production is not due to hastened maturation i.e. excessively rapid differentiation of cells with early delivery into the circulation, but is due to increased stem cell differentiation and /or increased proliferative

activity of the erythroblasts during their maturation period. (237) (817) (559) (240) (829) (240) (527)

III-b-4 Erythroblast Proliferation (mitosis).

Erythroblast proliferative activity can be defined as a process in which, from a limited number of primitive red cell precursors, a vastly greater number of red cells is formed by a process of sequential mitotic division. This occurs during the maturation period while the erythroblast is capable of DNA synthesis, and for this reason can only occur in nucleated erythroblasts. Mitotic activity has ceased in polychromatic normoblasts.At this stage DNA synthesis has ceased and the nucleus is becoming polychromatic.(817) (201) (463) (889) (18) (19) (167) (488) The process is most easily explained diagrammatically. (see Figure III-2)

Figure III-2 Diagrammatic Representation of Proliferative Activity.



We have in a later section, while developing our model, referred to the basic unit of erythropoiesis. For our purposes, this refers to all the cells in the recognisable erythrocyte precursor compartment in the bone marrow derived from a single erythroblast committed stem cell during the maturation period. This is mentioned here only for convenience of description at a later stage.

Interpretation of the literature would appear to suggest that there may be a number of variables influencing proliferative activity.

(a) Any erythroblast unit, as described above, may undergo a variable number of mitoses during its maturation period. Some pro-erythroblasts may mature to reticulocytes with no demonstrable intervening mitotic activity.(817)(486)(559)

By means of DNA labelling with tritiated thymidine, studies of mitotic indices, and standard morphological classification of cell maturity, the normal pro-erythroblast has been shown to undergo three to four mitotic divisions during maturation. Each pro-normoblast will therefore give rise, on the average, to between eight and sixteen red cells.(463)(271)(559)(240)(18)(19)(168)(436)(464)(811)(889)(560)(239)

Assuming that each red cell produced is viable and contains normal amounts of haemoglobin, it is easy to understand how variable proliferative activity will proportionately vary total red cell production

as has been postulated by many workers.[817][889][237][559][240][829][240][527][803][804][18][19]

(b) Some ten to twenty percent of all cells in the erythroblast series normally die during the period of maturation.[52][418][725][628][728] This process of destruction of bone marrow erythroblasts during their maturation period is referred to as ineffective erythropoiesis - i.e. wasted erythropoietic activity. In various disease states (see later under ineffective erythropoiesis; section III-b-7) the proportion of total erythropoietic activity that is wasted by this process of intramedullary destruction is vastly increased. These conditions are associated with 'maturation arrest', abnormally delayed mitotic activity, and a decrease in the effective red cell haemoglobin yield.

It has been shown that there is normally no diurnal variation in erythropoietic activity.[560]

III-b-5 Erythroblast iron uptake.

Iron uptake by erythroblasts has been studied both 'in vivo' and 'in vitro'. Two different types of erythroblast iron uptake have been described although, for practical purposes, uptake of iron from transferrin by maturing erythroblasts is sufficient for all the maturing erythroblast's iron requirements. The

absence of transferrin demonstrates this well, being associated with a severe microcytic anaemia caused by impaired delivery of iron to the developing erythroblast.[432] [60] [227] [606] [609] [14] [824] [283] [84] [479] [828] [231] [486] [697] [789] [721] [367] [368] [369] [388] [21] [22] [846] [730] [79] [164] [686] [688] [864]

Studies utilising transferrin labelled with ^{59}Fe injected intravenously have universally shown almost immediate localisation of the injected radioactivity within the bone marrow[21] [846] [22] [730] [79] [164] [686] [688] [864]

In vitro studies using doubly labelled transferrin have conclusively shown a two stage uptake of iron from transferrin by reticulocytes, and this finding seems to apply equally to other red cell precursors.[606] [609] [84] [434] [479] [59]
Initially, transferrin is taken up onto specific erythroblast membrane receptor sites. This uptake has been shown to occur onto 'ghost cells' of haemolysed reticulocytes, is stimulated by ATP and ascorbic acid, and is necessary for iron uptake into the maturing erythroblast from transferrin. This has been shown by trypsin digestion of the membrane binding sites prior to incubation with ^{59}Fe labelled transferrin.[434] [227]
Uptake of transferrin onto the specific membrane binding sites may also be prevented by metabolic inhibitors.[432]

Transferrin has been shown to be essential for normal iron uptake by erythroblasts. Freely ionised iron can be non specifically taken up onto the membranes of both erythroblasts and erythrocytes but this iron is readily eluted with mild chelating agents such as EDTA and is poorly utilised for

haemoglobin synthesis. Non specific metallic cation uptake is well described and has been shown by Jandl to cause increased cell agglutinability. Uptake of iron from transferrin does not have this effect, and this iron is readily available for haemoglobin formation.(432)(609)(231)(824) During cellular uptake of iron from transferrin no freely ionised iron could be recovered from the incubating medium,(432)(609) and none of the iron taken up by the cells could be eluted with chelating agents such as EDTA. Membrane uptake of transferrin iron appears to be independent of haem synthesis (as in lead poisoning where membrane uptake of transferrin is not blocked but haem synthesis is impaired(432)).

Release of iron from membrane bound transferrin is temperature and pH dependent, and is also dependent upon oxidative metabolism, and the presence of free sulphydryl groups.(432)(434)(566)(14)(227) Total erythroblast iron uptake is largely dependent upon the iron content of the transferrin in the incubating medium up to serum iron levels of 100 µg% or ± 20% transferrin saturation. At levels of transferrin bound iron above this, the rate and amount of iron uptake by the reticulocytes was independent of the iron level, implying an active process of iron uptake limited by the metabolic activity of the reticulocyte membrane. Iron uptake is independent of the transferrin concentration in the incubating medium, being related to its iron content.(434)(432)(609)(384)

Ready exchange between free and membrane bound transferrin has been shown to occur. During the period of attachment to the erythroblast membrane, transferrin undergoes only one change, the loss of its iron. In this way it acts as a true carrier.(609) The size of the membrane bound transferrin pool is minute relative to the total body transferrin content and does not constitute a significant 'local pool'. The turnover time of membrane bound transferrin is extremely rapid.

The experimentally observed rapidity with which ^{59}Fe labelled transferrin is cleared from the plasma plus the demonstration by external counting, γ and positron camera techniques and autoradiography supports the concept that erythroblast iron uptake probably comes completely from transferrin which is constantly bathing the maturing erythroblasts.(459)(486)(201)(202)(21)(846)(22)(730)(79)(686)(864) Studies with bone marrow cultures have shown that erythroblast iron uptake occurs at differing rates which depend upon the state of maturity of the erythroblast. Iron uptake from transferrin has been demonstrated to occur in the earliest recognisable erythroblast, and the amount of iron uptake taken up by the maturing erythroblast seems to decrease as the cell matures and is thought to cease once cellular RNA synthesis stops.(789)(379)(486)(434)(14)(201)(202)(639)(297)(637)(609)(227)(432)

Erythroblast iron uptake appears therefore to be dependent upon three factors:-

(a) cell maturity

(b) the concentration of transferrin bound iron in the plasma, up to levels of 100 µgm%

(c) the presence of transferrin.

Another source of erythroblast iron has been postulated on the basis of morphological studies. Here it is suggested that reticulum cells pass on iron to erythroblasts.(60)(459)(839)(862)(59) It has been shown that reticulum cells are incapable of taking up iron from transferrin under normal circumstances (II-e-2), and no bone marrow 'pool' of transferrin iron, as postulated by Pollycove, has been demonstrated.(686) It would seem that this is not a major source of erythroblast iron on the basis of this evidence.(452)(258)

Electron microscopic studies have shown rhopheocytosis of ferritin particles by erythroblasts. There has been some discussion about the direction in which this procedure is going. Bessis et al have suggested that reticulum 'nurse' cells may be a major source of erythroblast iron on the basis of their electron microscopic studies. They postulate that this iron (within the reticulum cells) is derived from haemoglobin catabolism, is converted within the reticulum cell to ferritin granules, extruded from the reticulum cell and then taken up by the maturing erythroblast by rhopheocytosis.(59)(60)

III-b-6 Haemoglobin Formation.

Once taken up by the erythroblast, the subsequent intracellular distribution of iron appears to be dependent upon the adequacy of a number of intracellular processes. For this reason, erythroblast iron uptake and haemoglobin formation are not

identical processes although they are closely related. Thorell, using ultra violet light microscopy demonstrated that haemoglobin was first seen, in maturing erythroblasts at a later stage than erythroblast iron uptake has been shown to occur. de Carvalho however, using identical techniques, demonstrated haemoglobin synthesis in early normoblasts.(486)(789)(191)(192)(190)(835)

It would seem reasonable to assume some time lag between membrane uptake of iron and its eventual insertion into protoporphyrin at a mitochondrial level to form haem.(59)(303)(896)(764)(302)(226) Initially, as previously described (III-b-5) the iron is removed from transferrin at the membrane, passes next into the soluble intracellular cytoplasmic compartment where it is believed to complex as low molecular weight Fe^{2+} complexes with cytoplasmic constituents. These complexes are thought to serve as an intracellular iron pool for both haemoglobin and ferritin synthesis.(14)(486)

Ferritin concentration within the erythroblast decreases as the cell matures.(60) This may be because it is extruded as the cell matures (excessive erythroblast iron uptake) or because it forms a true intracellular precursor store of iron for subsequent heme synthesis. The latter view is supported by a number of observers although this is disputed by Zail.(908)(486)(566)(14)(448) Ferritin synthesis within erythroblasts has been demonstrated in bone marrow cultures(486) although the amount of iron normally utilised for ferritin synthesis within the erythroblast is much smaller than the amount incorporated directly into haem.

Under exceptional circumstances where protoporphyrin synthesis is impaired and cellular iron uptake not effected (i.e. lead poisoning) the majority of erythroblast iron may be present as ferritin.(486)(303)(848)(499)(887)

A number of factors are thought to be important in directing the intracellular localisation of erythroblast iron. Copper deficiency results in aggregation of ferritin granules within cytoplasmic vacuoles in the erythroblast, and a decrease in the amount of iron incorporated into mitochondria in the absence of any defect in haemoglobin synthesis.(303) Copper or caeruloplasmin seem, because of this, to be important in increasing intracellular utilisation of iron, whether this effect be by directing the iron to mitochondria or by oxidising the Fe^{2+} to Fe^{3+}. Haem synthetase and ferrochelatase are thought to be important in promoting the incorporation of iron into protoporphyrin.(568b)(764)(84)

The intracellular synthesis of protoporphyrin will not be reviewed here as I feel that it lies outside the scope of this thesis. Once formed in maturing erythroblasts, the haem and globin are retained inviolate within the red cell until its death. Globin is synthesised 'de novo' within the maturing erythroblast from precursor amino acids or short polypeptide chains taken up by the erythroblast during its development.(885)(886)(101)(37)(773) The formation of globin is dependent upon DNA dependent RNA synthesis at the ribosomal level.(698b)(199)(458)(835)(110)(109) Haem is not added to the globin chains until after they have been synthesised.(613b)

Globin synthesis within the erythroblast appears to precede haem synthesis, the two substances being synthesised independently.(208)(352)(632)(635)(644) The synthesis of haem is stimulated by the presence of globin and globin synthesis is stimulated by the presence of haem.(526b)(325)(795)(9)(917) Eventually, haem combines with globin chains to form haemoglobin consisting of four globin chains plus four haem groups. This lies within the soluble protein fraction of the cell. (526b)(831)(895)

III-b-7 Ineffective Erythropoiesis.

This can be defined as wasted bone marrow erythropoietic activity. Here the bone marrow forms erythroblasts which synthesise haemoglobin and mature to variable stages but due to intramedullary destruction, never enter the circulation as viable red cells. In the normal individual approximately 10 - 15% of total erythropoietic activity is calculated to be ineffective, (52)(418)(725)(728)(628)(44)(724)(321)(869)(329) and in certain anaemias the proportion of total erythropoietic activity that is ineffective is grossly increased. Conditions in which this occurs are:-

1. megaloblastic anaemias.(329)(636)(854)(232)(321)(724)(80)(289)(657)(504)(44)(253)(754)(211)(383)(288)(258)
2. pyridoxine deficiency anaemias(297a)(358)(853)(286)(533)(258)(852)
3. lead poisoning(44)(448)(258)

4. erythropoietic porphyria (724) (321)
5. thallassaemia (815) (370) (258) (289) (724) (854) (504) (657) (232)
6. severe iron deficiency anaemias (258) (106) (164) (687) (688) (726) (727)
7. sideroachrestic anaemias (67) (724) (387) (386) (135) (318) (179) (297a) (528) (533) (597) (781) (636) (183) (258)
8. myelofibrosis (902) (258) (232)

In these conditions, bone marrow samples show hyperplastic erythropoiesis, (179) (92) (528) (182) (253) (852) a 'dyshaematopoietic' picture of 'maturation arrest' (67) (182) (286) (486) (889) and erythroblast destruction. (553) (499) (902) (636) (183) (258)

A considerable proportion of the iron delivered to the bone marrow will, in the presence of ineffective erythropoiesis, probably not find its way into the circulating blood even though the amount of iron taken up by the bone marrow will be increased because of increased total erythropoietic activity.

III-b-8 Erythropoietin.

(1) History.

The existence of erythropoietin was postulated long before its presence was proven. Carnot and Deflandre in 1906 (125) published evidence suggesting the presence of an erythropoietic factor in the serum of anaemic rabbits, this being confirmed in 1957 by Gray and Erslev. (322) With the development of erythropoietin assays, erythropoietin levels were found to be roughly proportional to the levels of the haematocrit in normal individuals. (614) In uraemic individuals, levels were found

to be inappropriately low[7][627] suggesting a renal site for erythropoietin production.[427][807] Studies by a number of workers have shown the production by the kidney of a substance capable of reacting with a constituent of normal plasma to produce erythropoietin.[482][306][244][911] This factor has been called renal erythropoietic factor (R.E.F.) and is found in the mitochondrial fraction of the renal cortex and medulla.[912] It is thought that this substance is generated by microsomes and stored in mitochondria.[121] The active erythropoietin produced by the enzymatic action of REF on the plasma substance can be neutralised by anti-erythropoietin antibody although this does not neutralise REF.[913][572][762]

(2) Production of REF is accelerated by factors known to increase erythropoietic activity (such as hypoxaemia, cobalt therapy, anaemia, testosterone administration) and depressed by factors decreasing erythropoietic activity (such as hyperoxia, polycythaemia).[305][307][910] The substrate in the plasma acted upon by the REF is produced in the liver. Erslev et al have questioned this concept of erythropoietin production.[244] It is clear, from studies on adequately dialysed, anephric man, that erythropoietin must be produced by sources other than the kidney. It is, however, unclear whether these sites of production of erythropoietin are normally present, or whether they develop as an adaptive response to the nephrectomised state.[633][574][587][591][590][588][627][589][276]

The production of REF by the kidney appears to be a direct

response to hypoxia. The denervated transplanted kidney is capable of responding to vascular changes or rejection phenomena causing local hypoxia, as well as to perfusion with hypoxemic blood, cobalt and testosterone.[195][665][591][834][3][642][261]

(3) Elucidation of the chemical composition of erythropoietin has been fraught with difficulty due to technical problems in its purification and instability of the purified product. It has however been characterised as a glycoprotein.[301][530][650][655][787] It appears to be species specific both in biological activity and antigenic reactivity.[496][498][759][761] A number of body proteins have been demonstrated not to be erythropoietin (i.e. aldosterone, caeruloplasmin, angiotensin, renin) but may act to promote erythropoiesis by inducing production of erythropoietin.[85][260][537][546]

(4) Erythropoietin titres have been shown to be roughly inversely proportional to the haematocrit,[614] increasing normally as the haematocrit falls. The importance of erythropoietin in day to day regulation of erythropoiesis has been well demonstrated by Schooley et al, who, by neutralising circulating erythropoietin with injections of anti-erythropoietin antibody, produced anaemia and bone marrow erythroid hypoplasia.[761] A number of workers have shown that the primary action of erythropoietin is the induction of stem cell differentiation to early erythroblasts although it may act only upon erythroblast committed stem cells.[18][19][241][251][651][671][830]

In addition, under conditions of abnormal stress, erythropoietin may act upon differentiated recognisable erythroblasts to cause

(a) skipping of mitoses resulting in premature release of immature red cells into the circulation.[123][380][259]

(b) an increase in the rate of haemoglobin synthesis in cells capable of RNA synthesis.[487][806][808] This acceleration of terminal maturation with skipped mitoses and an increased rate of haemoglobin synthesis results in the release of macrocytes into the peripheral circulation.[18][19] Where terminal maturation is prolonged due to deficiency of precursor material (e.g. iron deficiency), Hb synthesis is decreased, additional mitoses occur and microcytes are produced.[93][510][802][805]

(5) In vivo studies have shown that erythropoietin stimulates the formation of RNA and DNA in erythropoietic tissue[390][391][651][736] as well as the synthesis of enzymes required for the synthesis of these nucleic acids. It also stimulates the formation of ALA synthetase, an enzyme having a rate limiting effect upon haem synthesis.[83][630]

In vitro studies have shown that erythropoietin stimulates haem synthesis,[215][242][243][402][477] stimulates the incorporation of amino acids into the erythroblast stroma and lipid fractions[216] and stimulates the pentose phosphate pathway of glucose utilisation thus providing ribose for nucleic acid synthesis.[670] The effects of erythropoietin are inhibited by actinomycin D which blocks DNA dependent RNA synthesis.[707]

In conclusion, erythropoietin appears to act by a permissive effect upon DNA dependent RNA synthesis. This results in the production of messenger RNA, so providing the templates required for the synthesis of enzymes needed by the maturing erythroblast for cell division, maturation and haemoglobin formation. In practical terms, it acts to maintain the circulating red cell mass at a level sufficient to provide optimal tissue oxygen requirements.

(6) Regulation.

Erythropoietin is excreted into the urine in amounts directly proportional to circulating levels which in turn depend upon the rate of erythropoietin production.[6][12] Increased production of erythropoietin occurs when tissue hypoxia is produced,[124][247][714][784] following phlebotomy,[5][4][8][124] and in most anaemias,[333][441][495][497][570][629][645][695][304] excepting those associated with starvation, infection, neoplasia or uraemia where normal or low levels may be found. Under normal circumstances an increased production of erythropoietin will increase red cell production, although in some anaemias (i.e. aplastic anaemia, pernicious anaemia, iron deficiency, sickle cell disease, leukaemia, sidero-achrestic anaemias) bone marrow response may be suboptimal.

Decreased erythropoietin production occurs in conditions associated with increased tissue oxygenation - e.g. hyperoxia, individuals descending from high to low altitudes or transfusional polycythaemias.[8][6]

CHAPTER IV. NORMAL FERROKINETICS.

Introduction

There is no doubt that the ferrokinetic technique has been the basic research rock upon which a vast amount of the knowledge of iron metabolism has been built. As a research tool, the ferrokinetic approach has aided in a basic understanding of physiological and pathological iron metabolism, as well as in an understanding of the pathophysiology of most haematological states involving the red cell series. This aspect has been covered, in some detail, in my review of iron metabolism. There remains the problem of assessing the relevance of ferrokinetic investigation to the clinical situation. In summary, ferrokinetic investigation of a haematological patient aims to achieve:

(1) A measurement of total erythropoietic activity.

(2) Information regarding the effectiveness of this erythropoietic activity

(3) Information concerning the red cell lifespan of the individual being studied (when combined with chromium studies).

(4) Some idea of the state of the iron stores of the patient being studied.

This quantitative information is used to characterise the haematological status of the patient being studied, with reference to the erythroid series only. As a result of basic research, it is known that different groups of diseases affecting the red cell series can be characterised by a pattern of ferrokinetic parameters. The results obtained in the study

of an individual patient can be compared with those characterising different disease states, so contributing to the functional and aetiological diagnosis.

It must be acknowledged that ferrokinetic investigation of a patient involves additional expense, time and expertise, and one must ask whether this is justifiable. In order to answer this question, one must review the diagnostic capabilities of other basic haematological investigations which attempt to perform the same task, bearing in mind the ease with which ferrokinetic investigation can be performed in a prepared laboratory.

A. Clinical Assessment of Erythropoietic Activity.

Every clinician is aware of the clinical features of anaemia, and in addition, many conditions causing anaemia can be readily recognised after careful history taking and examination of a patient. Very few anaemias however, present in such a characteristic way that a clinician would make a solely clinical diagnosis. The amount of investigation the clinician would request would most likely be related to the availability of investigative services, but certain basic investigations would be required. With the aid of these basic investigations, the presence of anaemia may be confirmed, and in many cases, the cause of anaemia will be apparent. The basic haematological investigations will be:-

(1) The haemoglobin - this will show the presence or absence of anaemia (Normal : Male 13.5 - 18G%, Female 11.5 - 16.4G%)

(2) The haematocrit - this is usually more accurately measured than the haemoglobin, and will give one similar information. (Normal : Male 40 - 54, Female 36 - 47)

These two measurements will confirm the presence of anaemia, but give one no idea as to the underlying mechanism.

(3) Other red cell values:

(i) $MCV = \frac{Hct}{RCC} \times 10$ (Normal 76 - 96 cμ)

(RCC = Red cell count in millions)

(Hct = Haematocrit)

(MCV = Mean corpuscular volume)

(ii) $MCH = \frac{Hb}{RCC} \times 10$ (Normal 27 - 32 μμg)

(MCH = Mean corpuscular haemoglobin)

(Hb = Haemoglobin concentration)

(iii) $MCHC = \frac{Hb}{Hct} \times 100$ (Normal 32 - 36%)

(MCHC = Mean corpuscular haemoglobin concentration)

The accuracy of these measurements depends upon accurate measures of Hb, Hct and RCC. Mechanisation has not totally removed problems associated with experimental error. The MCHC is probably the most accurate of these measurements, being uninfluenced by the red cell count.

Examination of the blood smear.

With the aid of (1) - (4) above, the anaemia can be characterised as normo, micro or macrocytic

normo, hypo or hyperchromic.

In many instances, these investigations contribute all of the information necessary to establish a clinically useful diagnosis. There are cases, however, when confirmation of a suspected aetiological diagnosis may require more elaborate tests e.g. measurements of plasma iron, B_{12} and folate concentrations. Examination of the peripheral smear will detect the presence of abnormal cells which may help further in the diagnosis of the underlying disease state.

Chemical investigations may produce clues to suggest the presence, site and severity of haemolysis (i.e. total and conjugated bilirubin, haptoglobin, haemoglobin electrophoresis, urinary urobilinogen, lactate dehydrogenase, alanine aspartate transaminase.)

In certain cases, an aetiological diagnosis is insufficient for complete patient management and some means is required of assessing the erythroid precursor response to the disease, as well as its response to therapy. Similarly the severity of haemolysis, if present, needs to be quantitated. A number of investigations may be used for this purpose. These include the following indicators of total erythropoietic activity, effective erythropoietic activity and red cell survival.

(5) Examination of bone marrow morphology.

This is probably the most useful of basic haematological investigations for assessing total erythropoietic activity but,

for a number of reasons, it is also probably the least precise. A hypoplastic marrow in the presence of anaemia will obviously mean decreased total erythropoietic activity and a hyperplastic marrow will, in the presence of anaemia, mean that haemolysis (either extra or intra medullary) or blood loss is present. An estimate of bone marrow erythropoietic activity however depends upon an assessment of the ratio of precursor myeloid to erythroid cells. (The M : E ratio). This assumes that the sample of bone marrow studied is representative of the bone marrow 'organ' as a whole, and that myelopoiesis is normal. The normal M : E ratio quoted is $\pm$ 3 : 1.(661) The M : E ratio gives very little indication of normality or abnormality in erythropoietic activity. This is measured by subtle morphological features(179)(92)(528)(182)(253)(852)(67)(286)(486)(889)(553)(499)(902)(636)(183)(258) which may be difficult to detect, and are not, in any case, quantitative. In addition, erythropoietic activity may be greatly increased with a normal M : E ratio if the bone marrow organ mass is greatly increased. With the advent of radioactive methods of measuring bone marrow mass, this change can be readily detected.(21)(846)(22)(730)(79)(164)(686)(688)(864)

(6) Faecal urbilinogen excretion.

Measurement of total faecal urobilinogen (UBG) excretion gives a measurement of total pyrrole pigment catabolism. This is mainly derived from haemoglobin but a small amount is derived from non haemoglobin sources - i.e. myoglobin, catalases,

cytochromes and peroxidases. A measure of total faecal UBG therefore is an indicator of total haem catabolism, reflecting in most cases, total haemoglobin synthesis.[654][755][867][866][416][509][774][728][525][52][418][725][511][905][628]

Most of the bilirubin excreted by the liver is finally excreted as UBG, although some is reabsorbed and enters in the enterohepatic bile pigment circulation. Very small amounts of bile pigment are normally lost in the urine. Faecal UBG measurements should therefore give a reliable measure of total haem synthesis, assuming a steady state where synthesic and catabolic rates remain equal. As 1 gm. of Hb gives rise to 35 mg. of UBG[289] one should, in normal patients, be able to estimate the difference between actual UBG excretion and that expected from Hb catabolism as measured from total RBC mass and lifespan measurements. This should reflect UBG derived from sources other than circulating erythrocytes. This should, therefore, measure total and ineffective erythropoiesis. In practise, this method is rarely sufficiently accurate. The largest problem is collection of a complete faecal sample, even under 'metabolic ward' conditions. The test is useless in patients taking antibiotics as these alter the bacterial flora of the gut.[334] There is some loss of UBG via routes other than the faeces. Finally, the test is cumbersome, time consuming, unpleasant to perform, and, even in the best hands, has been found to give an extremely wide range of normal results. As a measure of erythropoiesis, it appears to be infinitely less accurate and more unpleasant to perform than ferrokinetic investigation.

(7) The reticulocyte count.

This parameter appears to afford an accurate indication of effective erythropoietic activity.[385][384][38][153][256] The reticulocyte count, expressed as a percentage, gives a rough measure of effective erythropoietic activity providing that essential precursor material for haemoglobin synthesis is adequately supplied to the bone marrow. In the presence of anaemia, this parameter becomes less accurate as a measure of effective erythropoiesis when compared with other parameters, owing to changes in the total number of red cells and prolongation of the time required for reticulum to be lost from reticulocytes released at an earlier stage of maturation into the circulation. Corrections have been proposed by Hillman[385] for these aberrations and, when these are applied, the resulting reticulocyte index correlates very well with other parameters of effective erythropoiesis. The reticulocyte count may be corrected:

(a) by correcting for changes in total number of red cells by

(i) expressing the reticulocyte count as an absolute number per unit volume or

(ii) by expressing the reticulocyte count in a standardised way relative to the haematocrit

i.e. $\text{Corrected Reticulocyte Count \%} = \frac{\text{Patient's Hct}}{\text{Normal Hct}(45)} \times \text{Retic.Count \%}$

(for changes in Hct) (Hct = haematocrit)

Normal value is 1%

(b) by using a reticulocyte index in an attempt to quantitate effective erythropoiesis - i.e.

$$\text{Reticulocyte Index} = \frac{\text{Corrected reticulocyte count (\%)}}{\text{Normal reticulocyte count (\%)}}$$

(c) by correcting the reticulocyte index for changes in maturation time of reticulocytes prematurely released into the circulation because of stress, the stress being proportional to the drop in haematocrit.

i.e.

Hct	Reticulocyte Maturation Time
45	1
35	1.5
25	2
15	2.5

This will result in a production index of effective erythropoiesis i.e.

$$\text{Reticulocyte Production Index} = \frac{\text{Reticulocyte Index}}{\text{Maturation Time}}$$

This was found by Hillman et al[385] to be an excellent comparative method of assessing effective erythropoietic activity. It does not, however, measure total erythropoietic activity, and will not therefore measure any erythropoiesis that is ineffective.

In concluding this introductory chapter, I feel that the ferrokinetic technique is a quick and easy way of measuring total erythropoietic activity; this can be done in one day with minimal patient inconvenience or laboratory work, and gives quantitative results. The reticulocyte production index provides a good indicator of effective erythropoiesis but does not quantitate it in terms of amount of haemoglobin formed. The ferrokinetic technique is probably able to do this, and

therefore measures effective erythropoietic activity as well as the reticulocyte production index. Overall, a lot of information may be obtained using the ferrokinetic technique that can not be obtained by other methods. This is obviously of interest in characterising pathophysiological mechanisms, but, in the majority of anaemic patients, is not very helpful clinically. In situations where the haematological status remains unexplained after routine haematological investigation, ferrokinetic investigation may be of help in elucidating the cause and severity of the underlying abnormal process.

IV-1-a The Effect of Iron Status on Ferrokinetic Performance.

It became apparent at an early stage in this study that the presence or absence of iron deficiency or overload could exert a profound influence on ferrokinetic data obtained from any given patient. This is a well known observation and has been documented by others. Hillman and Henderson,[384] for example, have shown that completely 'normal' individuals have a fairly uniform bone marrow and ferrokinetic response to repeated phlebotomy, this response depending largely upon the haematocrit and the availability of precursor material, particularly iron.

Since, in the long view, it was my intention to apply ferrokinetic techniques to the investigation of erythroid disorder that might be complicated by various degrees of iron deficiency or excess, I felt it necessary to devise some means of "correcting" the data for the diagnostically trivial effect of variable iron status. This, it seemed, could only be done by defining, in quantitative terms, the relationship between ferrokinetic performance on the one hand and iron status (as reflected in the plasma iron concentration) on the other, in individuals whose bone-marrow function was, in all other respects, normal.

IV-1-b Selection of patients for this study.

Patients selected for this study were either normal in every haematological respect or those who appeared to have "pure" iron deficiency or overload with no other haematological abnormality. All patients with known causes for haemolytic anaemias were excluded, as were all patients with demonstrable folate or Vitamin B_{12} deficiencies. Appropriate steps were taken to exclude patients with hepatic, renal, infective, neoplastic or auto-immune diseases.

IV-1-c Method of Study:

All patients had full, routine ferrokinetic and radio-chromium studies as detailed in the appendix under Methods.

IV-1-d Results.

The findings in this group of patients are summarised in Table IV-1. As can be seen from the data, haemoglobin values varied over a wide range (8.1 to 20.4 G%) as did the haematocrits (23% to 64%). Plasma iron values and values for % iron saturation varied from frankly iron deficient to hyperferraemic. (9 µg% - 243 µg%)(2 - 100% saturation). In all cases the $t_{\frac{1}{2}}$ for ^{51}Cr-labelled autologous red cells was normal.

Table IV-1 Composite Ferrokinetic Findings in normal patients.

Abbreviations used in this table:-

Bl. Vol.	=	Blood volume
Pl. Vol.	=	Plasma volume
PI	=	Plasma iron
TIBC	=	Total Iron Binding Capacity
% Sat.	=	Percentage transferrin saturation
$t_{\frac{1}{2}}$PIC	=	$t_{\frac{1}{2}}$ Plasma Iron Clearance
PIT	=	Plasma Iron Turnover
RBCU	=	Red Cell Utilisation
$t_{\frac{1}{2}}{}^{51}Cr$	=	^{51}Cr Autologous Red Cell Survival.

Table IV-1. Composite Ferrokinetic Findings in normal patients.

Patient	Hb (G%)	Hct (%)	Bl.Vol (ml/kg)	Pl.Vol. (ml/kg)	P.I (µg%)	TIBC (µg%)	%Sat	$t_{\frac{1}{2}}$PIC (min)	PIT	RBCU (%)	$t_{\frac{1}{2}}{}^{51}$Cr days
deB	15.6	43	64	45	62	286	22	60	.6245	83	25
Web	16.2	52	54	33	88	264	33	91	.5044	93	29
Luk	12.8	38	84	52	209	209	100	134	1.0144	49	28
Cup	10.3	30	75	55	113	159	71	53	1.5436	53	24
Car	12.1	38	81	49	53	90	59	35	.9849	83	32
Hey	20.3	64	70	35	64	289	22	28	.9399	87	26
Bot	17.2	51	66	38	114	197	58	70	.8644	75	28
Rus	11.9	36	80	52	49	210	23	64	.5121	100	27
Kho	15.6	45	64	37	55	233	24	51	.6320	100	29
Thy	14.7	44	91	58	105	185	57	85	.7352	84	24
Arm	12.6	38	90	60	92	282	32	62	.9657	90	26
Sch	11.0	36	99	69	21	280	7	34	.4131	98	36
Bas	8.1	23	56	39	9	384	2	16	.4435	94	24
Hoo	9.9	29	66	49	16	243	6	29	.4045	94	23
Cha	10.9	36	65	44	19	563	3	18	.7060	96	22
Loz	13.2	36	79	49	9	232	3	35	.7720	90	24
Gog	16.8	46	81	48	196	205	95	90	1.2561	54	26
O'Co	13.1	35	65	40	243	270	90	210	.7845	40	28
Van	11.2	34	70	40	137	343	40	86	1.0941	82	24
Pet	16.4	42	62	39	29	302	10	51	.3489	92	27
Mgo	16.1	46	66	39	163	164	99	133	.7069	66	26
Tan	14.5	40	57	37	101	279	36	82	.7184	96	27
Wes	17.3	54	56	29	143	187	76	74	.9724	69	29
Nqw	16.3	49	60	35	206	246	84	101	1.1202	44	29
Mar	20.4	60	88	43	101	295	34	52	.8702	86	29
Ans	12.0	38	74	49	32	395	8	30	.6938	100	33
Wil	11.0	33	68	45	22	412	5	31	.7613	95	27
Pre	17.6	47	66	43	86	302	28	34	1.4357	91	22
Sam	16.1	44	69	44	123	287	43	61	1.2002	87	26
Jeg	11.4	34	73	50	45	267	17	33	.9371	89	24
Pax	13.3	29	61	44	41	272	15	54	.5561	100	27
Rob	9.1	28	72	59	80	249	32	42	1.4141	100	27
Tay	16.4	54	100	60	26	366	7	17	.7696	100	30
Sch	8.6	26	56	50	33	231	14	31	.8099	91	33

IV-2-a The $T_{\frac{1}{2}}$ Plasma Iron Clearance.

It was noticed at an early stage that patients with higher plasma iron concentrations took a longer time to clear radio-iron from their plasma than did patients with low plasma iron concentrations. This initial impression was studied further by taking this parameter, (the $t_{\frac{1}{2}}$PIC), and plotting it against both the plasma iron concentration per 100 ml. of plasma and the plasma iron concentration per 100 ml. of whole blood. This latter figure was obtained, as calculated by Cook[164] i.e. plasma iron concentration per 100 ml. whole blood = $\frac{100 - (0.92 \times Hct)}{100}$ x Plasma Iron(µg/100 ml. plasma) where 0.92 is a correction factor for plasma trapped in the red cells while reading the haematocrit.

Once plotted, these points were fitted, by the method of least squares, to a regression line, and the equation describing this line, the standard error of the estimate of the line, and its correlation coefficient were calculated. The results can be seen in Figure IV-1 and IV-2.

Figure IV-1 The Plasma Iron Clearance $t_{\frac{1}{2}}$ (y ordinate) plotted against the plasma iron concentration in µg/100 ml. plasma (x ordinate).

$t_{\frac{1}{2}}PIC = 0.5509 \times P.I.$ (µg/100 ml plasma) + 14.3389

$r = 0.8698$

$SE\ y/x = 19.5$

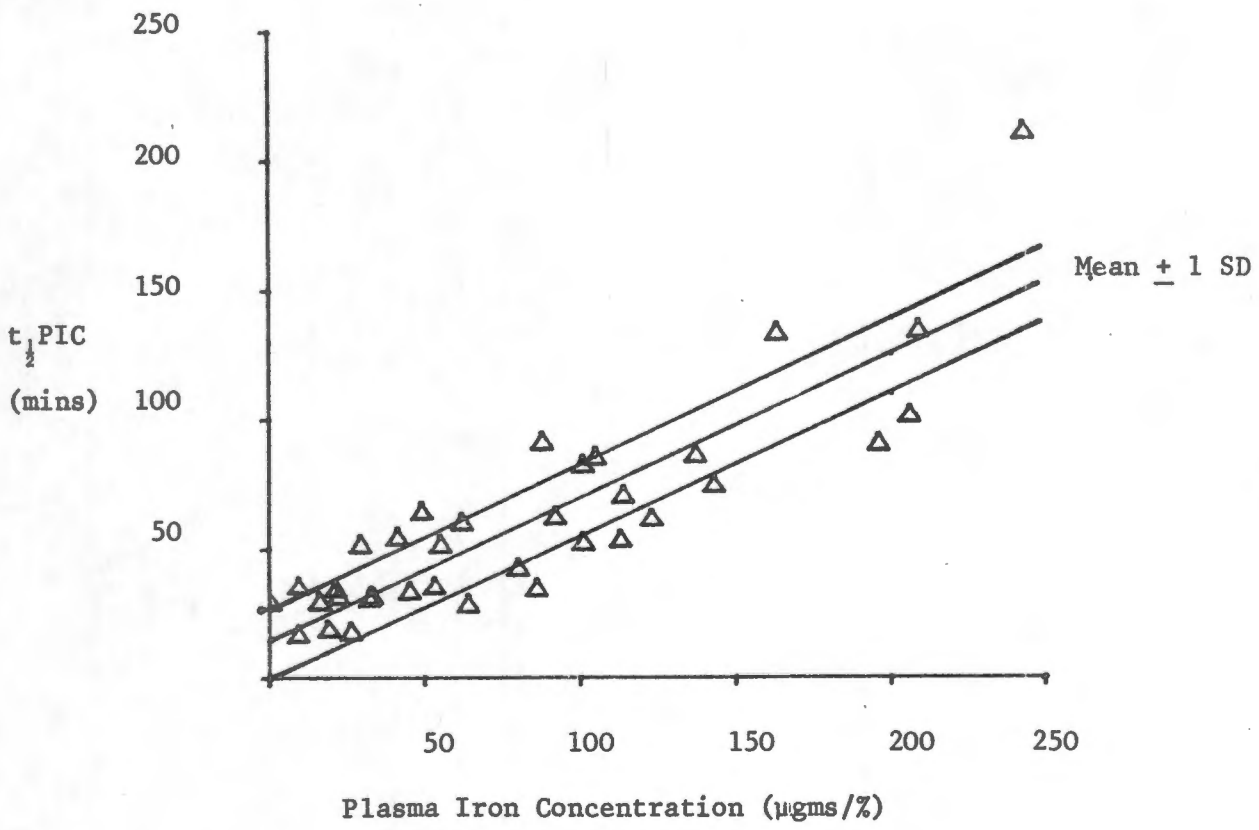
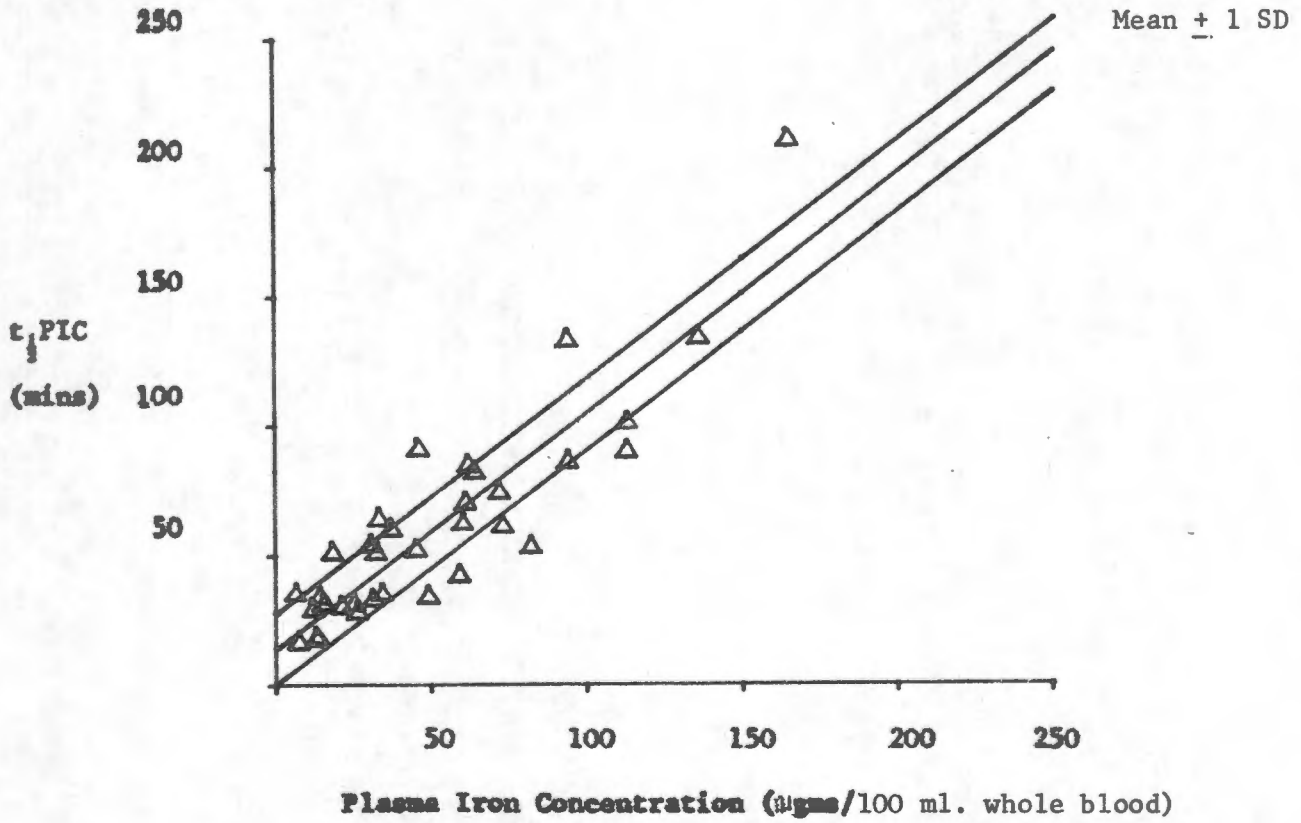


Figure IV-2 The plasma iron clearance $t_{\frac{1}{2}}$ (y ordinate) plotted against the plasma iron concentration in µg/100 ml. of whole blood (x ordinate).

$t_{\frac{1}{2}}PIC = 0.9114 \times P.I.$ (µg/100 ml w.b.) + 14.0171

$r = 0.8931$

$SE\ y/x = 17.8$



IV-2-b It can be seen from these graphs that as the plasma iron concentration increases, whether this be measured as µgm/100 ml. plasma or µgm/100 ml. of whole blood, there is a proportionate increase in the $t_{\frac{1}{2}}$PIC, as described by the regression equation, in different individuals. Allowing for some biological variation between individuals, this mathematical description of the variation in $t_{\frac{1}{2}}$PIC as a function of iron concentration seems to describe normal individuals fairly well (r = 0.8698 and 0.8931 respectively). This observation is not new, having been previously described by a number of workers.(62)(61)(863)(80)(258)(735)(371) Other authors however have stressed the relationship between the $t_{\frac{1}{2}}$PIC and total erythropoietic activity.(300)(872)(55)(848)(815)(443)(673)(635)(61)(735)(486)(900)(863)(284)(914) It is obvious then, that the $t_{\frac{1}{2}}$PIC depends upon both the plasma iron concentration and the erythropoietic activity being more rapid where plasma iron concentrations are low and/or bone marrow erythropoietic activity increased, and being prolonged where plasma iron concentrations are increased and/or bone marrow erythropoietic activity decreased. The problem therefore is to separate the effect of these two variables upon the $t_{\frac{1}{2}}$PIC.

IV-2-c The cause of the prolongation of the $t_{\frac{1}{2}}$PIC with increasing plasma iron concentrations in patients with the same amount of bone marrow activity appears to be that the bone marrow, to a major extent, regulates the rate of removal of iron from the plasma, as it is the major site of deposition of plasma iron.
(21)(846)(22)(730)(79)(164)(686)(688)(864)(459)(486)(201)(202)

The $t_{\frac{1}{2}}$PIC will largely reflect the rate of turnover of the plasma iron pool which in turn will depend upon a state of equilibrium between erythropoietic iron demand and available plasma iron supply. Should the erythropoietic demand remain constant, the rate of turnover of iron in the plasma, and hence the $t_{\frac{1}{2}}$PIC, will depend on the plasma iron concentration. Should this decrease, the $t_{\frac{1}{2}}$PIC will decrease proportionately, and the reverse will occur when the plasma iron concentration increases. That this relationship is linear is well demonstrated by our normal subjects. Similarly, should erythropoietic activity change and the plasma iron pool remain constant in size, the rate of removal of iron from the plasma and hence the $t_{\frac{1}{2}}$PIC will be proportionate to the change in erythropoietic activity.

IV-2-d Having defined, by regression equation, the relationship between plasma iron concentrations and $t_{\frac{1}{2}}$PIC in subjects with normal bone marrow function, this could be used to predict the normal $t_{\frac{1}{2}}$PIC that would be expected at any given concentration of plasma iron. A value for the $t_{\frac{1}{2}}$PIC significantly greater than that predicted would thus indicate depressed erythropoietic activity. Conversely, a value less than that predicted would indicate increased erythropoietic action. For convenience, I have chosen to combine the observed and predicted half time for the clearance of ^{59}Fe from the plasma as an "Erythropoietic Index" defined as the ratio of the predicted $t_{\frac{1}{2}}$PIC to the experimentally derived P.I.C.

i.e.

$$\text{Erythropoietic Index} = \frac{\text{Predicted } t_{\frac{1}{2}}\text{PIC at Plasma Iron Conc. of patient}}{\text{Experimentally derived } t_{\frac{1}{2}}\text{PIC of patient}}$$

An erythropoietic index of 1 would then mean normal total erythropoietic activity, a ratio significantly greater than 1 indicating increased, and a ratio significantly less than 1 indicating decreased total erythropoietic activity.

IV-2-e A theoretical flaw exists in the development of this parameter, and that is the influence of non erythroid iron uptake upon the $t_{\frac{1}{2}}$PIC. Should non erythroid iron uptake vary in different individuals independently of the variation in plasma iron concentration, it would be inappropriate to apply a correction for the $t_{\frac{1}{2}}$PIC based upon the assumption that there is a relationship. For reasons that I shall detail in the section on non erythroid iron turnover, I believe that the influence of non erythroid iron uptake upon the $t_{\frac{1}{2}}$PIC is constant in different disease states, depending only upon variations in plasma iron levels, and hence of minor importance as far as the $t_{\frac{1}{2}}$PIC is concerned. (See IV-3) In consequence, I believe that it is reasonable to insist that $t_{\frac{1}{2}}$PIC values be interpreted only in relationship to the patient's plasma iron concentration.

IV-2-f <u>Assessment of the Validity of the Erythropoietic Index Parameter in assessing Total Bone Marrow Erythropoietic Activity</u>. I have attempted to assess the validity of the erythropoietic index as a parameter of total bone marrow erythropoietic activity.

For this purpose I have taken two groups of patients in addition to my normal patients. The first group of patients was selected on the basis of known hypoplastic anaemia. These patients had been demonstrated histologically to have depressed erythropoiesis on bone marrow smears, and labelled clinically as hypoplastic or aplastic anaemias. The second group of patients were patients with hyperplastic bone marrow on histology, and anaemias characterised by increased total erythropoietic activity. Into this group I have placed patients with haemolytic anaemias, megaloblastic anaemias, sidero-achrestic anaemias and myelofibrosis. These conditions are known to be associated with increased total erythropoietic activity, although a variable proportion of this may be ineffective.(329)(636)(854)(232)(321)(724)(80)(289)(657)(504)(44)(253)(754)(211)(383)(288)(258)(815)(370)(832)(67)(387)(386)(135)(318)(179)(297a)(528)(533)(597)(781)(183)(902)

The $t_{\frac{1}{2}}$PIC is accepted by most workers as an index of total erythropoietic activity. (See IV-4-b) All these patients will have increased total erythropoietic activity. It will be obvious that a tremendous range of erythropoietic activity will be present in these patients, and this will be mirrored in the E.I. (erythropoietic index) results. All patients with increased erythropoietic activity should have E.I. values greater than 1 whereas all patients with depressed erythropoiesis should have E.I. values of less than 1.

Results.

Composite results for the normal subjects are given in Table IV-1 and Figure IV-1.

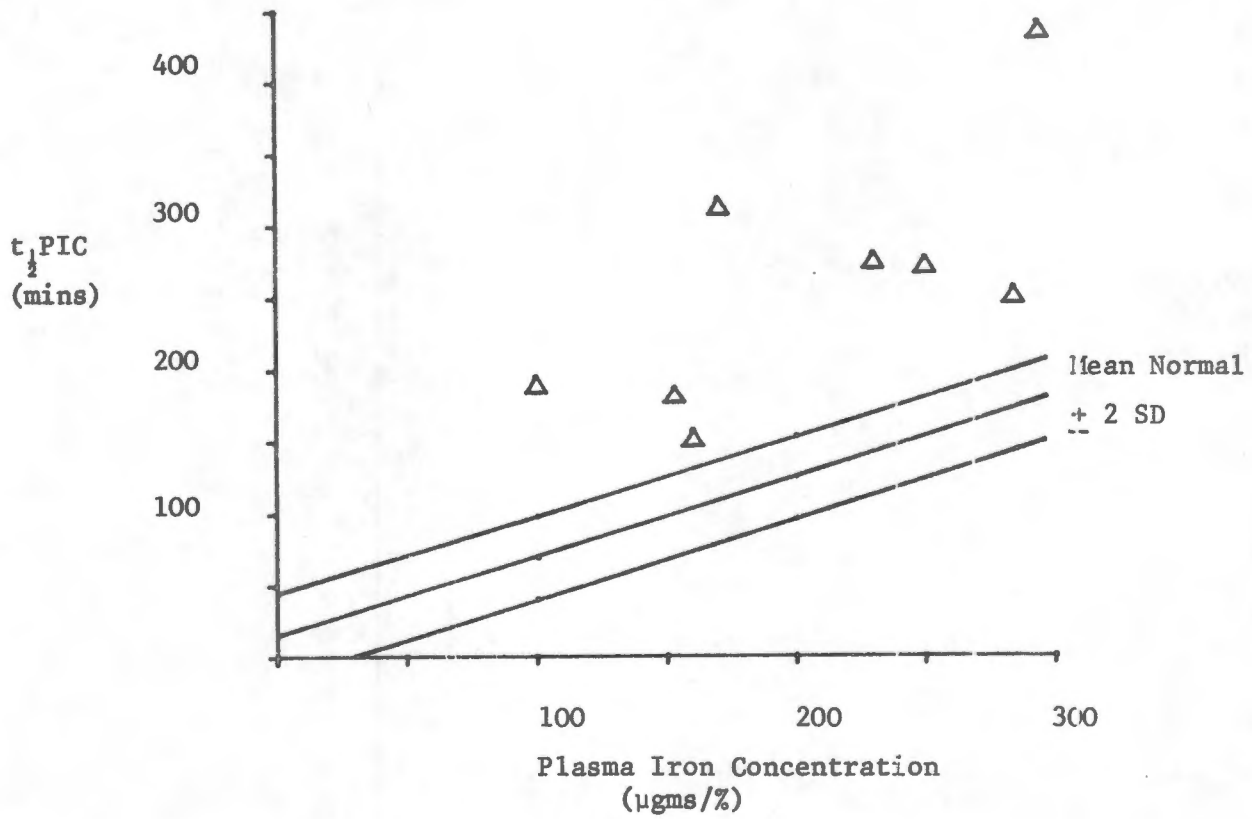
The results for the hypoplastic group of patients are summarised in Table IV-2 and Figure IV-3.

The results for the hyperplastic group of patients are summarised in Table IV-3 and Figure IV-4.

Table IV-2 Composite Ferrokinetic Findings in Patients with Hypoplastic Bone Marrow.

Patient	Hb G%	Hct %	Bl.Vol ml/kg	Pl.Vol ml/kg	SI µg%	TIBC µg%	%Sat	$t_{\frac{1}{2}}$PIC min	EI	PII	RBCU %	$t_{\frac{1}{2}}{}^{51}Cr$ day
Ery	11.5	32.0	60.5	41.6	100	198	51	188	.34	.3753	50	-
Coo	12.0	34.5	66.8	44.2	153	249	61	180	.55	.5802	-	-
Fra	11.2	36.0	-	-	230	336	68	273	.52	.5635	25	19
All	10.6	35.0	-	-	250	250	100	270	.56	.6278	25	-
Bai	9.4	27.0	56.9	42.1	160	323	49	150	.68	.8017	26	29
Pep	11.8	34.0	67.8	47.1	294	294	100	433	.41	.4666	9	40
Das	11.4	32.0	59.2	42.2	170	174	98	311	.35	.3857	6	45
duT	11.5	31.0	64.3	47.5	284	307	93	249	.69	.8153	15	38

Figure IV-3 Graphical representation of $t_{\frac{1}{2}}$PIC values (y ordinate) plotted against plasma iron concentrations in µgms/100 ml. of plasma (x ordinate) in patients with hypoplastic bone marrows.

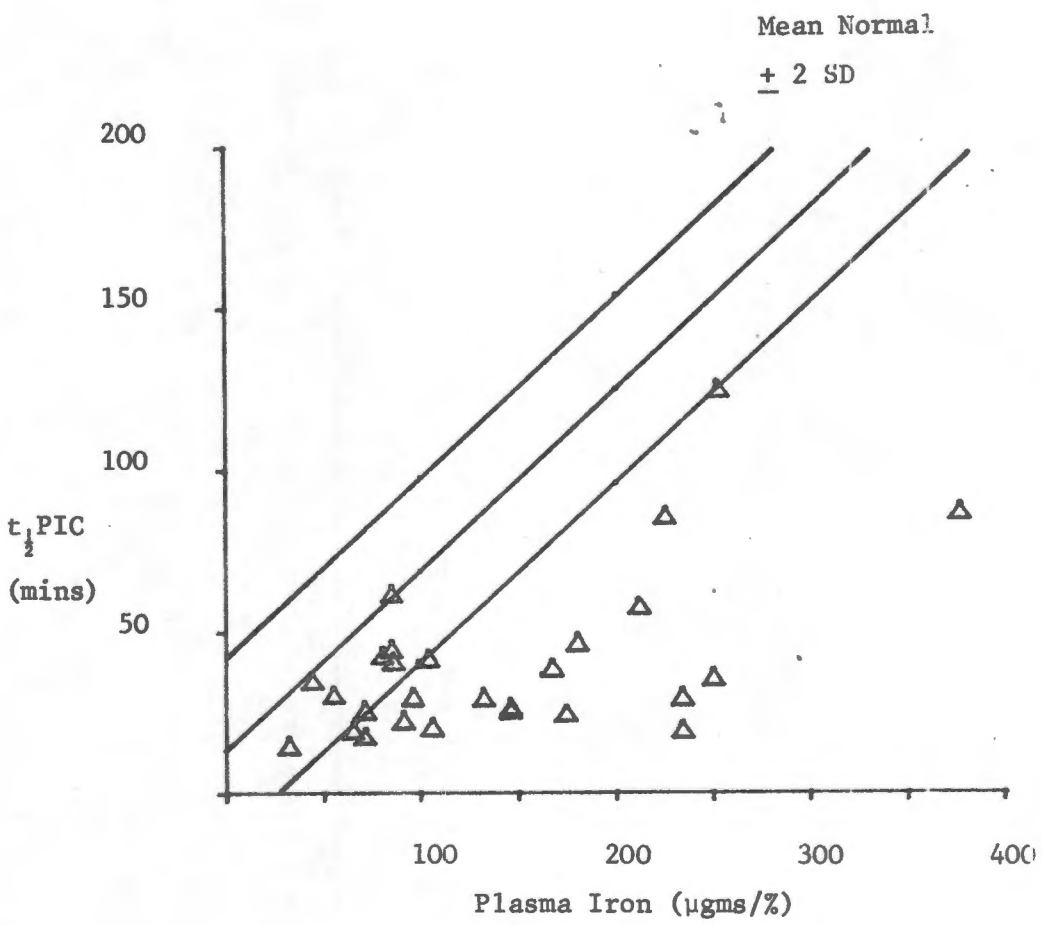


The shaded area represents predicted normal $t_{\frac{1}{2}}$PIC values (as in Figure IV-1) with 95% confidence limits. Plotted points represent the results in the hypoplastic patients with hypoplastic bone marrow.

Table IV-3 Composite Ferrokinetic Findings in Patients with Hyperplastic Bone Marrows.

Patients	Hb	Hct	Bl.Vol ml/kg	Pl. Vol. ml/kg	SI	TIBC	%Sat	$t_{\frac{1}{2}}$	PIC	EI	PIT	RBCU	$t_{\frac{1}{2}}$ Cr^{51}	Diagnosis
Mte	9.6	30	86	63	132	229	58	29	3.00	3.2954	22	6		Myelofibrosis
Kot	10.3	33	77	61	234	264	89	19	7.54	8.5767	27	14		di Gugliemos
Jac	9.0	32	83	59	146	203	72	26	3.65	3.9622	34	15		Thallassaemia
Rob	5.2	24	-	-	180	189	96	46	2.47	3.0490	23	9		Myelofibrosis
Isr	9.4	30	97	69	234	240	98	29	4.94	5.8419	-	-		Thallassaemia
Bot	10.9	33	55	38	253	253	100	124	1.24	1.4209	13	16		Sideroachestic Anaemia
Liv	10.3	29	54	39	225	253	89	85	1.63	1.9408	15	16		Lymphosarcoma
Mar	9.0	27	70	54	91	264	34	22	2.93	3.1089	28	14		Myelofibrosis
Ism	6.9	21	83	67	250	239	100	35	4.34	5.7629	-	-		Thallassaemia
Sil	9.6	30	66	48	55	299	18	30	1.49	1.3273	76	12		Ca.Liver
Lou	13.7	36	65	42	85	185	46	44	1.39	1.2920	77	18		Hered. Spherocytosis
Han	8.4	25	108	57	71	230	31	17	3.14	3.2159	58	13		Myelofibrosis
vAa	12.0	35	-	-	106	-	-	20	3.64	3.5934	-	20		AIHA
Sto	11.7	28	71	52	146	281	52	25	3.79	4.3356	31	10		Hered.Spherocytosis
Bor	7.7	18	65	52	86	295	29	40	1.54	1.7940	94	10		AIHA
Kin	10.4	33	83	64	32	369	9	14	2.28	1.5918	-	15		CML
Bro	10.4	28	73	54	104	228	46	41	1.75	1.8832	70	17		?
Jac	8.8	25	88	64	174	189	92	24	4.59	5.5825	32	11		Thallassaemia
vNi	14.4	38	74	50	212	277	77	57	2.30	2.4190	37	14		Lymphoma
Sev	14.0	44	-	-	71	230	31	25	2.14	1.6904	62	17		Folate def.
Hoe	13.9	40	-	-	65	269	24	19	2.64	2.1621	47	26		AIHA
Jan	7.9	24	59	46	96	279	34	29	2.32	2.5794	46	24		B_{12} def.
Rob	9.1	28	72	59	80	249	32	42	1.39	1.4141	100	11		Myelofibrosis
Ste	10.0	30	86	63	167	212	79	38	2.80	3.1818	-	17		AIHA
Wil	9.2	30	77	34	44	200	22	34	1.13	.9369	-	-		Valve HA
Koc	8.6	25	89	66	85	246	35	61	1.00	1.0730	29	16		AIHA
Ber	6.3	18	56	40	377	425	89	86	2.58	3.6578	9	9		Sideroachrestic Anaemia

Figure IV-4 Graphical Representation of $t_{\frac{1}{2}}$PIC values (y ordinate) plotted against plasma iron concentrations in µgms/100ml. of plasma (x ordinate) in patients with hyperplastic bone marrows.



The shaded area represents predicted normal $t_{\frac{1}{2}}$PIC values (as in Figure IV-1) with 95% confidence limits.

Plotted points represent the results in the hyperplastic patients with hyperplastic bone marrows.

Discussion

The hypoplastic group of patients all demonstrated marked prolongation of the $t_{\frac{1}{2}}$PIC relative to normal values at the same plasma iron concentrations. The E.I. values ranged between 0.34 and 0.69 and gave a good indication of the decrease in total erythropoietic activity.

The reverse occurred in the 'hyperplastic' group of patients where the $t_{\frac{1}{2}}$PIC, in most cases, was markedly decreased relative to predicted normal $t_{\frac{1}{2}}$PIC values for the same plasma iron concentrations. Once again, the E.I. values, ranging between 1.00 and 7.54 gave a clear indication of the increase in total erythropoietic activity. This was not as well demonstrated at low plasma iron concentrations as it was at higher plasma iron concentrations. This is probably explained by the greater avidity of the normal bone marrow for iron at low plasma iron concentrations.

From this study, it appears justifiable to use the E.I. value as an index of total erythropoietic activity. The figure obtained appears to show, reasonably accurately, the extent of change of erythropoietic activity for an individual compared with the range of normal patients, and expresses the change in what appears to be a reasonably quantitative manner.

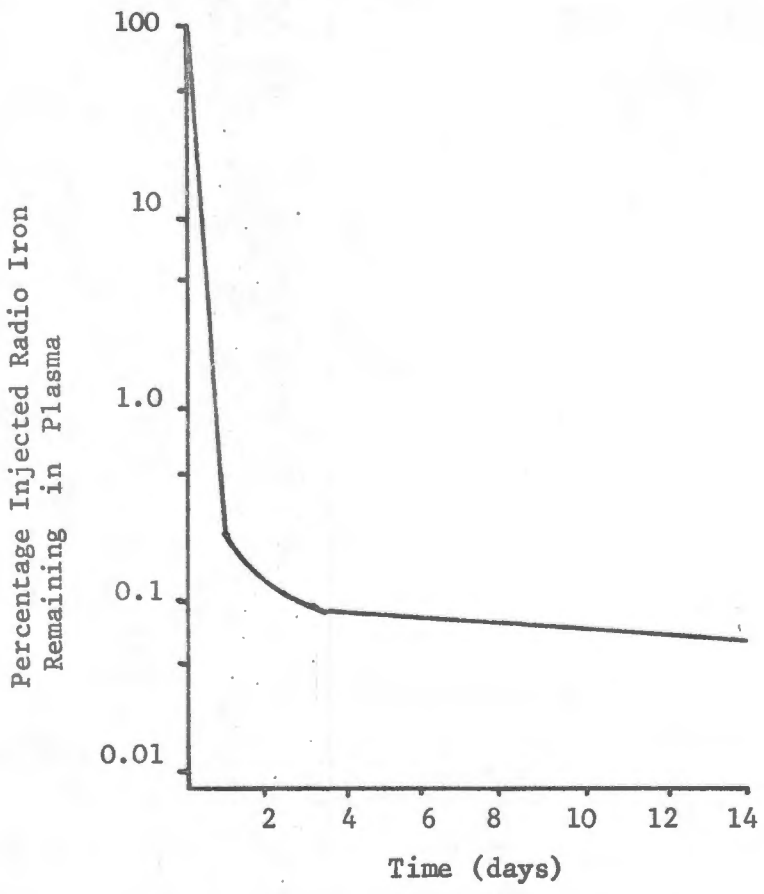
IV-2-g Prolonged Plasma Iron Clearance Curves.

A number of authors have based models for quantitating erythropoiesis from ferrokinetic data upon a study of plasma radio-iron clearance curves over a prolonged period of time.(550)(864)(399)

(258)(164)(686)(288) By this technique, plasma is repeatedly sampled over a period of approximately fourteen days, and the percentage of radio-iron injected that remains in the plasma, bound to transferrin, is calculated. We attempted to duplicate this technique in a number of patients, in an attempt to obtain meaningful data. Our attempts were largely unsuccessful, and a number of reasons may be given for this.

The concentration of transferrin bound radio-iron in the plasma is initially high, but with the passage of time, the radio-iron gets taken up at its various sites of deposition, and the concentration progressively decreases. All workers seem to agree that the initial clearance of radio-iron from the plasma is a single exponential function, but as time progresses the line becomes a curve when plotted upon semi-logarithmic paper. This is best illustrated diagrammatically as in Figure IV-5.

Figure IV-5 Theoretical changes in plasma radio-iron concentration as a function of time.



The resultant curve can be mathematically described in terms of a number of exponential functions or by probability analysis and integro-differential equations. Initial clearance of radioactivity from the plasma is extremely rapid. Subsequent points, as quoted by the various authors mentioned, are all taken at times where the residual plasma radioactivity is approximately 0.1% of the initial concentration.

Where 4 ml. samples of plasma are being counted, one can calculate the amount of radioactivity that this represents. If we assume that the average plasma volume is 2.5 litres, for each microcurie of injected radio-iron, the amount of radioactivity being counted at t_o will be:-

$$3.7 \times \frac{10^{10}}{10^6} \times \frac{4}{2500} \quad \text{disintegrations/second (dps)}$$

where $1\ \mu c = 3.7 \times \frac{10^{10}}{10^6}$ disintegrations/second

This equals 59.2 dps at t_o.

By the time that residual radioactivity is down to 0.1% of the t_o value, the count rate will be:-

$$59.2 \times \frac{1}{10^3} \text{ dps} = 0.592 \text{ dps}$$

Normal counting efficiency for ^{59}Fe, as quoted by Hosain et al[399] is $\pm$ 25%. Thus each microcurie injected would give a count rate of:-

$$59.2 \times \frac{1}{10^3} \times \frac{25}{10^2} \quad \text{cps}$$

$$= 1.405 \times 10^{-3} \quad \text{cps}$$

In their experiments, the dose quoted was 0.5μc per kg. which represents 35μc for a 70 kg. man. This would give a count rate, after day one, of:-

$$35 \times 1.405 \times 10^{-3} \quad \text{cps}$$

$$\text{or} \quad 35 \times 1.405 \times 10^{-3} \quad \text{cpm}$$

$$= 2.9505 \times 10 \quad \text{cpm} \quad (\pm \ 30 \text{ cpm})$$

They counted each sample with a background count of 220 cpm. for a total of 10,000 counts on each sample, this being done in duplicate. The ratio of sample counts to background counts is therefore 30 : 270 or 1 : 7, with a total count rate of 250 cpm. Each sample would therefore need to be counted for $\frac{10,000}{250}$ minutes to give a total count rate of 10,000, which is approximately 40 minutes. In practice, most samples would be counted at the end of the fourteen day period, when only 80% of the initially injected radioactivity would be present due to decay ($t_{\frac{1}{2}} Fe^{59}$ = 45 days), so that this period would probably be slightly longer with an even less favourable ratio of sample to background counts.

In a paper published by Loeringer and Berman in 1951, a method for calculating the intrinsic error of low counting samples was proposed.(522) According to them, the percentage error of a

sample count with a confidence limit of 68% is expressed by the equation:-

$$N_t = \frac{(100)}{V_s} \cdot \frac{\sqrt{r} + 1}{(1-r)^2} \cdot r^{3/2}$$

where N_t = total counts (10^4 in this case)

V_s = percentage error of sample count with 68% confidence limits

$r = \frac{R_t}{B_b} = \frac{\text{total count rate}}{\text{background count rate}}$ (= 1.08 in this case)

By this calculation, $V_s = \pm 18\%$ for a confidence limit of 68%. To get a 95% confidence limit on the count rate as described, V_s will be $\pm$ 2 x 18% i.e. $\pm$ 36% or a range of 72% around the experimental point.

These statistical considerations indicate, therefore, that there are serious limitations upon the accuracy with which plasma radioactivity can be measured after the first 24 hours following a standard intravenous dose of ^{59}Fe. This fact imposes correspondingly serious limitations upon the valid interpretation of kinetic parameters derived from the fitting of experimental curves to data obtained after the first day.

If this holds when relatively high doses of ^{59}Fe are used (i.e. $\pm$ 35μc), the results must be even more questionable when the dose is scaled down in accordance with the practice of most laboratories. In our technique, we have been counting radio-iron over a narrower 'window' than that quoted by Cook et al,[164] in order to exclude counts due to radioactive chromium. This is because nearly all of our patients have had double isotope

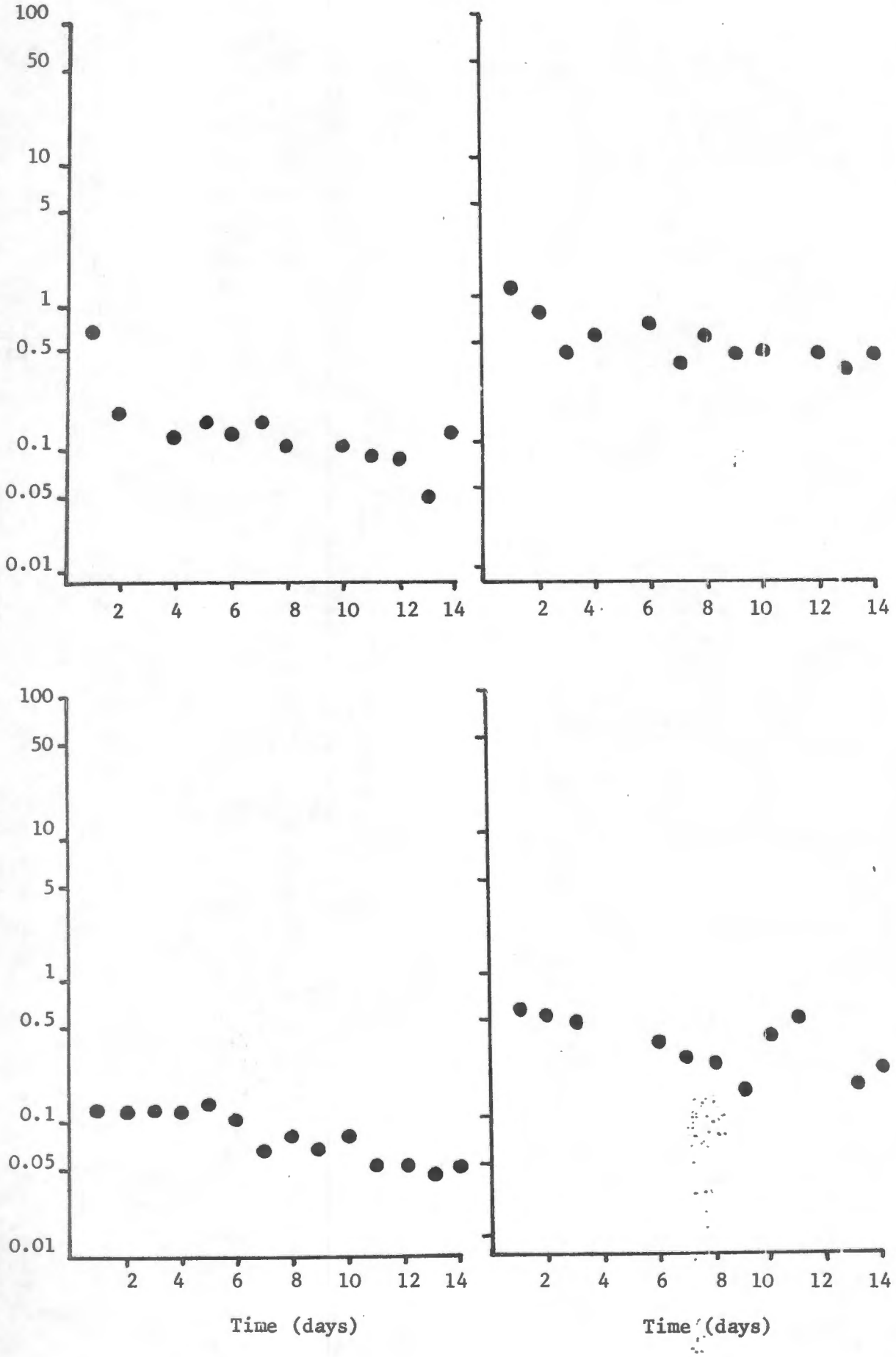
studies, in order to accurately measure red cell volume and in an attempt to assess red cell survival. For this reason, our counting efficiency for radio-iron is less than that of the above authors, as is our count rate. We have an average background count rate of 60 cpm with a sample count rate, after day one, of between 0 and 10 cpm. Our ratio of sample to background counting rate is accordingly much the same as that quoted by other authors, but we would have to count for much longer periods in order to get counts of 10,000 i.e. $\pm$ 250 minutes per sample. This has just not been practical with our limited facilities and pressure on our counter. Because of this, we have not been able to count our plasma samples as accurately as we would wish to, and the resultant curves are accordingly poor. In discussion with Cook, we have realised that this problem is not peculiar to our department, and we have now discontinued the practice of counting plasma samples after day one.

It is clear that the derivation of meaningful data from prolonged plasma iron clearance curves is difficult, and even when these points are corrected for a standardised plasma iron level, as recommended by Hozain et al[399] the fitting of meaningful curves is hazardous. This applies particularly in our experiments where the counting conditions were so unsatisfactory, after day 0.

Some curves were, however, derived. In general these all showed deviation from a single exponential function within a few hours of injection. I will not dwell upon any results in the measurement of this parameter as I believe them to be highly

suspect, but a few curves have been demonstrated.

(Figures IV-6, IV-7, IV-8, IV-9)



I must stress that in some subjects, usually patients with severe iron deficiency, we were unable to demonstrate any radioactive iron in the plasma after day one.(207) My experimental results are set out in Tables IV-4 and IV-5. I believe that it is important to scan the results from left to right in order to see how great the variation is in day to day sample counts above background. The accuracy of least squares fitting of an exponential function to these points is obviously poor, and even "eyeball" fitting gives unsatisfactory results.

Table IV-4 Percentage of initially injected radioactivity remaining in the plasma as a function of time.

	% Initially injected ^{59}Fe activity remaining in Plasma														
Patient	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Day
Gog	1.09	0.73	0.44	0.55	0.73		0.39	0.54	0.45	0.47			0.32	0.43	
Ser	1.94	0.61	0.58	0.53			0.32	0.37		0.24					
Luk	0.99	0.52	0.90		0.46	0.09		0.39		0.30					
Thy		0.36	0.12	0.21	0.39	0.07	0.51	0.09		0.32					
Bot	0.29	0.02		0.12			0.29	0.29	0.22						
Vlo	0.46	0.55	0.10	0.12	0.19		0	0.19	0.21		0.12				
Bot	1.78	1.27	1.78	1.33	0.76		0.19	0.10	0.33	0.39					
Rus	0.80	0.06	0.15	0.05		0.07	0.15	0.06	0.00		0.23			0.37	
Smi	0.57	0.45	0.26		0.13	0.09	0.26		0.05	0.02			0.02	0.02	
Jac	0.70	0.55	1.13				1.21		0.66		0.39			0.90	
Cup	0	0		0.73	0	0		0.31	0.78		0		0.31		
Car	0		0.27	0	0.53		0	0		0.13		0			
Pep	18.95	6.45	3.28	1.53		1.21	1.06	1.26	0.96	0.64	0.87		0.84		
Nqi	0.62	0.20		0.16	0.19	0.17	0.20	0.11		0.12	0.09	0.08	0.05	0.14	
Loz	1.32	1.31	1.44	1.41	1.58	1.03	0.63	0.76	0.58	0.77	0.52	0.50		0.47	
Wal	1.19	0.25	0.22			0.55	1.33	0.55	0.38	0.20					
Tan		0.32	0.17	0.30	0.22		0.19	0.18							
Mat	1.27			0.34	0.17		0.12		0.13						
Pet	0.80	0.04	0.05	0.11	0.01		0.05	0.04	0.05						
Bas	0	0	0.50	0.11	0.24	0.12	0.06		0.07	0.02	0.02		0.02		
Cha	0.30	0.07		0.46	0.42	0.43		0.55	0.57	0.58	0.37	0.60	0.51	0.10	
Lou	0.27	0.16	0.15	0.23		0.28	0.20	0.14	0.22	0.10					

Table IV-5 Actual counts per minute per four ml. plasma sample above background as a function of time.

	Counts per minute above Background/4 ml. sample.													
Patient	1	2	3	4	5	6	7	8	9	10	11	12	13	14 Day
Gog	14.5	9.75	5.90	7.30	9.75		5.20	7.15	5.95	6.25			4.30	5.70
Ser	39.63	12.38	11.88	10.73			6.48	7.43		4.78	2.98			
Luk	0.07	0.04	0.06		0.03	0.01	0	0.03		0.02				
Thy		3.45	1.10	2.00	3.70	0.65	4.85	0.85		3.00				
Bot	4.55	0.35		1.95			4.60	4.60	3.55					
Vlo	4.63	5.54	1.01	1.17	1.92		0	3.70	4.16		2.42			
Bot	14.07	10.07	14.07	10.49	6.03		1.49	0.81	2.58	3.06				
Rus	6.24	0.48	1.20	0.40		0.51	1.20	0.44	0.01		1.79			2.92
Smi	0.37	0.31	0.20		0.09	0.08	0.04		0.00	0.02			0	0.08
Jac	0.02	0.01	0.03				0.03		0.02		0.01			0.02
Cup	0	0		0.05	0	0		0.02	0.05		0		0.02	
Car	0		0.02	0	0.04		0	0		0.01		0		0.01
Nqi	26.89	8.54		6.84	8.34	7.34	8.49	2.69		5.04	3.84	1.94	2.04	5.84
Loz	8.20	14.05	15.40	15.05	16.90	11.00	3.90	8.15	3.60	8.25	3.20	5.40		5.05
Wal	16.84	3.58	3.08			7.72	13.88	7.78	5.42	2.86				
Tan		18.43	9.73	17.03	12.83		6.83	10.33						
Mat	27.13			4.98	4.48		1.93		2.38					
Pet	0	0.28	1.12	0.23	0		3.48	0.83	2.97					
Bas	0	0	4.63	0.99	2.37	1.19	0.63		0.71	0.21	0.09		2.21	
Cha	2.30	0.90		3.55	3.25	3.30		4.20	4.40	4.45	2.85	4.60	3.95	0.75
Lou	6.60	3.95	3.75	5.55		6.95	4.95	3.40	5.45	2.55				

Had our counting time been greater, we would perhaps have been able to derive curves agreeing more closely with those published. In an attempt to increase the statistical accuracy of low level counting, I arranged with Dr. M. Peisach, at the Southern Universities Nuclear Institute, for the analysis of 4 ml samples of plasma taken from a patient with mild haemolytic anaemia (Skelton) using a gamma ray spectrometer with a 3" x 3" NaI(Tl) scintillation detector; gamma ray spectra were recorded on a 400 channel analyser and compared with spectra obtained from distilled water. Under the conditions of this experiement, count rates as low as 2 dpm would have been observed. No counts were observed within the statistical validity of the method, even when samples were counted individually for up to 12 hours.(207) Using the criteria that photopeak counts in excess of three times the standard deviation of the background count would have been measureable, the content of radioactivity in all samples submitted was calculated to be less than 4 dpm. Subsequent to this we applied to the South African Nuclear Institute for permission to use larger doses of ^{59}Fe (up to this time we had been using maximum doses of 10 μc) and received permission to use up to 25 μc per patient. Although this gave us higher count rates, the accuracy of our late points on the plasma iron clearance curve was still dubious.

Even without good data on delayed plasma iron clearance curves we found deviation from a single exponential clearance function within a few hours of injection of radio-iron labelled transferrin.

The sources of the changed slope appears to be multiple, as detailed by Cook [164]

i.e.

(1) recycling of transferrin from extravascular extracellular fluids (i.e. lymphatics, ascites etc.) back into the plasma.

(2) recycling of iron from ineffective erythropoiesis

(3) return to the plasma of radio iron taken up initially by parenchymatous iron stores.

It appears likely that the initial deviation in the first portion of the plasma iron clearance curve is produced by re-entry into the plasma of labelled transferrin from lymphatics and other extracellular, extravascular fluids.[258][686][399][164] The subsequent curve appears to be influenced by feedback of radioiron into the plasma from ineffective erythropoiesis.[164][288] The subsequent single exponential fall off in plasma radioactivity appears to represent equilibration between plasma and storage iron, with gradual loss of iron from the body.

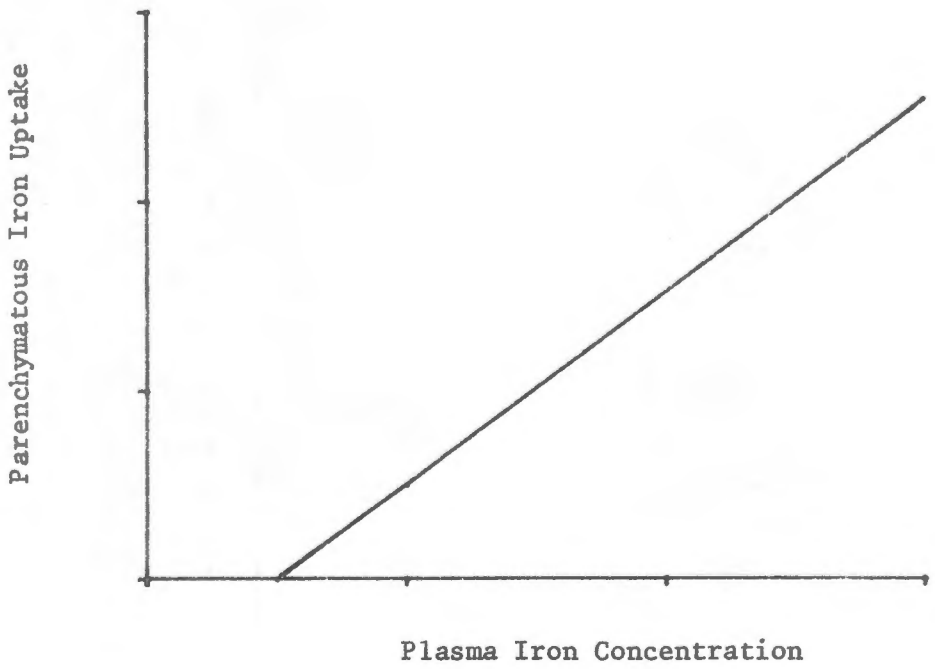
IV-3 Non Erythroid Iron Uptake.

IV-3-a Theoretical considerations.

It would, theoretically, seem reasonable to propose that non erythroid iron uptake does not occur in severe iron deficiency, since repeated phlebotomy to render a patient iron deficient has been shown to remove iron from body stores[384][211][360][61][415][252][156] In addition, patients with severe iron deficiency do not take up radioactivity into storage sites as

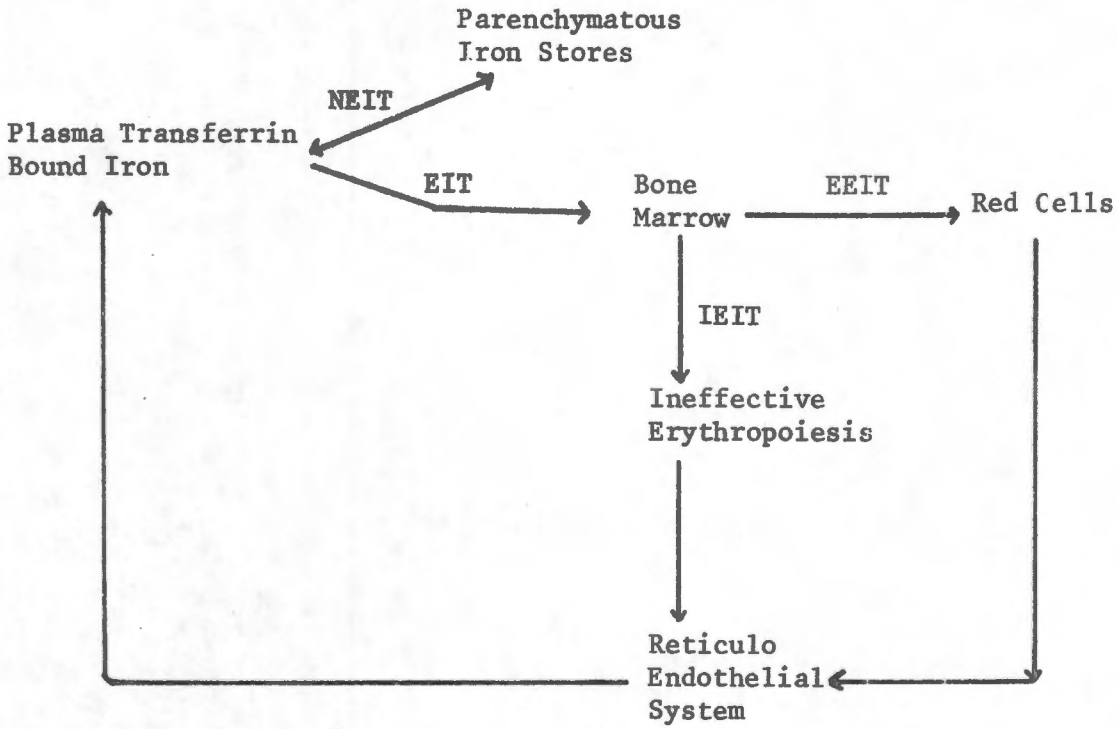
measured by external counting and have absent iron stores as assessed by histological techniques.(252)(61)(387)(130)(698)(815)(415) Patients with iron overloaded states however, show fairly marked uptake of radioactive iron from intravenously injected transferrin labelled with radio-iron, this uptake being predominantly into liver parenchymal cells. From this, one intuitively supposes that parenchymatous iron uptake is determined, to a certain extent, by the plasma iron concentration. It will be low or absent where iron deficiency exists and will probably increase as the plasma iron concentration increases. This is represented diagrammatically in Figure IV-10.

Figure IV-10. Diagrammatic Representation of Postulated Parenchymatous Iron Uptake as a function of Plasma Iron Concentration.



IV-3-b I have noticed, empirically, that as the plasma iron concentration increases, in normal patients, the percentage of injected radio-iron incorporated into circulating red cells appears to decrease. This would mean, according to our postulated model of iron kinetics as detailed in the review section, that as the plasma iron concentration increases, more iron goes to sites other than the bone marrow, providing that the fraction of erythropoietic activity devoted to ineffective haemoglobin formation remains constant as the plasma iron concentration increases. The only other site to which this radio-iron can go is the parenchymatous iron stores (See Figure IV-11)

Figure IV-11 Graphical Representation of Internal Iron Kinetics demonstrating the possible routes of distribution of transferrin iron.



NEIT = Non Erythroid Iron Turnover

EIT = Erythroid Iron Turnover

IEIT = Ineffective Erythroid Iron Turnover

EEIT = Effective Erythroid Iron Turnover

IV-3-c There appears to be no reason why the percentage of ineffectively formed haemoglobin should vary with changes in plasma iron concentration. On this basis, I decided to adopt an empiric approach in an attempt to quantitate non erythroid iron uptake. The normal patients previously described (Table IV-1) were studied. Measurements of red blood cell utilisation (RBCU) were made, as detailed under methods. It was assumed that non erythroid iron uptake (NEI uptake) was represented by the percentage of injected radio-iron not getting into the circulating red cells by day 14. (i.e. 100-RBCU%). The RBCU was plotted against plasma iron concentration per 100 ml. of plasma and against plasma iron concentration per 100 ml. of whole blood calculated as previously described.(IV-2-a)

IV-3-d We have postulated, (in paragraph IV-3-a), that non erythroid iron uptake would be zero at low plasma iron concentrations, and would increase in proportion to increase in the plasma iron concentration.(Figure IV-10) Some experimental evidence exists to show that bone marrow erythropoietic activity becomes limited by inadequate iron supply at plasma iron concentrations less than 70 µgms%[385][609] and we have plotted RBCU against plasma iron concentrations regardless of plasma iron level, and against plasma iron levels of more than 70 µgms/100 ml. plasma or more than 40 µgms/100 ml. whole blood in an attempt to see whether a better fit for the data could be obtained using a 'cut off' point for plasma iron concentration. The results are summarised in Figures IV-12 - IV-15.

Figure IV-12 : RBCU plotted against plasma iron concentration/100 ml. plasma in all patients.

Least squares regression equation.

RBCU% = - 0.232 (PI/100 ml) + 104.422

r = 0.8898

SE^{y}/x = 6.8432

(100 - RBCU)% = 0.232 (PI/100 ml) - 4.422

Equation 1.

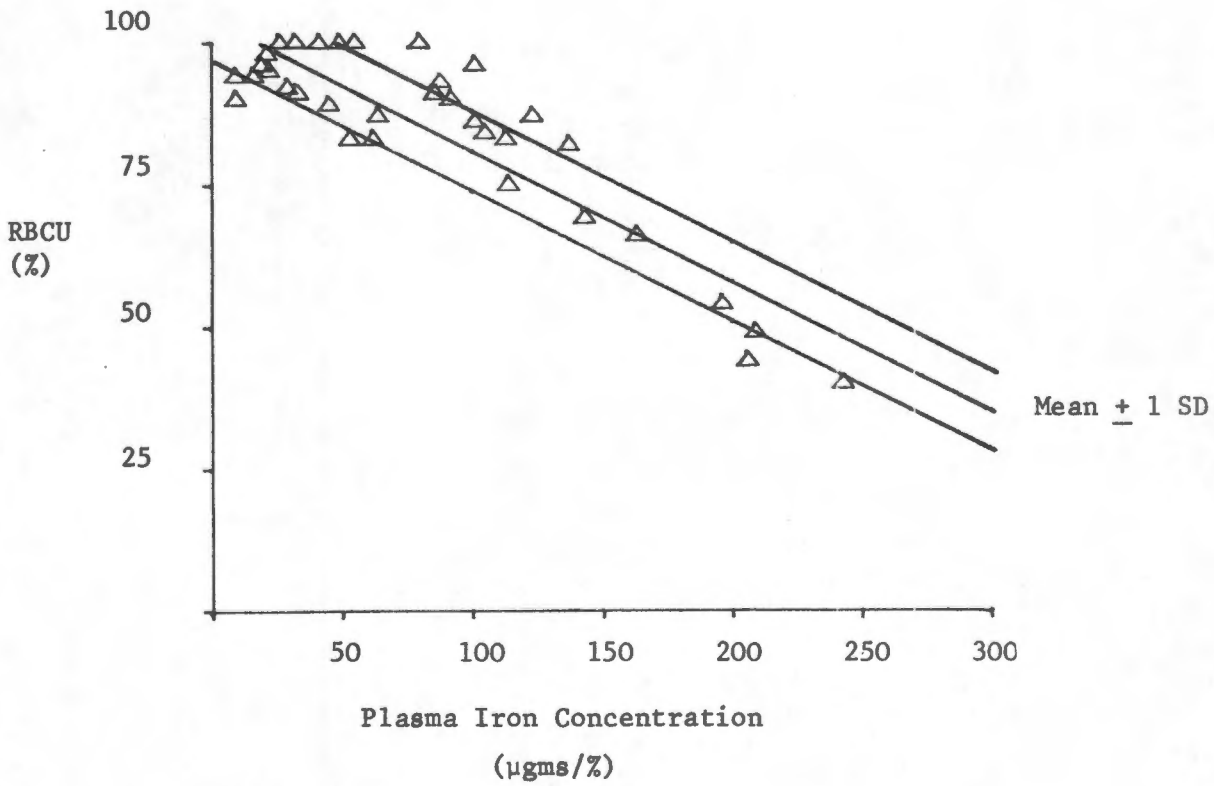


Figure IV-13 : RBCU plotted against plasma iron concentration/100 ml. plasma in patients with plasma iron concentrations exceeding 70 µgms %.

Least squares regression equation:

RBCU % = - .3654 (P.I./100 ml) + 125.2567

r = 0.9709

SE^{y}/x = 4.4019

(100 - RBCU)% = 0.3654(P.I./100ml) - 25.2567

Equation 2.

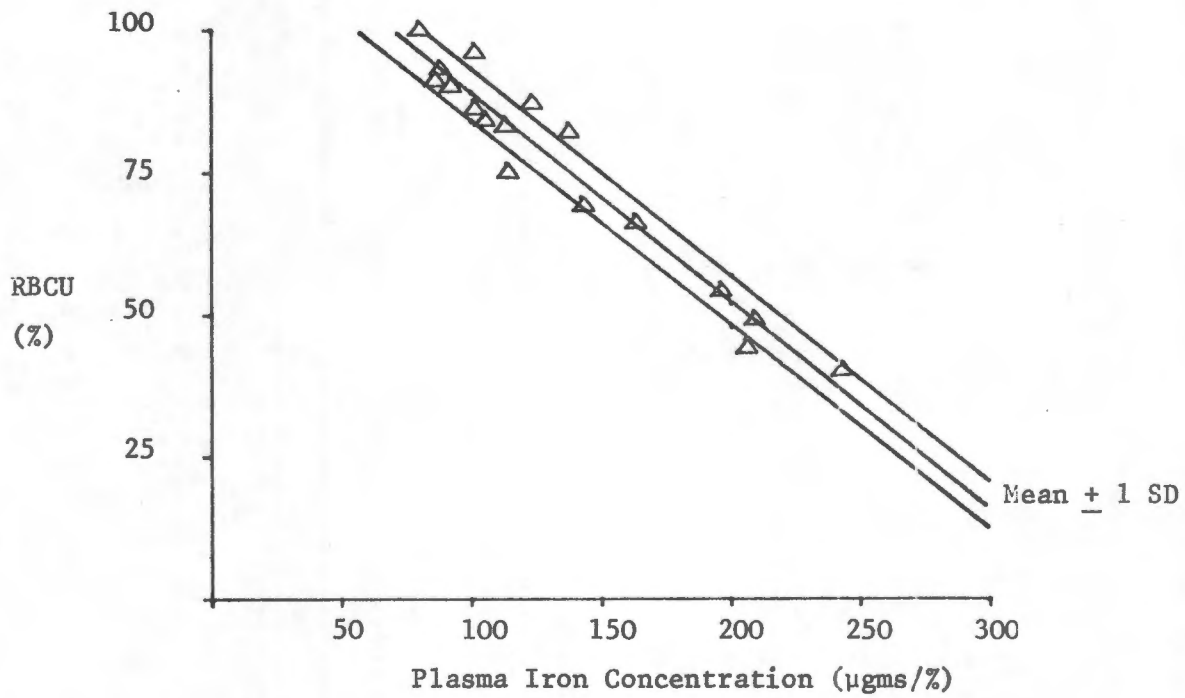


Figure IV-14: RBCU plotted against plasma iron concentration/100 ml. whole blood in all patients.

Least squares regression equation:

RBCU % = -.3633(P.I./100 ml wb) + 103.5096

r = 0.8640

SE $^{y}/_{x}$ = 11.22

(100 - RBCU)% = 0.3633(P.I./100 ml. wb) - 3.5096

Equation 3.

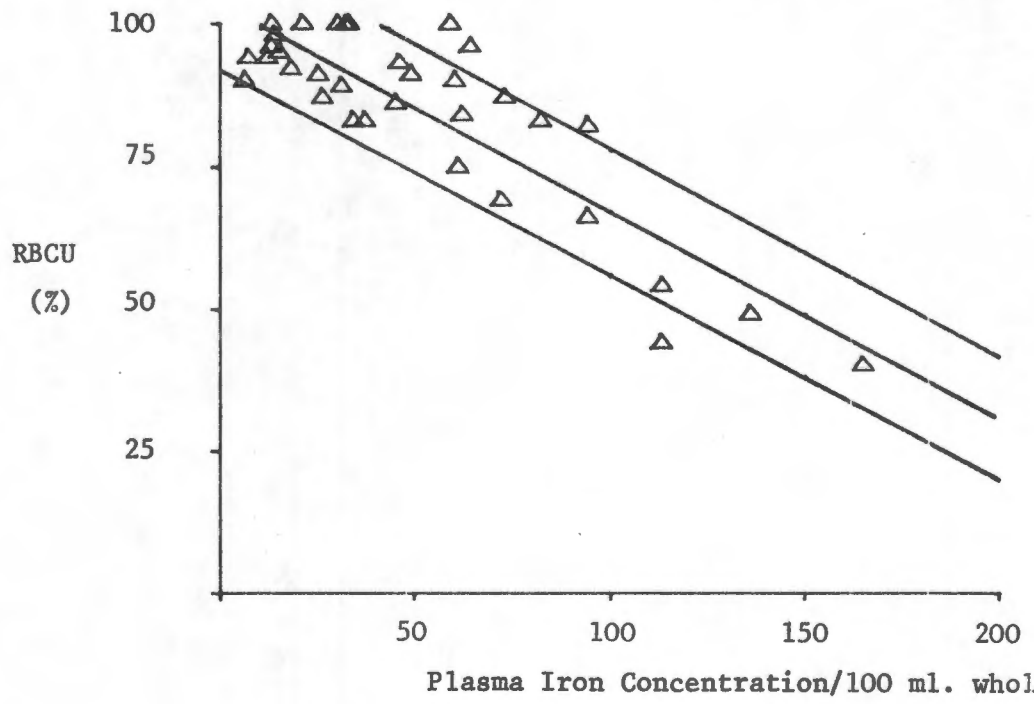


Figure IV-15 : RBCU plotted against plasma iron concentration/100 ml. whole blood in patients with plasma iron concentrations exceeding 40 µgms/100 ml. whole blood.

Least squares regression equation:

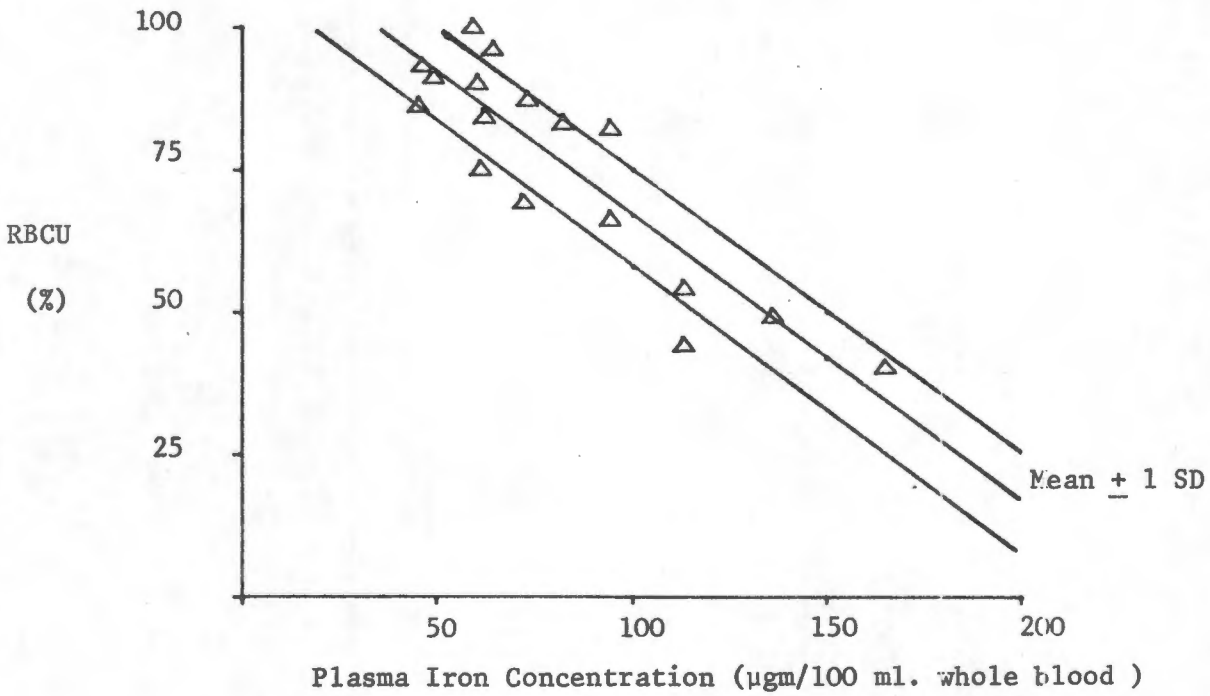
RBCU% = -0.4996(P.I./100 ml. wb) + 116.7352

SE $_{y/x}$ = 8.4857

r = 0.8875

(100 - RBCU)% = 0.4996(P.I./100 ml.wb) - 16.7352

Equation 4.



IV-3-e As can be seen from these graphs, the RBCU values decreased as plasma iron concentrations increased. This decrease was linearly related to the plasma iron concentration and could be fitted by a regression equation, with good correlation between the two parameters. This tended to support my theoretical postulate that as the plasma iron concentration increased, the amount of iron going to non erythroid sites increased proportionately. From the graphs, it can be seen that the best correlation between RBCU and plasma iron value is in Figure IV-13, where r = 0.9709, suggesting that non erythroid iron uptake probably only starts at plasma iron concentrations of approximately 70 µgms%.

IV-3-f By extrapolation from this data, the percentage of iron going to non erythroid sites can be expressed as 100 - RBCU%. When these points for individual patients are plotted against the plasma iron parameters described (IV-3-d), four equations describing the resultant least squares fit regression equation can be derived (See Figures IV-12 to IV - 15). Using these equations numbered 1 - 4, one would be able to calculate the non erythroid iron turnover, because the fraction of iron being taken up by non erythroid sites would be the same fraction of the plasma iron turnover. Expressed mathematically this would be:-

$$NEIT = (100 - RBCU)\% \times PIT$$

where NEIT = Non erythroid iron turnover

PIT = Plasma iron turnover.

It seems reasonable to me to express some reservations about this empiric method of calculating NEIT values.

This approach obviously required accurate measurement of the RBCU and plasma iron. Since the scatter of my data is relatively small, and the correlation between RBCU and plasma iron concentration good, I felt justified in presuming that this requirement had been met. I am also aware that I have assumed a constant rate of ineffective erythropoiesis, independent of plasma iron concentration. This is not a strictly justifiable assumption, since ineffective erythropoiesis has been shown to increase in iron deficiency.[106] [164] [258] [687] [688] [727] [766] This probably explains why the fit for the data is best where plasma iron cut off points are used. I am aware that ineffective erythropoiesis and variations in red cell survival affect the RBCU but have no reason to believe that red cell lifespan was sufficiently abnormal in any of these patients to influence the RBCU. There is no obvious reason, therefore, for believing that the variation in RBCU with changes in plasma iron concentration was due to any factor other than change in NEIT.

IV-3-g The non-erythroid iron uptake is probably relatively unaffected by changes in parenchymal store avidity for transferrin iron. My data, and that of Cook[164] would suggest that any variation in NEIT between individuals depends more upon variations in plasma iron concentration than upon any other factor, and I feel that the dependence of NEIT upon plasma iron concentration is reasonably accurately described by the regression equation derived from my experimental data. (Figure IV-12 to IV-15).

Cook,[164] using a "reflux" model, defined the relationship between NEIT and plasma iron concentration by the following equation.

$$\text{NEIT (mg/100 ml w.b/day)} = .0035 \times \text{P.I. } (\mu g/100 \text{ ml.wb})$$

$$r = 0.873$$

As his was the only study I knew of in which any attempt had been made to measure NEIT, I have compared the values for NEIT in my patients calculated by my analytical technique with corresponding values obtained by substituting Cook's equation. The results are compared in Table IV-6. It can be seen that at low plasma iron values Cook's formula gives slightly higher values for NEIT than do my equations, the opposite being the case at higher plasma iron concentrations.

IV-3-h Acting on the assumption that the preferred analytical technique for defining the relationship between plasma iron and NEIT would be that giving the highest degree of correlation between these values, I examined the nature of the relationship when NEIT is calculated by my method or Cook's, and expressed as a function of plasma iron or the iron content of whole blood. The resultant regression lines are shown in Figures IV-16 and IV-17.

Table IV-6 NEIT Values Derived by Different Methods.

Patient	N.E.I.T.(mg/100 ml wb/day)					PI
	Cook	French				(mg/100
		1	2	3	4	ml.wb)
deB	.1312	.0622	*	.0630	*	37
Web	.1601	.0807	.0348	.0674	.0313	46
Luk	.4758	.4470	.5185	.4650	.5192	136
Cup	.2863	.3364	.2475	.4096	.3726	82
Car	.1206	.0776	*	.0887	*	34
Hey	.0921	.0980	*	.0581	*	26
Bot	.2118	.1904	.1418	.1598	.1167	61
Rus	.1141	.0356	*	.0430	*	33
Kho	.1128	.0521	*	.0518	*	32
Thy	.2187	.1466	.0964	.1411	.1065	62
Arm	.2094	.1634	.0807	.1759	.1270	60
Sch	.0942	.0019	*	.0066	*	14
Bas	.0248	*	*	*	*	7
Hoo	.0411	*	*	.0030	*	11
Cha	.0445	*	*	.0018	*	12
Loz	.0217	*	*	*	*	6
Gog	.3957	.5156	.5823	.4720	.4993	113
OCo	.5766	.4076	.4984	.4420	.5145	164
vWy	.3295	.2994	.2714	.3357	.3317	94
Pet	.0623	.0080	*	.0103	*	18
Mgo	.3291	.2361	.2425	.2167	.2137	99
Tan	.2234	.1366	.0831	.1414	.1180	64
Wes	.2519	.2796	.2625	.2200	.1868	72
Nqw	.3960	.4854	.5603	.4209	.4457	113
Mar	.1584	.1654	.1014	.1125	.0511	45
Ans	.0728	.0208	*	.0281	*	21
Wil	.0536	.0052	*	.0156	*	15
Pre	.1708	.2230	.0885	.2044	.1099	49
Sam	.2562	.2894	.2365	.2170	.2381	73
Jeg	.1082	.0564	*	.0724	*	31
Pax	.1052	.0283	*	.0414	*	30
Rob	.2079	.1999	.0562	.2553	.1829	59
Tay	.0458	.0124	*	.0096	*	13
Sch	.0819	.0262	*	.0455	*	25

* The points represent zero values for NEIT, due to plasma iron values being markedly depressed.

Figure IV-16 : Comparison of least square fits for NEIT plotted against plasma iron concentration using different formulas to calculate NEIT (see text).

Cook: $y = 0.0021x + .0005$ ($r = 0.9811$)

French (1) $y = 0.0024x + .0521$ ($r = 0.9410$)

(2) $y = 0.0035x - .2354$ ($r = 0.9445$)

(3) $y = 0.0022x - .0431$ ($r = 0.9161$)

(4) $y = 0.0029x - .1418$ ($r = 0.8132$)

y = NEIT (mg/100 ml wb/day)

x = Plasma Iron Concentration (µg%)

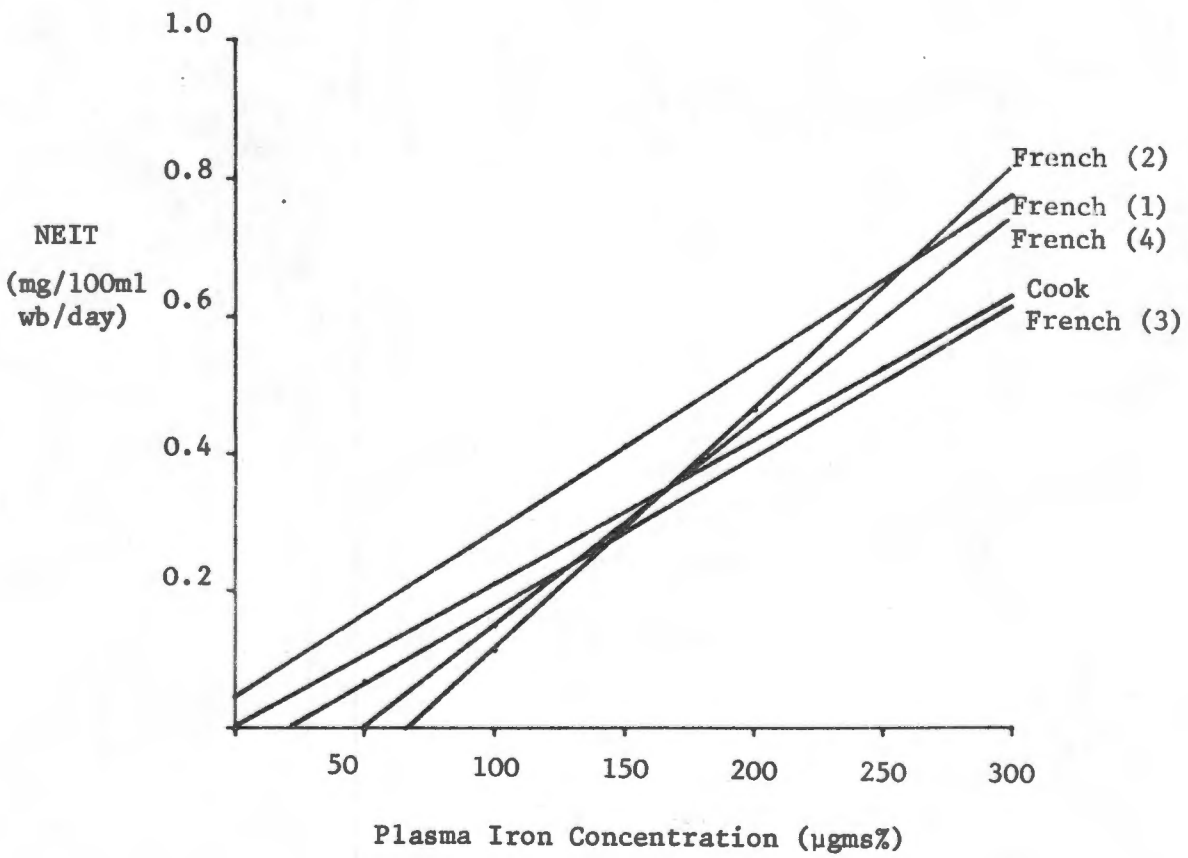


Figure IV - 17 : Comparison of least squares fits for NEIT plotted against plasma iron concentration per 100 ml. whole blood using different methods to calculate NEIT (see text).

Cook : $y = 0.0035\,x$ ($r = 0.873$)

French (1) $y = 0.0031x - 0.0421$ ($r = 0.9218$)

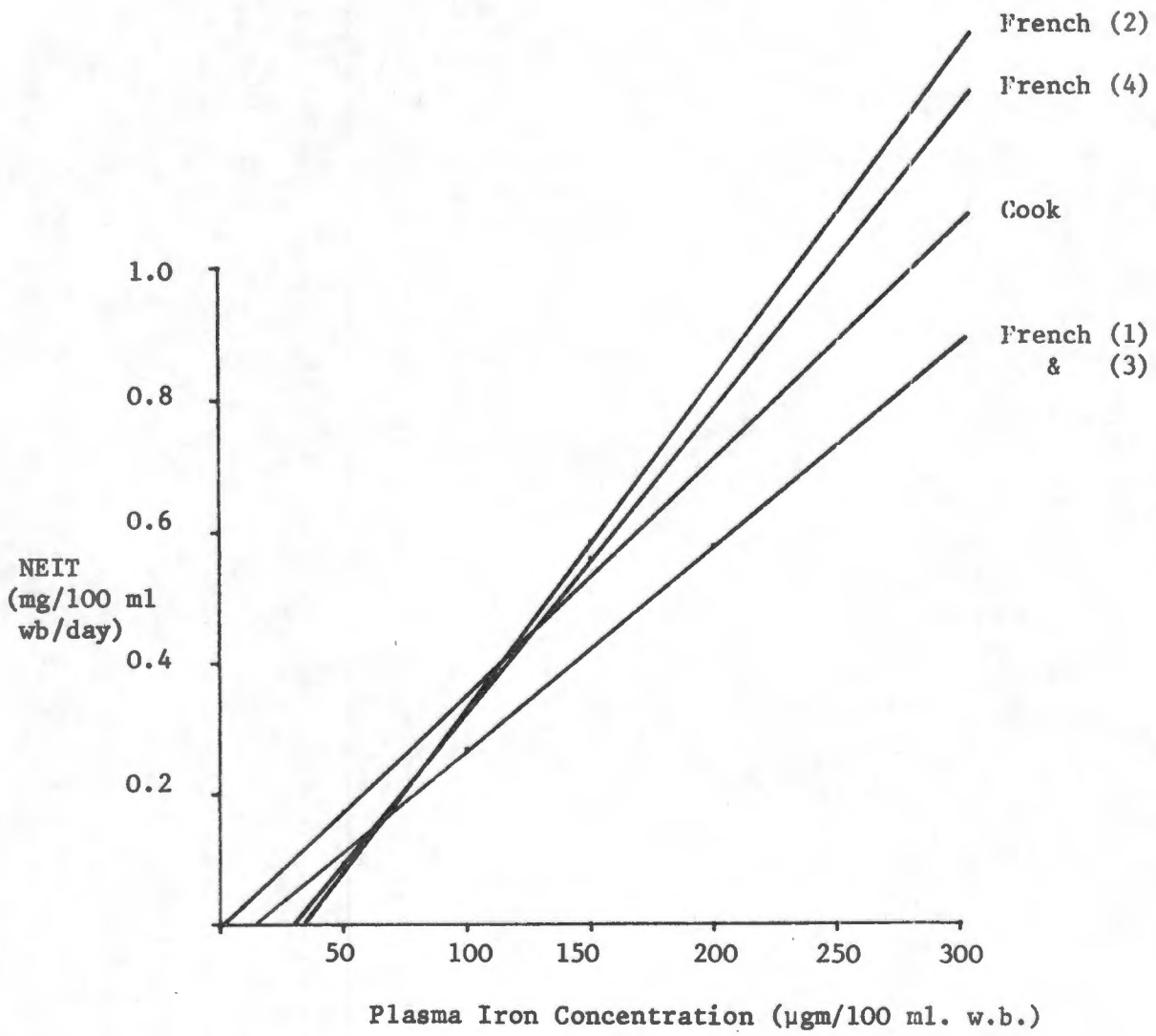
(2) $y = 0.0050x - 0.1651$ ($r = 0.8901$)

(3) $y = 0.0031x - 0.0431$ ($r = 0.9382$)

(4) $y = 0.0046x - 0.1315$ ($r = 0.9203$)

y = NEIT (mg/100 ml wb/day)

x = plasma iron/100 ml wb (µg/100 ml wb)



From simple inspection of these figures, it can be seen that values for the NEIT tend to be lower when calculated by my method at low plasma iron concentrations, and higher at high plasma iron concentrations. This is particularly noticeable when 'cut off' points are used. It is of interest that the slopes and equations derived using my method, when no 'cut off' points are used agree closely with those derived using Cook's equation. (i.e..0021, 0.0024 and 0.0022 when x is plasma iron concentration per 100 ml. of serum, and 0.0035, 0.0031 and 0.0031 when x is plasma iron concentration per 100 ml. of whole blood). It would seem therefore that my analytical techniques yield results that are essentially similar to those obtained when Cook's method is used. In defining a functional relationship between NEIT and plasma iron it seems 'a priori' more physiological to use the concentration of iron to which erythroid and non erythroid iron uptake sites in the body would be exposed. The correlation coefficients obtained using my method were slightly better than those obtained using Cook's method, although both were good.

IV-3-i I now had ten equations describing the relationship between NEIT and plasma and blood iron concentration. I then had to decide which of these seemed physiologically most appropriate. The only way in which I felt that I could do this was by comparing the calculated erythroid iron turnover values (i.e. PIT - NEIT) with predicted EIT values, assuming that each patient had a constant proportion of ineffective erythropoiesis and a normal red cell lifespan. This assumption is probably not entirely valid because the ^{51}Cr method of measuring red cell survival

used by us gives a relatively crude estimate of red cell survival, and, as previously described, some variation between individuals with regard to the percentage of their total erythropoietic activity that is ineffective is to be expected. For these reasons it was realised that these values would not necessarily correlate well. I, nevertheless, decided to use the best statistical fit between these two values as an indicator of which equation best described non erythroid iron turnover.

IV-3-j Predicted EIT was calculated assuming a red cell lifespan of one hundred days, and assuming that 20% of total erythropoietic activity was ineffective.

$\therefore$ Predicted EIT = $\frac{Hb}{L}$ x 3.4 x 1.2 mg/100 ml. wb/d

where Hb is the haemoglobin concentration/100 ml. wb.

L is the red cell lifespan (100 days)

3.4 represents the amount of iron, in mgms, required for the synthesis of one gram of haemoglobin,

and 1.2 represents a correction factor to correct erythropoietic iron turnover for ineffective erythropoietic activity. This figure was derived by assuming that total erythropoietic activity will be 1.2 x greater than effective erythropoietic activity if the amount of ineffective erythropoiesis is approximately 17% of total erythropoiesis.

Table IV-7 Comparison of indirectly calculated EIT values with ferrokinetically calculated EIT values using ten different equations to calculate NEIT (see text.)

Patient	Ind EIT	Ferrokinetic EIT (P.I.T. - N.E.I.T.)									
		Plasma Iron/100 ml. wb.					Plasma Iron/100 ml. plasma				
		Cook	French 1	French 2	French 3	French 4	Cook	French 1	French 2	French 3	French 4
deB	.6365	.4933	.5836	.5290	.5504	.6022	.4938	.5865	.5312	.5278	.6245
Web	.6610	.3437	.4248	.3717	.4042	.4400	.3191	.3910	.3539	.3453	.4318
Luk	.5222	.5386	.5206	.5545	.6351	.4998	.5750	.5501	.5977	.5649	.5183
Cup	.4202	1.2573	1.2988	1.2840	1.3321	1.2996	1.3058	1.3577	1.3381	1.3245	1.3835
Car	.4937	.8643	.9578	.9005	.9201	.9776	.8731	.9730	.9114	.9098	.9849
Hey	.8282	.8478	.9399	.8856	.9004	.9399	.8050	.8961	.8422	.8384	.9399
Bot	.7018	.6526	.7175	.6836	.7189	.7269	.6245	.6756	.6567	.6429	.7008
Rus	.4855	.3974	.4929	.4339	.4526	.5121	.4087	.5118	.4474	.4466	.5121
Kho	.6365	.5192	.6152	.5558	.5742	.6320	.5160	.6143	.5541	.5521	.6320
Thy	.5998	.5165	.5792	.5471	.5836	.5878	.5142	.5725	.5473	.5353	.6031
Arm	.5141	.7563	.8220	.7874	.8223	.8316	.7720	.8407	.8064	.7970	.8791
Sch	.4488	.3639	.4131	.4042	.4117	.4131	.3685	.4131	.4100	.4131	.4131
Bas	.3305	.4187	.4435	.4435	.4435	.4435	.4241	.4435	.4435	.4435	.4435
Hoo	.4039	.3634	.4045	.4042	.4045	.4045	.3704	.4045	.4045	.4045	.4045
Cha	.4447	.6615	.7060	.7021	.7060	.7060	.6656	.7060	.7060	.7060	.7060
Loz	.5386	.7509	.7720	.7720	.7720	.7720	.7526	.7720	.7720	.7720	.7720
Gog	.6854	.8604	.8676	.8809	.9477	.8559	.8440	.8295	.8680	.8378	.8055
oCo	.5345	.2079	.1581	.2180	.3159	.1258	.2737	.2216	.2930	.2534	.1694
vWy	.4570	.7646	.7925	.7889	.8443	.7885	.8059	.8386	.8358	.8174	.8500
Pet	.6691	.2866	.3985	.3262	.3358	.4250	.2875	.4066	.3282	.3314	.4828
Mgo	.6569	.3778	.4059	.4021	.4575	.4019	.3641	.3760	.3914	.3678	.3718
Tan	.5916	.4950	.5563	.5253	.5626	.5643	.5058	.5673	.5393	.5281	.6003
Wes	.7058	.7205	.7729	.7493	.7914	.7777	.6716	.6995	.7009	.6813	.7073
Nqw	.6650	.7242	.7313	.7447	.8116	.7196	.6871	.6646	.7101	.6779	.6346
Mar	.8323	.7118	.7936	.7459	.7720	.8091	.6576	.7191	.6911	.6779	.7521
Ans	.4896	.6210	.7296	.6599	.6714	.6938	.6261	.6938	.6665	.6691	.6938
Wil	.4488	.7077	.8223	.7477	.7559	.7613	.7146	.7613	.7560	.7606	.7613
Pre	.7181	1.2649	1.3427	1.2982	1.3265	1.3567	1.2546	1.3281	1.2896	1.2814	1.3701
Sam	.6569	.9440	.9949	.9724	1.0154	.9993	.9414	.9853	.9727	.9571	1.0051
Seg	.4651	.8289	.9263	.8658	.8833	.9476	.8421	.9371	.8812	.8812	.9371
Pax	.5426	.4509	.5493	.4880	.5050	.5561	.4695	.5561	.5090	.5098	.5561
Rob	.3713	1.2062	1.2724	1.2374	1.2721	1.2822	1.2456	1.3239	1.2812	1.2742	1.3695
Tay	.6691	.7238	.8409	.7643	.7711	.8693	.7145	.7696	.7555	.7593	.7696
Sch	.3509	.7720	.8259	.7601	.7742	.8495	.7401	.8099	.7804	.7828	.8099

IV-3-k Table IV-7 compares, in tabular form, the indirectly calculated EIT values with EIT values derived ferrokinetically by subtracting the calculated NEIT (derived by 10 different equations as previously described) from the PIT. Statistical analysis of the results compared are seen in Table IV-8. It can be seen that the correlation between indirectly calculated EIT and ferrokinetically derived EIT values is very poor. It seemed obvious to me that while we were completely normalising indirectly calculated EIT values by assuming a constant value for red cell lifespan and percentage of ineffective erythropoiesis, no such normalisation of the ferrokinetic data was being attempted. I have previously shown (IV-2-f) that the erythropoietic index can be used as an indicator of variable erythropoietic activity. If one divides the ferrokinetically derived EIT value by the same patient's E.I. value, the ferrokinetic EIT should be normalised for variations in ineffective erythropoiesis and red cell lifespan, as all these patients were in a steady state for the duration of the experiment. Table IV-9 details a statistical comparison of indirectly calculated EIT values compared with ferrokinetically derived EIT values divided by the EI to normalise for variable total erythropoietic activity, produced by changes in red cell lifespan or variable proportions of ineffective erythropoiesis.

Table IV-8 Statistical analysis when comparing indirectly calculated EIT values (x axis) with ferrokinetically derived EIT values (y axis).

Equation	SE^{y}/x	r	P
1. Plasma Iron/100 ml. w.b.			
Cook	.3013	.0221	< .45
French (1)	.3302	.0363	< .40
(2)	.3128	.0206	< .45
(3)	.3242	.0416	< .40
(4)	.3328	.0489	< .35
2. Plasma Iron/100 ml. plasma			
Cook	.3110	.0516	< .35
French (1)	.3454	.0689	< .30
(2)	.3375	.0532	< .35
(3)	.3102	.0663	< .35
(4)	.3454	.0163	< .45

IV-3-1 When this is done, i.e. the ferrokinetic EIT is corrected for variable erythropoietic activity, the correlation between the indirectly calculated EIT and the ferrokinetic EIT improves. However, there are exceptions to this, and one must assume that the EI is not totally correcting or normalising the EIT in these individuals. For the majority of patients, this parameter is definitely of use, as can be seen from the improvement in the statistical significance that can be assigned to its use. The correction produced by using our experimental EI value is, however, far from perfect. Using this approach, it would appear that my equations using plasma iron/100 ml. plasma as x or independent variable, and equations 2 or 3 for NEIT, best fit the two parameters. As can be seen, the fit is still poor.

Table IV-9 Statistical analysis when comparing indirectly calculated EIT values (x axis) with ferrokinetically derived EIT values divided by EI (y axis).

Equation	SE^{y}/x	r	P
1. Plasma Iron/100 ml. wb			
Cook	.2537	.2029	< .10
French (1)	.2801	.1759	< .15
(2)	.2623	.2012	< .10
(3)	.2685	.1879	< .10
(4)	.2814	.1524	< .15
2. Plasma Iron/100 ml. plasma			
Cook	.2630	.2710	< .05
French (1)	.2844	.2371	< .05
(2)	.2758	.2816	< .025
(3)	.2751	.2900	< .025
(4)	.2946	.2042	< .10

IV-3-m In view of this, I have attempted another approach to the problem of deriving the best equation for calculation of NEIT. The equation for indirect measurement of EIT can be written as previously (IV-3-j).

i.e.

$$\text{EIT (indirect)} = \frac{Hb}{L} \times 3.4 \times \frac{EIT}{EEIT}$$

where EEIT = Effective Erythropoietic Iron Turnover

EIT/EEIT is normalised as 1.2 as previously discussed.

L = 120 days (normal rbc lifespan).

Any variation in L or EIT/EEIT will obviously have tremendous effects upon the indirectly calculated EIT, which will in turn affect any comparison of ferrokinetic and indirectly calculated EIT values. I have suggested that correction of ferrokinetic EIT values for variable erythropoiesis (which may be a result of variation in red cell lifespan or percentage ineffective erythropoiesis) by an EI value should normalise the ferrokinetic value for variation in these two parameters.

i.e.

$$\frac{\text{EIT (ferrokinetic)}}{\text{EI (experimental)}} = \frac{Hb}{L} \times 3.4 \times 1.2$$

$$= \frac{Hb \times 3.4}{100} \quad \text{(when L = 120 days)}$$

I have shown that my experimentally derived EI value fails to do this satisfactorily. However, one can calculate a theoretical EI value which will match these two values.

i.e.

$$EI = \text{EIT (ferrokinetic)} \times \frac{100}{Hb \times 3.4}$$

We can call this our theoretical E.I. value. I have previously shown that the E.I.can be expressed as the ratio of predicted $t_{\frac{1}{2}}$PIC (at the individual plasma iron concentration) to the experimentally derived $t_{\frac{1}{2}}$PIC value.

i.e.

$$\text{E.I.} = \frac{\text{Predicted } t_{\frac{1}{2}}\text{PIC (at individual's P.I. concentration)}}{\text{Experimentally derived } t_{\frac{1}{2}}\text{PIC}}$$

By calculating a theoretical E.I. value as described, we can also calculate a theoretical $t_{\frac{1}{2}}$PIC value for each individual and each equation.

i.e.

$t_{\frac{1}{2}}$PIC theoretical = $t_{\frac{1}{2}}$PIC experimental x E.I. (theoretical)

This can be done for all the patients in the different groups (i.e. the ten equations derived for calculating NEIT). The line giving the best correlation between plasma iron values and theoretical $t_{\frac{1}{2}}$PIC values would represent, I believe, the equation best describing NEIT. The reason for my believing this is that all the ferrokinetic data in this group of normal patients that I have studied, seems to be primarily dependent upon changes in plasma iron concentration. I have previously stated (IV-3-h) that I feel that it is more physiological to express plasma iron concentration in terms of its concentration/100 ml. of whole blood, as this probably represents the iron concentration 'seen' by the sites taking up iron from transferrin. In Table IV-9 however, it can be seen that P values are most highly significant where plasma iron concentrations/100 ml. of plasma are considered, and the correlation coefficient between indirectly calculated EIT values and ferrokinetically derived EIT values is also best

(although still poor) using these calculations.

In Table IV-10, the results of theoretically calculated EI and $t_{\frac{1}{2}}$PIC values as derived from ferrokinetic EIT values (see above) are tabulated. When the correlation coefficients between the theoretical $t_{\frac{1}{2}}$PIC and plasma iron concentrations (either as µgm% or as µgms/100 ml. wb) are calculated, it can be seen that the best correlation coefficient is obtained with equation French (3) where x is the plasma iron concentration/ 100 ml. wb.

Table IV-10 Theoretically calculated $t_{\frac{1}{2}}$PIC and EI values, as derived from ferrokinetic EIT values compared with indirectly calculated EIT values (see text) using the various equations to calculate EIT (PIT - NEIT)

Least squares regression equations:- ($y = t_{\frac{1}{2}}$PIC)

1. Plasma Iron/100 ml. whole blood (= x axis)

a)	Experimental :	$y = 0.9114x + 14.0171$	(r = 0.89)
b)	Cook :	$y = 0.87x + 34.67$	(r = 0.75)
c)	French (1)	$y = 0.78x + 45.10$	(r = 0.63)
	French (2)	$y = 0.89x + 37.21$	(r = 0.76)
	French (3)	$y = 1.08x + 32.48$	(r = 0.84)
	French (4)	$y = 0.72x + 47.63$	(r = 0.64)

2. Plasma Iron/100 ml. plasma (= x axis)

a)	Experimental :	$y = 0.5509x + 14.3389$	(r = 0.87)
b)	Cook :	$y = 0.47x + 39.18$	(r = 0.70)
c)	French (1)	$y = 0.46x + 46.20$	(r = 0.64)
	French (2)	$y = 0.55x + 37.94$	(r = 0.71)
	French (3)	$y = 0.58x + 35.76$	(r = 0.68)
	French (4)	$y = 0.42x + 49.94$	(r = 0.58)

TABLE IV-10

Patient	Hb	Hct	PI	$t_{\frac{1}{2}}$ PIC ex.	Plasma Iron/100 ml. whole blood								Plasma Iron/100 ml. plasma											
					Cook		(1)		(2)		(3)		(4)		Cook		(1)		(2)		(3)		(4)	
					$t_{\frac{1}{2}}$ (th)	EI (th)	$t_{\frac{1}{2}}$ (th)	EI (th)	$t_{\frac{1}{2}}$ (th)	EI (th)	$t_{\frac{1}{2}}$ (th)	EI (th)	EI (th)	$t_{\frac{1}{2}}$ (th)	EI (th)	$t_{\frac{1}{2}}$ (th)	EI (th)	$t_{\frac{1}{2}}$ (th)	EI (th)	$t_{\frac{1}{2}}$ (th)	EI (th)	$t_{\frac{1}{2}}$ (th)	EI (th)	$t_{\frac{1}{2}}$ (th)	EI (th)
deB	15.6	43	62	60	56	.93	66	1.10	60	1.00	62	1.04	68	1.14	56	0.93	66	1.11	60	1.00	60	1.00	71	1.18
Web	16.2	52	88	91	57	.62	70	.77	62	0.69	67	0.73	73	0.80	53	0.58	65	0.71	58	0.64	57	0.63	71	0.78
Luk	12.8	38	209	134	166	1.24	160	1.20	171	1.27	196	1.46	154	1.15	177	1.32	169	1.26	184	1.37	174	1.30	160	1.19
Cup	10.3	30	113	53	190	3.59	197	3.71	194	3.67	202	3.80	197	3.71	198	3.73	205	3.88	203	3.82	200	3.78	209	3.95
Car	12.1	38	53	35	74	2.10	81	2.33	77	2.19	78	2.24	83	2.38	74	2.12	83	2.37	78	2.22	77	2.21	84	2.39
Hey	20.3	64	64	28	34	1.23	38	1.36	36	1.28	37	1.30	38	1.36	33	1.17	36	1.30	34	1.22	34	1.21	38	1.36
Bot	17.2	51	114	70	78	1.12	86	1.23	82	1.17	86	1.23	87	1.24	75	1.07	81	1.16	79	1.12	77	1.10	84	1.20
Rus	11.9	36	49	64	63	0.98	78	1.22	69	1.07	72	1.12	81	1.27	65	1.01	81	1.26	71	1.11	71	1.10	81	1.27
Kho	15.6	45	55	51	50	0.98	59	1.16	53	1.05	55	1.08	61	1.19	50	0.97	59	1.16	53	1.04	53	1.04	61	1.19
Thy	14.7	44	105	85	88	1.03	99	1.16	93	1.09	99	1.17	100	1.18	87	1.03	97	1.15	93	1.10	91	1.07	103	1.21
Arm	12.6	38	92	62	109	1.77	119	1.92	114	1.84	119	1.92	120	1.94	118	1.80	122	1.96	117	1.88	115	1.86	127	2.05
Sch	11.0	36	21	34	33	0.97	38	1.10	37	1.08	37	1.10	38	1.10	34	0.99	38	1.10	37	1.10	38	1.10	38	1.10
Bas	8.1	23	9	16	24	1.52	26	1.61	26	1.61	26	1.61	26	1.61	25	1.54	26	1.61	26	1.61	26	1.61	26	1.61
Hoo	9.9	29	16	29	31	1.08	35	1.20	35	1.20	35	1.20	35	1.20	32	1.10	35	1.20	35	1.20	35	1.20	35	1.20
Cha	10.9	36	19	18	32	1.78	34	1.91	34	1.89	34	1.91	34	1.91	32	1.80	34	1.91	34	1.91	34	1.91	34	1.91
Loz	13.2	36	9	35	59	1.67	60	1.72	60	1.72	60	1.72	60	1.72	59	1.68	60	1.72	60	1.72	60	1.72	60	1.72
Gog	16.8	46	196	90	136	1.51	137	1.52	139	1.54	149	1.66	135	1.50	133	1.48	131	1.45	138	1.52	132	1.47	127	1.41
oCo	13.1	35	243	210	98	0.47	75	0.35	103	0.49	149	0.71	59	0.28	129	0.61	104	0.50	138	0.66	119	0.57	80	0.38
vWy	11.2	34	137	86	173	2.01	179	2.08	178	2.07	191	2.22	178	2.07	182	2.12	189	2.20	189	2.19	185	2.15	192	2.23
Pet	16.4	42	29	51	26	0.51	36	0.71	30	0.59	31	0.60	39	0.76	26	0.52	37	0.73	30	0.59	30	0.59	44	0.87
Mgo	16.1	46	163	133	92	0.69	99	0.74	98	0.73	111	0.84	98	0.73	88	0.67	91	0.69	95	0.72	89	0.67	90	0.68
Tan	14.5	40	101	82	82	1.00	93	1.13	87	1.07	94	1.14	94	1.14	84	1.03	94	1.15	90	1.09	88	1.07	100	1.22
Wes	17.3	54	143	74	91	1.22	97	1.31	94	1.27	100	1.35	98	1.32	84	1.14	88	1.19	88	1.19	86	1.16	89	1.20
Nqw	16.3	49	206	101	132	1.31	133	1.32	136	1.34	148	1.46	131	1.30	125	1.24	121	1.20	129	1.28	124	1.22	116	1.15
Mar	20.4	60	101	52	53	1.03	60	1.14	56	1.08	58	1.11	61	1.17	49	0.95	54	1.04	52	1.00	51	0.98	56	1.08
Ans	12.0	38	32	30	46	1.52	54	1.79	49	1.62	49	1.65	51	1.70	46	1.53	51	1.70	49	1.63	49	1.64	51	1.70
Wil	11.0	33	22	31	59	1.89	68	2.20	62	2.00	63	2.02	63	2.04	59	1.91	63	2.04	63	2.02	63	2.03	63	2.04
Pre	17.6	47	86	34	72	2.11	76	2.24	74	2.17	75	2.22	77	2.27	71	2.10	75	2.22	73	2.16	73	2.14	78	2.29
Sam	16.1	44	123	61	105	1.72	111	1.82	108	1.78	113	1.85	111	1.83	105	1.72	110	1.80	108	1.78	107	1.75	112	1.84
Jeg	11.4	34	45	33	71	2.14	79	2.39	74	2.23	75	2.28	81	2.44	72	2.17	80	2.42	75	2.27	75	2.27	80	2.42
Pax	13.3	29	41	54	54	1.00	66	1.21	58	1.08	60	1.12	66	1.23	56	1.04	66	1.23	61	1.13	61	1.13	66	1.23
Rob	9.1	28	80	42	163	3.90	173	4.11	168	4.00	173	4.11	174	4.14	169	4.03	180	4.28	174	4.14	173	4.12	186	4.43
Tay	16.4	54	26	17	22	1.30	26	1.51	23	1.37	24	1.38	27	1.56	22	1.28	23	1.38	23	1.36	23	1.36	23	1.38
Sch	8.6	26	33	31	82	2.64	88	2.82	81	2.60	82	2.65	90	2.91	78	2.53	86	2.77	83	2.67	83	2.68	86	2.77
				r	0.75		0.68		0.76		0.84		0.64		0.70		0.64		0.71		0.68		0.58	

The most similar slope to the experimentally derived slope for $t_{\frac{1}{2}}$PIC plotted against plasma iron concentration is from equation French (2) where x = plasma iron concentration/100 ml. plasma.

Comparing the experimentally derived $t_{\frac{1}{2}}$PIC curves with the theoretical curves, it can be seen that the slope of the regression equation is much the same, but that the line lies above that of the experimentally derived regression. This probably represents an error in patient selection on our part i.e. an excess of patients with iron deficiency and markedly raised E.I. values, perhaps reflecting subclinical haemolysis or increase in ineffective erythropoiesis.

IV-3-n By all the parameters that we have used in an attempt to assess the best equation describing the NEIT function, it would appear that

(a) from comparing indirectly calculated EIT values with ferrokinetically derived EIT values, (i.e. PIT - NEIT using all the formulae mentioned) divided by the experimental E.I. value :- the best equation seen to fit the data is my equation (3).

i.e. $y = 0.0022x - 0.0431$ $(r = 0.9161)$

where x = plasma iron concentration/100 ml. plasma

y = NEIT (mgms/100 ml wb/day).

In this comparison, when indirectly calculated EIT is plotted against ferrokinetically derived EIT,

$SE^{y}/x = 0.2751$

$r = 0.2900$

$P = < 0.025$

(b) When calculating a theoretical E.I. value by forcing the ferrokinetically derived EIT value to equal the indirectly calculated EIT (see text), and from this calculating a theoretical $t_{\frac{1}{2}}$PIC value which correlates best with the plasma iron concentration the best equation fitting the data is my equation (3) viz.

$y = .0031x - 0.0431 \quad (r = 0.9382)$

where x = plasma iron concentration/100 ml. wb.

y = NEIT (mg/100 ml. wb/day)

In this comparison, when plasma iron concentration is plotted against the theoretically derived $t_{\frac{1}{2}}$PIC, r = 0.84. It should be pointed out that both these equations have been derived from the same equation;

i.e. (100 - RBCU)% = 0.3633 (PI/100 ml wb.) - 3.5096

$r = 0.8640$

$SE^{y}/x = 11.22$

(c) $\therefore$ NEIT = $\frac{(100 - RBCU)}{100}$ x P.I.T. (mg/100 ml. wb/d)

The derived equations described in (a) and (b) above have been obtained by plotting the NEIT values calculated from (c) against plasma iron concentration per 100 ml. of plasma and against plasma iron concentration per 100 ml. of whole blood respectively, and calculating the least squares best fit regression equation to give equations (a) and (b) respectively. This data would suggest that it does not really matter which parameter of plasma iron concen-

tration is used, both being equally valid.

IV-3-o Data derived by my method and that of Cook gives closely similar values. For reasons of convenience, as Cook's equation is already published and better known, I have used his method to calculate NEIT in subsequent work, believing that my method probably gives results of the same order of accuracy. It is interesting to me that my experimentally derived EI should fit so closely to a theoretically derived EI value. I believe that my regression, experimentally derived, of $t_{\frac{1}{2}}$PIC against plasma iron concentration, probably calculates too rapid a predicted $t_{\frac{1}{2}}$PIC for reasons already stated, and because of this prefer to use the theoretically derived EI value as a measure of total body erythroid activity.

In summary to this section, therefore, I propose, in future work in this thesis, to use the following equations for calculating various ferrokinetic parameters.

(1) Predicted $t_{\frac{1}{2}}$PIC = $0.87x + 34.67$ (x = PI/100 ml.wb)

(2) NEIT = $0.0035x$ (mg/100 ml. wb/d) (x = PI/100 ml. wb)

(3) EIT = PIT - NEIT (mg/100 ml. wb/d)

(4) $$PIT = \frac{PI}{100} \times \frac{100 - (Hct \times 0.92)}{t_{\frac{1}{2}}PIC \text{ (experimental)}}$$ (mg/100 ml. wb/d)

(5) $$EI = \frac{\text{Predicted } t_{\frac{1}{2}}\text{PIC at patient's PI level}}{\text{Experimental } t_{\frac{1}{2}}\text{PIC}}$$

(6) Predicted RBCU = $-0.3633x + 103.5096$ % (x = PI/100 ml wb)

Thus, all the equations measuring total erythropoietic activity have been derived from Cook's equation describing NEIT; the equation describing predicted RBCU is derived from my own work (see IV-3-d). These equations will characterise normal ferrokinetic performance at variable plasma iron levels.

IV-4 The Plasma Iron Turnover

IV-4-a Conventional methods of estimating the plasma iron turnover (PIT), utilising the formulae proposed by Huff et al[404] have been shown to overestimate erythropoietic demands for iron as shown by studies of red cell lifespan[405][232][685][684][686][79][80][289][113][769][23][218] in normal patients. In an attempt to remove this discrepancy, a number of mathematical models have been devised, based mainly upon data derived from study of prolonged plasma iron clearance curves. As I was unable to duplicate this data (IV-2-g) I have developed an empiric approach utilising a concept of iron kinetics developed from my review of the literature (Table IV-9). This has been reviewed in the previous section. (IV-1-a to IV-3-o).

IV-4-b There is general agreement that PIT reflects not only total (i.e. effective plus ineffective) erythropoietic activity but also uptake of iron by non erythroid sites. (NEIT). i.e.

PIT = NEIT + EIT

EIT = EEIT + IEIT

effective + ineffective erythroid iron turnover.

NEIT has been quantitated as described in the previous section (IV-3-a to IV-3-o). It was comforting to discover that Cook's approach[164] and mine produced essentially similar results, indicating that we were reaching the same conclusions with different data and different methods.

The fact that the results were so similar suggests that the resultant equations are physiologically significant. As I have found NEIT to be totally dependent upon changes in plasma iron concentration, I do not believe that it significantly influences other parameters reflecting total erythropoietic activity, (i.e. PIT, $t_{\frac{1}{2}}$PIC, EI) in different disease states.

IV-4-c Factors influencing the PIT.

From the form of the formula used to calculate the plasma iron turnover i.e.

$$\text{PIT (mg/100 ml wb/d)} = \frac{\text{PI}}{100} \ (\mu g\%) \times \frac{100 - (\text{Hct} \times 0.92)}{t_{\frac{1}{2}}\text{PIC}}$$

it is obvious that the PIT will be influenced mathematically by change in plasma iron concentration, $t_{\frac{1}{2}}$ Plasma Iron Clearance and Haematocrit. We have shown (Section IV-2-a to IV-2-c) that the $t_{\frac{1}{2}}$PIC will vary with changes in plasma iron concentration, the change in both being proportional. As a result, in normal patients, the PIT should remain within very small limits of a 'normal' range. Mathematically, as the haematocrit decreases, the PIT should increase, if all other factors remain constant. The influence of these various parameters will now be discussed in greater detail.

IV-4-d Influence of changes in plasma iron levels.

I have previously stated that:-

PIT = EIT + NEIT

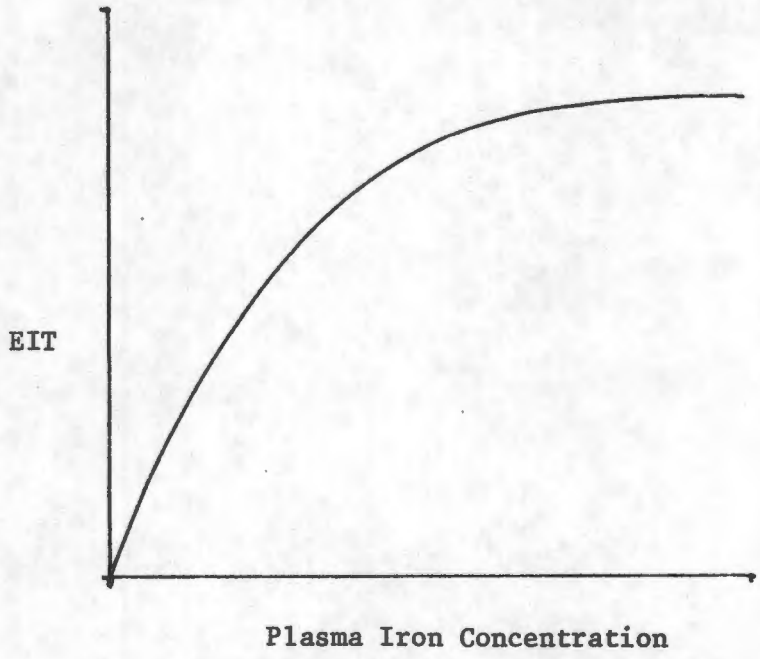
and have shown that NEIT increases as plasma iron concentration increases, in a directly proportional way. Consequently, with no change in EIT values, should the plasma iron concentration increase, the plasma iron turnover will likewise increase, by the amount of increase in NEIT. This probably accounts for the often normal PIT values in patients with aplastic anaemia, where plasma iron concentrations tend to be high (see section VI-d-5) and EIT values will obviously be depressed. This phenomenon will obviously occur in all disease processes.

IV-4-e Influence of changes in erythropoietic activity.

As stated in IV-4-d, PIT = NEIT + EIT.

Changes in total erythropoietic activity will cause proportionate changes in the amount of iron used for haemoglobin synthesis. Thus, with hyperplastic erythropoiesis, EIT and PIT values will increase, and the reverse will occur in hypoplastic erythropoiesis. It seemed important to me to attempt to define the normal range of EIT values in normal patients. Theoretically, because erythroblast iron uptake has been found to be dependent upon plasma iron concentrations up to levels of approximately 70 - 100 µgms%,[434][432][609][384] it would seem logical to suppose that EIT will also vary with plasma iron concentration in normal patients. This may be illustrated diagrammatically as in Figure IV-18 and discussed in Section III-b-5.

Figure IV-18: Theoretically expected changes of EIT with variation in plasma iron concentration.



Theoretically, EIT should increase as plasma iron concentration increases until a critical point is reached at which the amount of haemoglobin being formed, instead of being dependent upon the amount of iron available, will become dependent upon the body haemoglobin requirements.

In order to eliminate, as far as possible, variations in EIT due to variable erythropoietic activity, and thus to normalise body erythropoietic activity, the EIT has been calculated in each individual and divided by his EI, and then plotted against his plasma iron concentration. The resultant graph is shown in Figure IV-19, with the results of the individuals, in

tabulated form, shown in Table IV-11. EIT and EI have been calculated as previously described (IV-3-o).

Figure IV-19 : EIT/EI values (y axis) plotted against plasma iron concentration (x axis) showing the changes in EIT with changes in plasma iron concentrations.

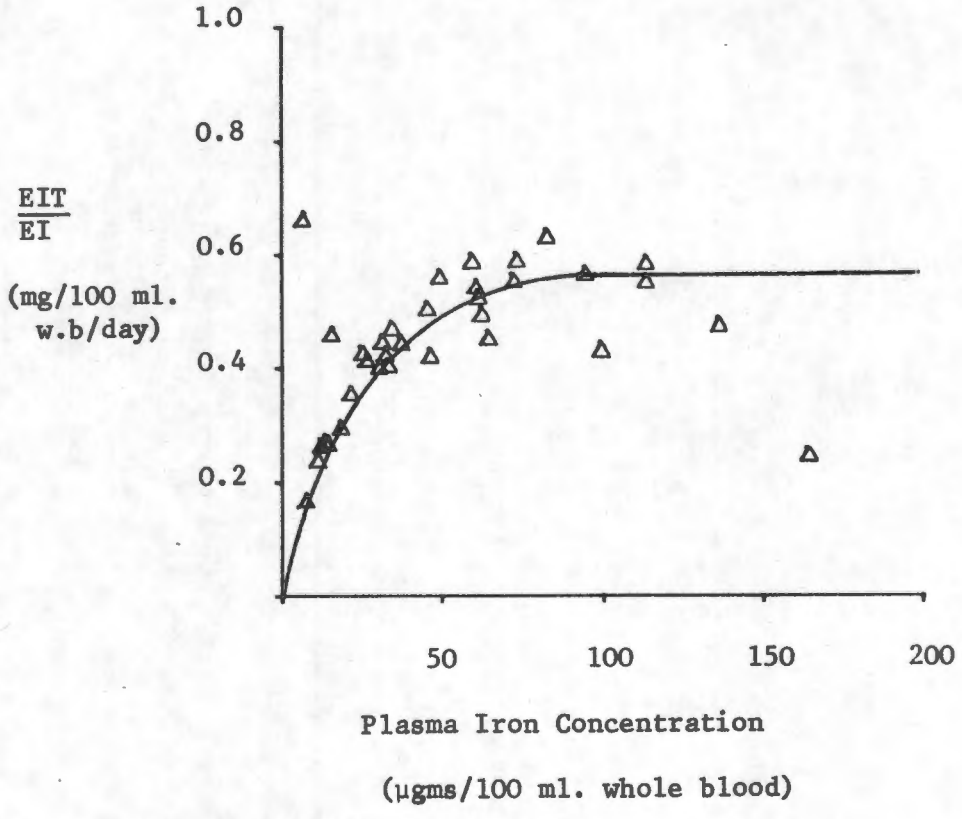


Table IV-11 Results of EIT/EI in Normal Patients.

Patient	PI / 100 ml.wb	$t_{\frac{1}{2}}$ exp.	EI	EIT	EIT / EI
deB	37	60	1.12	.4933	.4404
Web	46	91	0.82	.3437	.4191
Luk	136	134	1.14	.5386	.4725
Cup	82	53	2.00	1.2573	.6287
Car	34	35	1.85	.8643	.4672
Hey	26	28	2.06	.8478	.4116
Bot	61	70	1.25	.6526	.5221
Rus	33	64	0.99	.3974	.4014
Kho	32	51	1.23	.5192	.4221
Thy	62	85	1.05	.5165	.4919
Arm	60	62	1.40	.7563	.5402
Sch	14	34	1.38	.3639	.3637
Bas	7	16	2.55	.4187	.1642
Hoo	11	29	1.55	.3634	.2345
Cha	12	18	2.54	.6615	.2604
Loz	6	35	1.14	.7509	.6587
Gog	113	90	1.48	.8604	.5814
oCo	164	210	0.85	.2079	.2446
vWy	94	86	1.36	.7646	.5622
Pet	18	51	0.98	.2866	.2924
Mgo	99	133	0.88	.3778	.4293
Tan	64	82	1.10	.4950	.4500
Wes	72	74	1.31	.7205	.5500
Nqw	113	101	1.32	.7242	.5486
Mar	45	52	1.42	.7118	.5013
Ans	21	30	1.76	.6210	.3528
Wil	15	31	1.55	.7077	.4566
Pre	49	34	2.27	1.2649	.5572
Sam	73	61	1.61	.9440	.5863
Jeg	31	33	1.87	.8289	.4433
Pax	30	54	1.13	.4509	.3990
Rob	59	42	2.06	1.2062	.5855
Tay	13	17	2.71	.7238	.2671
Sch	25	31	1.82	.7720	.4242

As can be seen from Figure IV-19, there is a gradual increase of EIT as the plasma iron concentration increases, leveling off to a plateau at plasma iron levels of approximately 60µgms/100 ml. of whole blood. With no restriction imposed upon the EIT by shortage of precursor iron, the normalised EIT appears to be ± 0.5 mg/100 ml. wb/day. With limitation in precursor iron supply however, it can be seen that EIT will vary from zero to maximum normal levels. This will occur where the issue is not confused by variations in red cell lifespan or proportions of ineffective erythropoiesis. Limitation in precursor iron supply appears not to be rate limiting in normal haemoglobin production above plasma iron concentrations of 50 µgms/100 ml. whole blood, a figure agreeing well with the results found by Hillman et al[384] and with experimental findings in vivo.[434][432][609]

Erythropoietic activity will also vary in different disease states, and the direction of this change will markedly influence the P.I.T. I have shown in section IV-2-a to IV-2-f, the dependence of the $t_{\frac{1}{2}}$PIC upon total bone marrow erythropoietic activity. Thus, the $t_{\frac{1}{2}}$PIC decreases, regardless of the plasma iron level, as erythropoietic activity increases, and the reverse occurs with decrease in total erythropoietic activity. From the equation used to calculate PIT (IV-4-c), a shortened $t_{\frac{1}{2}}$PIC will increase PIT, while the reverse will occur with prolongation of the $t_{\frac{1}{2}}$PIC.

i.e. $$PIT \propto \frac{1}{t_{\frac{1}{2}}PIC}$$

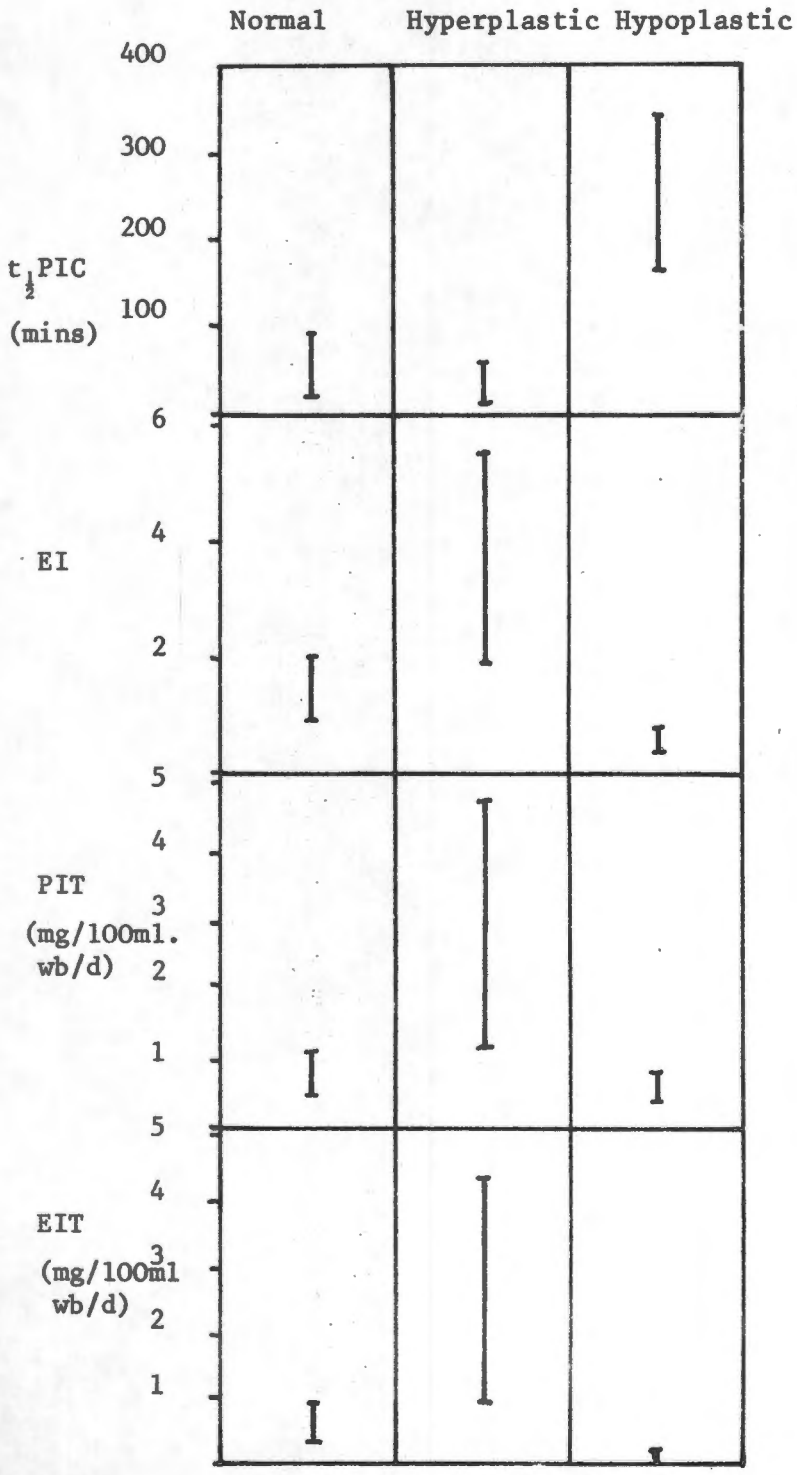
Where PIT values are elevated, the PIT will reflect increased total erythropoietic activity. For reasons stated previously however, normal PIT values may occur in hypoplastic bone marrow states (because of increased NEIT). Subtraction of NEIT values from the PIT to give EIT values will give fairly accurate representation of total body erythropoietic activity.

I have attempted to test the effect of total erythropoietic activity upon this parameter using the same two groups of patients used previously to test the $t_{\frac{1}{2}}$PIC and EI parameters (IV-2-f). The results for $t_{\frac{1}{2}}$PIC, EI, PIT and EIT have been compared in patients with normal, hyperplastic or hypoplastic erythropoiesis, as described in IV-1-b and IV-2-f. The results are shown in Table IV-12, Figure IV-20 and Figure IV-21. The test not only tests the PIT and EIT as parameters of total erythropoietic activity, but attempts to distinguish which parameter best measures or reflects changes in total erythropoiesis. The parameters have been calculated as detailed in section IV-3-o.

Table IV-12 Statistical comparison of ferrokinetic parameters of total erythropoiesis in normal patients, patients with proven hyperplastic erythropoiesis and patients with proven hypoplastic erythropoiesis. Results taken from patients previously described (Table IV-1, Table IV-3 and Table IV-2) with ferrokinetic parameters calculated as detailed in IV-3-o.

	$t_{\frac{1}{2}}$PIC	EI	PIT	EIT	
(1) Normals					
$\bar{x}$	61	1.52	.8324	.6517	from Table IV-1
SD	40	0.51	.3062	.2643	
(2)Hyperplastics					
$\bar{x}$	39	3.75	2.9883	2.6245	from Table IV-3
SD	25	1.81	1.8037	1.6965	
P	< .0005	< .0005	< .0005	< .0005	
(3)Hypoplastics					
$\bar{x}$	257	.65	.5770	.1110	from Table IV-2
SD	90	.17	.1688	.1319	
P	< 0.01	< .0005	< .0005	< .0005	

Figure IV-20 Histogram showing the differences, in ferrokinetic parameters, of patients with normal, hypoplastic and hyperplastic erythropoiesis. (lines represent the mean ± 1 S.D.)



From the computed statistical values, it can be seen that all the parameters mentioned are excellent indicators of total erythropoietic activity. The EI value tends to be more helpful than any of the others in distinguishing more subtle changes in erythropoietic activity because of its more comparative nature in stating the degree of change of erythropoietic activity relative to normal values. It is also obvious that all of the parameters are markedly influenced by changes in total erythropoietic activity. The EIT appears to be better than the PIT in this regard, as would be expected once the contribution of the NEIT to the PIT has been removed.

IV-5 <u>The utilisation of transferrin-bound radioiron by the erythroid bone marrow for incorporation into haemoglobin</u>. (RBCU)

IV-5-a The red blood cell utilisation (RBCU) measures the percentage of injected transferrin-bound radio-iron appearing in the circulating red cells as haemoglobin after approximately fourteen days.

IV-5-b A number of factors will obviously influence this parameter. I have reviewed the uptake of iron by erythroblasts from transferrin, and the subsequent release of mature erythrocytes in the circulation (Chapter III). I have also shown, in normal patients, that the RBCU will be dependent upon plasma iron concentrations (IV-3-a to IV-3-f), and how this dependence can be mathematically expressed. The reason for this phenomenon appears to be that a fairly constant proportion of the plasma iron turnover goes to non erythroid sites (IV-3),

the amount increasing as plasma iron levels increase. For this reason, it would appear reasonable to suggest that similarly, a constant proportion will exist in different disease states. It will become obvious, however, (as will be discussed later) that RBCU values are not solely influenced by changes in plasma iron concentration. Other factors influencing this parameter are the relative rates of effective and ineffective erythropoiesis, the erythroid activity of the individual and the red cell lifespan.

IV-5-c <u>The effects of variable ineffective erythropoiesis.</u>

It would seem reasonable, on physiological grounds, to assume that iron taken up by maturing erythroblasts is taken up non selectively - i.e. that it is taken up equally by erythroblasts that will mature normally and those that will not. It is well known that variable proportions of erythropoiesis are effective in different disease states, and that, in normal patients, approximately 10 - 20% of total erythropoietic effort is wasted. (III-b-7). The exact figure may vary in different normal patients, and may change enormously in different disease states. If radio-iron is non selectively taken up by maturing erythroblasts, it will be obvious that any variation in the proportion of ineffective erythropoiesis will cause an opposite change in the amount of radio-iron being effectively utilised for haemoglobin production and release into the circulation.

Mathematically, we can say that:-

EIT = EEIT + IEIT

+

RBCU

Where EIT = erythroid iron turnover

EEIT = effective erythroid iron turnover

IEIT = ineffective erythroid iron turnover

It will be obvious that, as IEIT increases, EEIT will decrease, and so will RBCU values. Assuming, for the present, that RBCU is unaffected by red cell lifespan, it is clear that the fraction of EIT used for effective erythropoiesis could be estimated by the ratio of RBCUe : RBCU predicted. In other words, if this ratio were unity, all erythroid iron turnover could be ascribed to iron leaving the plasma pool and being used for viable red cell synthesis. If the ratio were less than unity, this would indicate ineffective erythropoiesis. Expressed mathematically, we may write:-

$$\frac{RBCUe}{RBCUp} \times EIT \quad (mg/100\ ml.\ w.b./day) = \text{Effective EIT } (mg/100ml.\ w.b./day)$$

and EIT - EEIT will equal IEIT.

This mathematical expression has been used to compare indirectly calculated EEIT values with ferrokinetically derived EEIT values, in the same way as EIT/EI values were compared (see IV-3-j), in an attempt to assess the validity of this assumption in normal patients. Indirect EIT values were derived as previously described, EIT and RBCU predicted values were derived using the equation given in IV-3-o. When these two parameters i.e. $(\frac{RBCUe}{RBCUp} \times EIT)$ and (Indirect EEIT) were

compared with one another, a poor correlation was found ($r = 0.0719$, $SE^{y}/x = 0.3086$, $P = 0.30$). This presumably reflected the effect, in this group of normal individuals, of variations in red cell survival that were not adequately detected by $t_{\frac{1}{2}}{}^{51}Cr$ red cell survival measurements. It may also have reflected how poorly this equation measures changes in effective and ineffective erythropoietic activity. One may, however, say that, should this ratio drop considerably in the absence of changes in red cell survival, the proportion of ineffective erythropoiesis is increased or effective erythropoietis is decreased compared to normal patients.

Mathematically, if we assume that EIT/EEIT remains constant (i.e. $\pm$ 1.2 as previously discussed in section IV-3-j) in normal individuals, we can solve for ($\mathcal{l}$) using the equation:-

$$EIT \times \frac{RBCUe}{RBCUp} = \frac{Hb}{(\mathcal{l})} \times 3.4 \times 1.2$$

$$(\mathcal{l}) = \frac{Hb \times 3.4 \times 1.2 \times RBCUp}{EIT \times RBCUe}$$

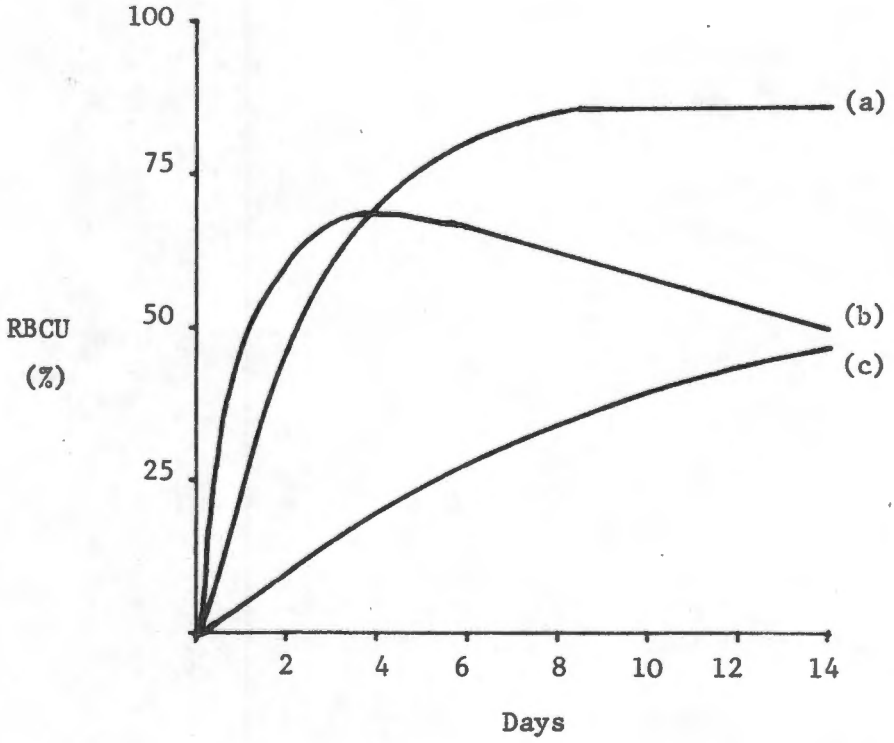
Where ($\mathcal{l}$) is red cell lifespan.

I have shown a reasonably good correlation between RBCU and plasma iron levels in my group of normal individuals and have shown that the relationship between these may be used to derive an equation for calculating NEIT. (Section IV-3). This equation describes a relationship very similar to that postulated by Cook.[164] This suggests in our normal group of patients, that ($\mathcal{l}$) did not influence the RBCU and that the

ratio of EIT/EEIT remained fairly constant. The poor correlation between indirectly calculated EEIT and RBCUe/RBCUp x EIT values must have been due mainly to changes in (Z) which I was unable to detect adequately. In this group of patients, therefore, a calculation of (Z) by the method detailed above may be valid (see later under IV-5-d).

Not only would increase in ineffective erythropoiesis cause a lower plateau level for the RBCU, it would also alter the shape of the RBCU curve. This would occur due to recycling of radio-iron from the erythroblast bone marrow pool (during erythroblast maturation) to the reticulo-endothelial system (RES) as a result of early catabolism, and from there back to the plasma. The period of recycling is thought to be extremely rapid so that the delay period would depend upon the stage of maturation at which intramedullary erythroblast destruction took place. This recycled iron might contribute significantly to the PIT, and would undergo the same process of uptake by erythroblasts and release into the circulation. Eventually, if red cell lifespan were normal, most of the label might appear in the circulation, but the time required for this to occur might exceed the duration of the experiment. As a result of this phenomenon the RBCU curve would be 'slurred' to the right, would fail to plateau early and might in fact not reach a plateau during the period of observation. This is diagrammatically illustrated in Figure IV-21.

Figure IV-21 Diagrammatic representation of the effects upon the RBCU curve of (a) normal erythropoiesis
(b) ineffective erythropoiesis and
(c) extramedullary haemolysis.



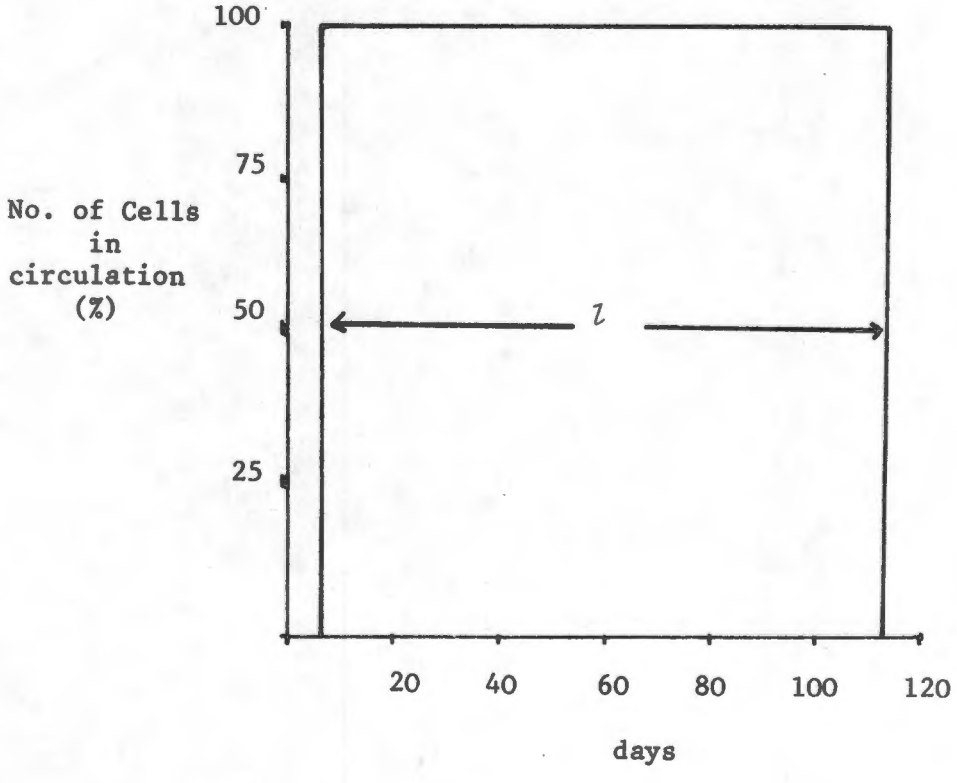
This effect would obviously depend upon the severity of the intramedullary haemolysis.

IV-5-d The effect of variable red cell survival upon RBCU.

Red cell lifespan ($\mathcal{l}$) may be shortened by a finite reduction of ($\mathcal{l}$), by random destruction, or by a combiration of these two processes:

(1) Where red cell survival ($\mathcal{l}$)is shortened because of a finite reduction in lifespan, all the cells delivered to the circulation would reach senescence before removal. Thus, all the cells delivered to the circulation at a particular time would, in the theoretical case, be removed from the circulation simultaneously after period ($\mathcal{l}$)in the circulation. This function will appear as detailed in Figure IV-22 if these cells are counted:

Figure IV-22



In this situation, the influence of (l) upon RBCU would be negligible unless (l) happened to be shorter than the duration of the experiment (i.e. 14 days). The influence of (l) upon EIT would, however, be considerable. In my patients, where the fate of ^{51}Cr labelled red cells was studied, a plot of ^{51}Cr activity against time should be linear (when corrected for elution) since constant numbers of labelled cells would die daily. The time at which ^{51}Cr disappeared from the circulation should equal (l). Simple as this may seem in theory, in practice it is more difficult. It is, however, of interest to compare (l) values derived by ^{51}Cr measurements and (l) values derived from our previously postulated equation,

i.e.

$$(l) = \frac{Hb \times 3.4 \times 1.2 \times RBCUp}{EIT \times RBCUe}$$

in normal patients. If we assume that, in these patients, ^{51}Cr elution occurred at a uniform rate, we can express ^{51}Cr (l) as :-

$$(l)_{^{51}Cr} = \frac{\text{exp. } t_{\frac{1}{2}}\,^{51}Cr}{\text{normal } t_{\frac{1}{2}}\,^{51}Cr} \times 120$$

These two parameters are compared in Figure IV-23 and Table IV-13.

Figure IV-23 Comparison of (τ) calculated by ^{51}Cr data and by ^{59}Fe data. (see text)

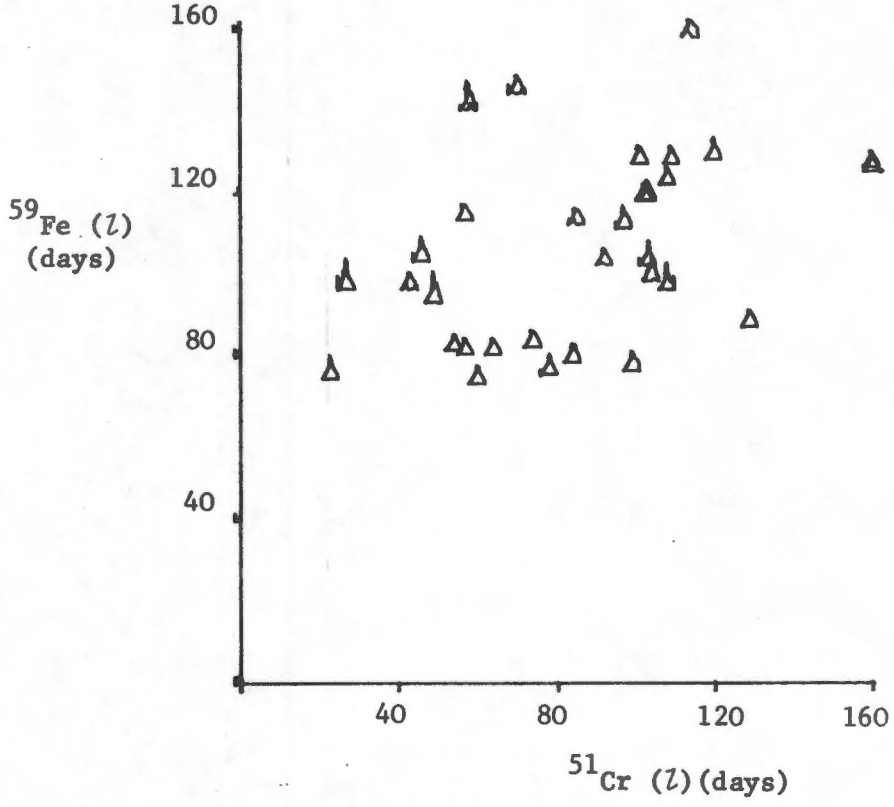


Table IV-13 Comparison of (τ) calculated by ^{51}Cr data and by ^{59}Fe data. (see text).

Patient	Ferrokinetic (τ) (days)	$^{51}Cr(\tau)$ (days)	EI
deB	129	89	1.12
Web	165	127	0.82
Luk	99	78	1.14
Cup	27	98	2.00
Car	58	142	1.85
Hey	97	113	2.06
Bot	108	124	1.25
Rus	102	120	0.99
Kho	104	100	1.23
Thy	103	104	1.05
Arm	57	82	1.40
Sch	114	160	1.38
Bas	78	77	2.55
Hoo	108	98	1.55
Cha	64	82	2.54
Loz	74	84	1.14
Gog	85	114	1.48
oCo	258	-	0.85
vWy	46	105	1.36
Pet	226	-	0.98
Mgo	168	128	0.88
Tan	92	104	1.10
Wes	101	129	1.31
Nqw	120	130	1.32
Mar	109	129	1.42
Ans	70	146	1.76
Wil	60	75	1.55
Pre	49	95	2.27
Sam	57	115	1.61
Jeg	54	83	1.87
Pax	103	120	1.13
Rob	23	76	2.06
Tay	84	80	2.71
Sch	43	98	1.82

From the figures it can be seen that in a number of patients correlation was excellent whereas in others it was poor. It would seem reasonable to suggest that in the latter group, the poor correlation was attributable to differences in EIT/EEIT ratios.

(2) Where red cell lifespan is shortened because of random haemolysis, each newborn cell has a chance of being destroyed early in its lifespan. The chance of newborn cells being destroyed will depend upon the number of these cells in the circulation, and because of this, removal of these cells from circulation will be an exponential function of time. It is obvious that this will greatly affect RBCUe results, both in terms of the height of the plateau and the shape of the curve with time.

(i) Because of a compensatory increase in erythropoiesis, stress reticulocytes containing radio-iron might be released at an earlier stage and in greater numbers than normal with the result that the initial phase of the RBCU curve would be 'shifted' to the left.

(ii) As the number of these cells in the circulation increased, the number removed by random destruction would also increase, so that the amount of circulating radioactivity would decrease with time in an exponential way. Thus, the later ascent of the RBCU curve would be slurred and the plateau would be lower. As a result of continued red cell destruction the plateau of the RBCU curve would not remain flat as it would where all the rbc's have a

finite lifespan exceeding the period of the experiment, but would fall exponentially.

(iii) ^{59}Fe released by haemoglobin catabolism would be recycled to the plasma via the reticulo-endothelial system, and would result in a late rise in RBCU values.

(3) Where a combination of random cell destruction and finite shortening of (l) is present, the effect upon the RBCU curve and figures would reflect the influence only of the random destruction if we assume that finite (l) exceeds fourteen days (i.e. the duration of the experiment).

IV-5-e Influence of variable effective erythropoietic activity upon the RBCU.

Where EIT is decreased because of depressed erythroid bone marrow function, the amount of iron taken up by the maturing erythroblasts should be less, and because of this less iron label would appear in the circulation. Similarly, where effective erythropoietic activity is increased, the amount of iron label taken up by the erythroblasts should be increased, and more should enter the peripheral circulation. This should occur where no change in (l)or EIT/EEIT values occur.

In concluding this theoretical discussion, one can see that any characterisation of the effectiveness of erythropoiesis depending upon the experimentally derived RBCU values must be based upon an assumed or proven finite rbc lifespan which exceeds

the period of the experiment. In addition, one must consider the effect of the plasma iron concentration, which we have shown to influence experimentally derived RBCU values, and the amount of effective erythropoietic activity present. I suggest, therefore, that should any value of RBCU be used to assess the effectiveness of erythropoiesis, it should be the ratio of RBCUe/RBCUp at the plasma iron level of the patient being studied. Any assessment based upon the RBCUe/100 must be incorrect for the reasons given, although this parameter has been used by a number of workers[404][164][253] including Cook, who has shown that it should be impossible in normal individuals, even if erythropoietic activity is totally effective, for all the injected iron label to enter the circulation unless a severe iron deficiency is present (I base this upon his equation describing NEIT). Where the ratio of RBCUe/RBCUp is less than 1, it would seem reasonable to assume a decrease in (Z) or EEIT. Where this ratio exceeds 1, it probably represents an increase in EEIT.

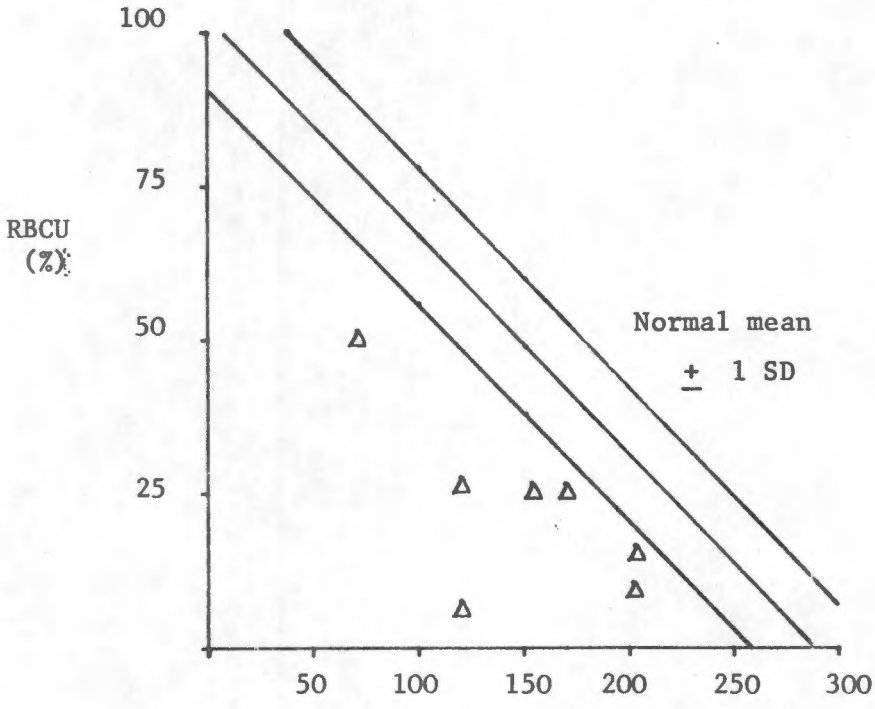
IV-5-f <u>Experimental results to show the influence of various disease states upon the RBCU</u>.

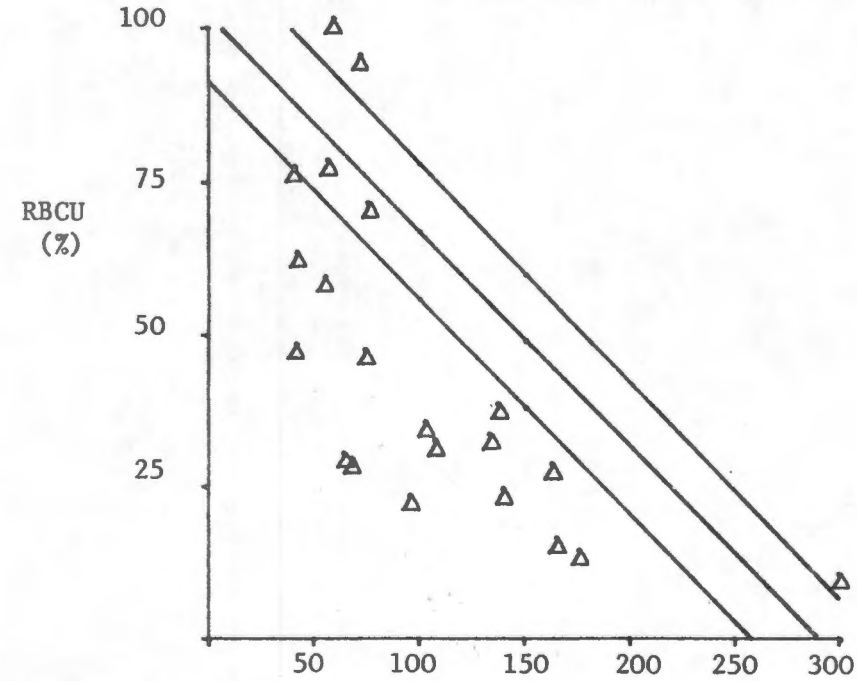
For the theoretical reasons given, any interpretation of RBCU curves is hampered by the variables already discussed. I have described a 'normal' RBCU response to changes in plasma iron concentration. (Figure IV-14, Section IV-3-d). It remains to describe the influence of different disease processes upon this parameter. I have used the same groups of patients previously described, i.e. the hyperplastic, hypoplastic and normal groups of patients. The results are shown in

Figures IV-24 and IV-25. Comparison of the data tends to confirm theoretically predicted results. RBCUe values tend to be lower than normal at similar plasma iron concentrations in all disease states except for fully compensated haemolytic states where (Z) is probably finite.

Figure IV-24: Comparison of RBCUe values (maximum) in normal patients, patients with hyperplastic erythropoiesis and patients with hypoplastic erythropoiesis.

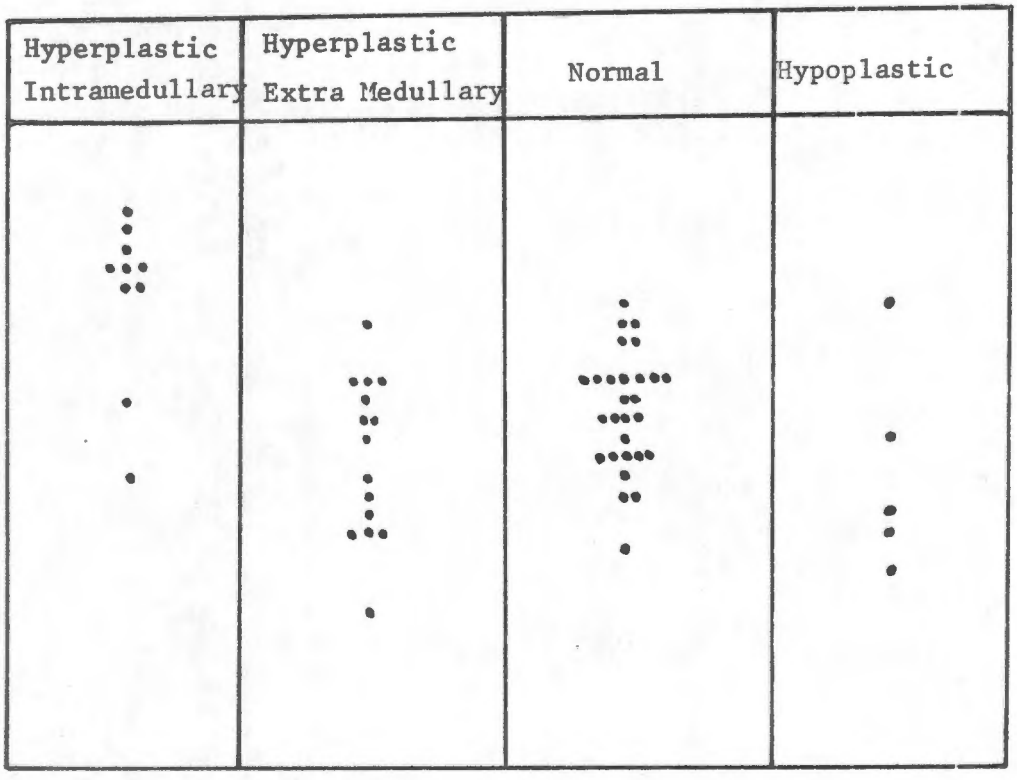
(a) Hypoplastic Patients





(b) Hyperplastic Patients.

Figure IV-25: Comparison of t(max) for RBCUe values in normal patients, patients with hypoplastic erythropoiesis, and patients with hyperplastic erythropoiesis. The patients with hyperplastic erythropoiesis have been subdivided into two groups, those thought to have principally intramedullary haemolysis and those thought to have mainly extramedullary haemolysis.



The time required for RBCU values to reach maximum height in normal subjects, varied between 6.0 and 12.0 days, with a mean of 9.2 and a standard deviation of 1.6 days. This time interval tended to be slightly shorter in extra-medullary haemolytic states, longer in intra-medullary haemolytic states, and probably unchanged in hypoplastic states (see Figure IV-25 and Table IV-14). It was decided not to use ferrokinetic criteria in deciding whether patients had predominantly extra or intramedullary haemolysis, but rather to choose, as representative of intramedullary haemolysis, those patients with disease processes known to be associated with an increase in this phenomenon (see III-b-7). These results are summarised statistically in Table IV-14.

Table IV-14 Comparison of t max. RBCU values between different groups of patients with variable erythropoietic activity.

Group	$\bar{x}$ t(max) RBCU	SD	P
1. Normals	9.2	1.6	
2. Hypoplastic	7.6	2.8	< .15
3. Hyperplastic			
a) Effective	7.2	1.6	< .0005
b) Ineffective	11.3	1.7	< .0025

IV-6 External counting:

IV-6-a The method that I have used for external counting is detailed in the methods appendix. Due to pressure upon equipment, not all patients had external counting performed, apart from a group of patients I studied while trying to describe bone marrow iron uptake. As the patients were getting both ^{59}Fe and ^{51}Cr, I attempted initially to externally count both isotopes. To a large extent, I was unhappy with my ^{51}Cr results, mainly because I was unable to obtain statistically satisfactory count rates in the short period of time at my disposal for each individual patient. With the ^{59}Fe data however, the statistics were very good, and this is reflected in the curves obtained. My main interest in organ counting has been in bone marrow counting, in an attempt to describe the uptake of iron label by this organ. The results of this have been detailed in the appendix.

It would be of doubtful value to attempt to draw any valid conclusion from my small experience with external counting. As has been the experience of other authors however, I have found ^{59}Fe a useful label for detecting extramedullary haemopoiesis, splenic sequestration of red cells, and hypoplastic bone marrow function(404)(686)(689) and have found it to be better than ^{51}Cr as an internal label for external counting in measuring these phenomena.

CHAPTER V ASSESSMENT OF FEEDBACK, UTILISING THE HADDINGHAM-FRENCH MODEL FOR THE MEASUREMENT OF INEFFECTIVE ERYTHROPOIESIS AND EARLY RANDOM RED CELL DESTRUCTION.

V-1 The theory and generation of this model are given in appendix B.

A total of 101 patients were studied by this method. Included in this group were patients who were assessed as being haematologically normal, whose only haematological abnormality, as far as could be assessed by us, was a restriction of iron supply and a number of patients with a variety of haematological disorders.

In an attempt to assess whether feedback, as measured by us, was related in any way to other ferrokinetic values (i.e. plasma iron concentration, $t_{\frac{1}{2}}$ plasma iron clearance, erythroid iron turnover, erythropoietic index or red cell utilisation values), the data for each individual patient for feedback was plotted against each of these values, and a correlation looked for. The results are presented graphically in Figures V-1 to V-5, and the compounded data for each patient are tabulated in Table V-1.

Figure V-1 Graphical representation of the comparison between iron/100 ml. whole blood and feedback values in individual patients with different disease states.

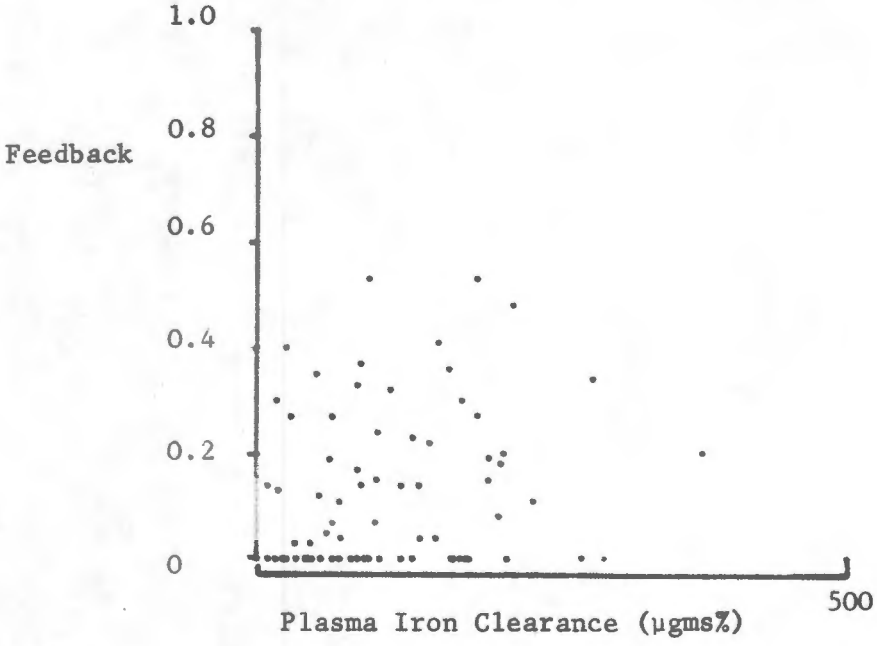


Figure V-2 Graphical representation of the comparison between $t_{\frac{1}{2}}$ Plasma Iron Clearance and feedback values in individual patients with different disease states.

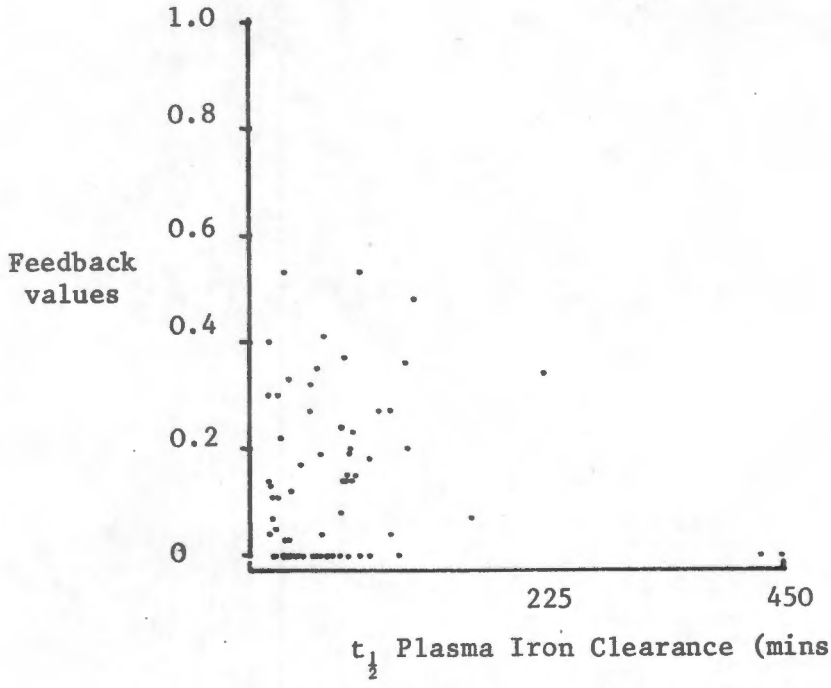


Figure V-3 Graphical representation of the comparison between Erythropoietic Indices and feedback values in individual patients with different disease states.

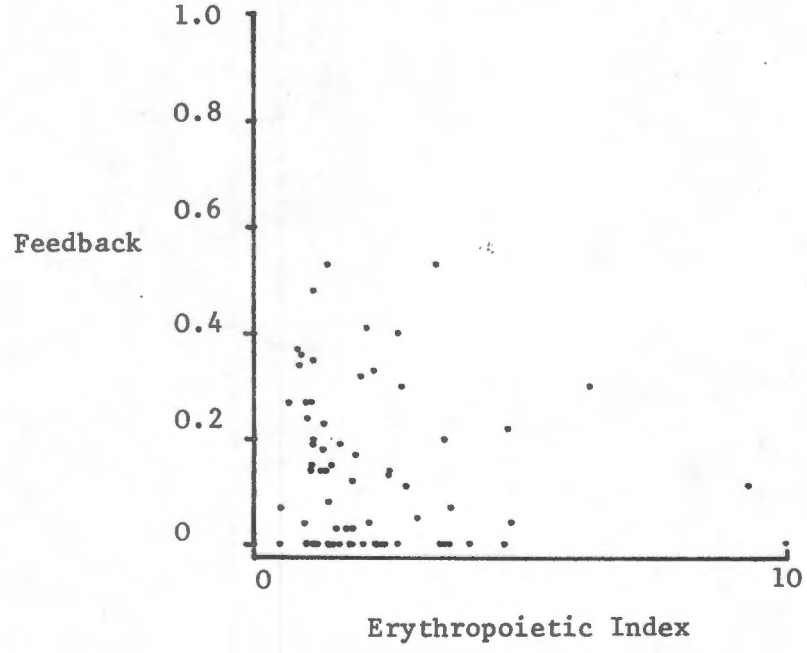


Figure V-4 Graphical representation of the comparison between erythroid iron turnover and feedback values in individual patients in different disease states.

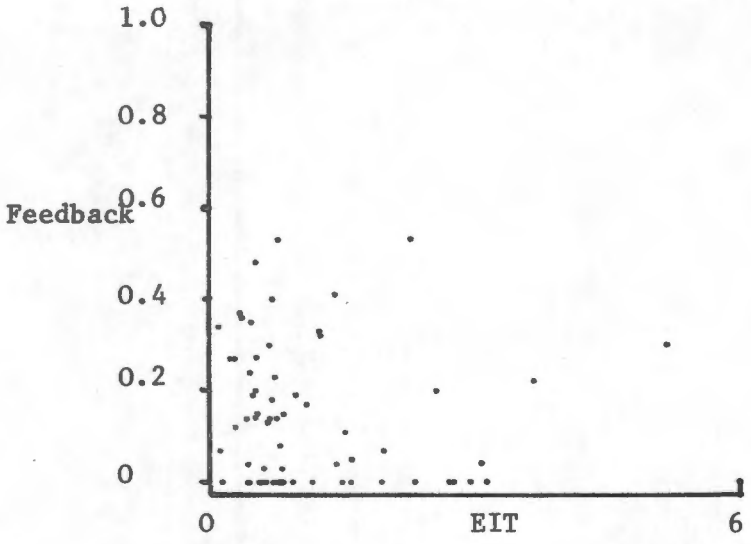


Figure V-5 Graphical representation of the comparison between Red Cell utilisation and feedback values in individual patients in different disease states.

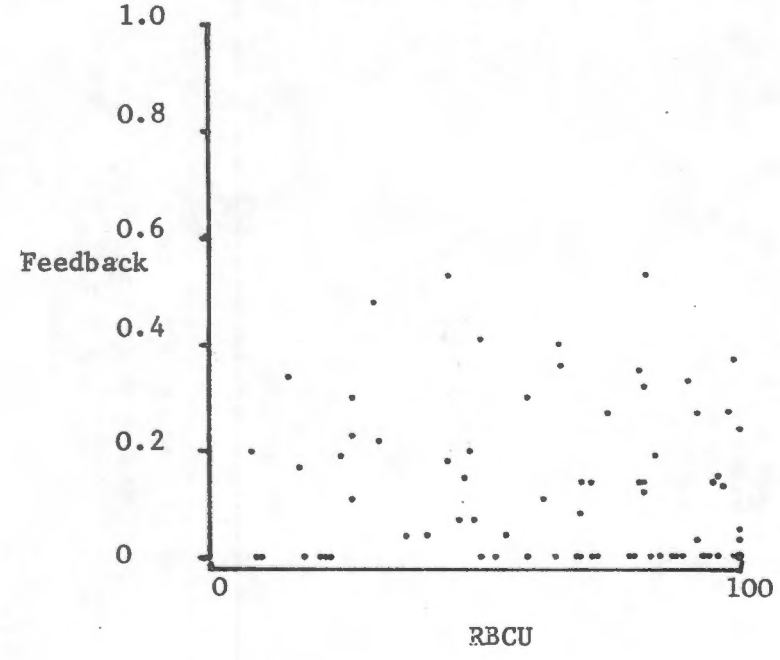


Table V-1 Tabulated values for ferrokinetic parameters and feedback values in individual patients with varying disease states.

* = Plasma Iron Concentration (µgms%)

+ = Haematocrit

** = Erythroid Iron Turnover in mgms/100 ml. whole blood/day.

Patient	PI*	Hct+	$t_{\frac{1}{2}}$PIC (mins)	EI	EIT**	RBCU (%)	f
Jan	-	44	66	-	-	83	0.00
Fis	-	42	65	-	-	96	0.00
Ase	-	44	45	-	-	72	0.00
Lou	172	47	84	1.42	0.8272	99	0.00
deB	62	43	60	1.12	0.4934	84	0.19
Key	165	41	102	1.22	0.6478	51	0.00
Joh	138	37	120	0.95	0.4381	37	0.04
Mte	132	30	29	4.06	2.9610	22	0.00
Eng	100	32	188	0.51	0.1284	50	0.07
Kot	234	33	19	9.29	8.0064	27	0.11
McN	204	62	78	1.42	0.8168	70	0.08
Web	88	52	91	0.82	0.3438	99	0.37
Smi	89	37	79	1.09	0.5376	70	0.14
Luk	209	38	134	1.14	0.5387	49	0.20
Jac	146	37	26	4.78	3.6793	32	0.22
Cup	113	30	53	2.00	1.2573	82	0.32
Car	53	38	35	1.85	0.2997	82	0.12
vNi	152	26	62	2.18	1.4604	41	0.04
Hey	64	64	28	2.06	0.8478	85	0.00
Col	180	48	34	3.59	2.7761	10	0.00
Bas	9	23	16	2.55	0.4271	95	0.14
Cha	19	36	18	2.54	0.6817	97	0.13
Sil	55	30	30	2.31	1.1792	79	0.00
Loz	9	36	34	1.17	0.1529	88	0.00
Gog	196	46	90	1.48	0.8605	48	0.15
Pep	294	34	433	0.49	0.0000	9	0.00
Lam	274	37	126	1.52	0.8018	54	0.00
vWy	137	34	86	1.36	0.7703	82	0.14
Pet	29	42	51	0.98	0.2853	92	0.27
Mag	163	46	133	0.88	0.3805	66	0.36

Table V-1 contd.

Patient	PI*	Hct+	$t_{\frac{1}{2}}$PIC (mins)	EI	EIT**	RBCU (%)	f
Tan	101	40	82	1.10	0.5579	96	0.15
Ser	217	37	140	1.14	0.5229	31	0.48
Nqw	206	49	101	1.32	0.7298	45	0.18
Wal	95	25	28	3.51	2.3379	18	0.00
Lou	85	36	44	1.91	1.1019	77	0.17
Han	71	25	17	4.84	3.0823	56	0.04
duT	284	31	249	0.85	0.1044	15	0.34
Ans	32	38	30	1.76	0.6303	100	0.03
Wil	22	33	31	1.55	0.4343	94	0.00
Pre	86	47	34	2.27	1.2565	90	0.33
Bog	86	18	40	2.43	1.5250	93	0.00
Sam	123	44	61	1.61	0.9439	87	0.00
Bro	104	28	41	2.48	1.6175	69	0.00
Gre	133	32	88	1.32	0.7404	27	0.23
Rix	16	16	20	2.33	0.6484	70	0.00
Swa	92	28	20	4.71	3.1422	23	0.00
Ash	64	35	110	0.66	0.2428	75	0.27
Jac	174	25	24	6.30	5.1842	27	0.30
vNi	212	38	57	2.71	1.9578	18	0.00
Bow	177	40	94	1.40	0.7998	80	0.00
Sco	153	27	63	2.14	1.4316	51	0.41
Jeg	45	35	33	1.86	0.8289	92	0.03
Pax	41	29	54	1.13	0.4567	73	0.00
Hoe	65	40	19	3.71	1.9847	47	0.07
Sav	71	44	25	2.86	1.5425	63	0.11
Ahr	123	39	82	1.26	0.6858	72	0.14
Cha	51	37	57	1.12	0.4724	81	0.35
Jan	96	24	29	3.44	2.2912	45	0.53
Sal	70	23	71	1.17	0.5874	96	0.00
vNi	59	38	22	3.09	1.6259	100	0.05
Lif	81	29	77	1.12	0.5634	65	0.00
Fer	46	35	33	1.87	0.8505	21	0.00
Ste	167	30	38	3.68	2.7255	60	0.00
vdM	25	31	22	2.28	0.7572	60	0.00
Kna	17	35	16	2.79	0.6800	60	0.30

Table V-1 contd.

Patient	PI*	Hct+	$t_{\frac{1}{2}}$PIC (mins)	EI	EIT**	RBCU (%)	f
Tay	26	54	17	2.71	0.7238	66	0.40
Ngo	187	45	120	1.08	0.5296	98	0.27
Wil	44	30	34	1.84	0.8254	93	0.00
Sch	33	26	31	1.82	0.7273	89	0.00
Ber	377	18	86	3.59	2.5739	8	0.20
deV	196	44	84	1.62	0.9835	25	0.19
leG	102	57	77	1.00	0.4620	100	0.24
Gor	187	44	94	1.40	0.7956	82	0.53

These figures show that there is no apparent correlation between feedback values, in this heterogenous group of patients, and any of the ferrokinetic data. The value for 'feedback' presumably measures some aspect of bone marrow function not measured by other ferrokinetic techniques.

Having shown that the feedback values of an individual was an independent measure of some function other than those measured by conventional ferrokinetic values, an attempt was made to assess the use of feedback results in describing or typifying different disease states. In the review section, I have reviewed conditions known to be associated with ineffective erythropoiesis (see chapter III-b-7). One would expect, from our description of the model, that diseases associated with a major degree of ineffective erythropoiesis or with early random red cell destruction, would be typified by high feedback values. The patients detailed in Table V-1 were accordingly split into their broad disease groups, and feedback values of the groups as a whole were analysed statistically. The results are shown in Table V-2. The groups described are

Table V-2 Feedback Results in different disease states.

Disease Group	Feedback Values			
	Mean	SD	NO	P
1) Normal	0.11	0.22	14	
2) Normal excluding S.P.	0.08	0.12	9	< .35
3) Haemolytic Anaemias	0.09	0.11	29	< .40
a)Thallassaemia	0.26	0.06	2	< .05
b) Hereditary Spherocytosis	0.17	0.17	3	< .35
c) + A.I.H.A's	0.05	0.10	5	< .25
d) Iron Deficiency	0.09	0.07	7	< .40
e) Myeloproliferative	0.05	0.07	8	< .20
4) *Aplastic				
a)	0.09	0.13	6	< .45
b)	0.18	0.15	14	< .20
5) ** Iron Deficiency				
a)	0.13	0.15	8	< .45
b)	-	-	-	-
c)	0.06	0.07	14	< .25
d)	0.13	0.14	12	< .40
6) Myelofibrosis	0.09	0.11	10	< .40
7) Reticuloses	0.12	0.22	10	< .475
8) Infection	0.11	0.14	11	< .499
9) Inflammation	0.08	0.09	8	< .35
10) Cirrhosis	0.20	0.14	10	< .15
11) ++ Meg. Anaemias	0.17	0.20	7	< .30
12) Symptomatic Prophyrics	0.14	0.13	12	< .35
13) Neoplasms	0.20	0.22	5	< .15

described separately under the individual disease groups.

Table V-2 Feedback Results in different disease states.

+ AIHA = Auto Immune Haemolytic Anaemias

++ Meg.Anaemias = Megaloblastic Anaemias

* Aplastics (a) = Clinically diagnosed true aplastic anaemias

(b) = Ferrokinetically diagnosed aplastic anaemias or presumptive clinically diagnosed patients in whom the diagnosis was not later confirmed.

** Iron deficiency (see Chapter VI-f-1)

(a) = Classical Iron deficiency anaemias

(b) = Iron deficiency unassociated with anaemia

(c) = Patients with plasma iron levels (<70µg%) behaving ferrokinetically in the same way as group (a)

(d) = Patients with low plasma iron levels (<70µg%) behaving suboptimally on ferrokinetic assessment.

Table V-2 shows that feedback values are of no real use as a basis for distinguishing different diagnostic categories of haematological diseases. The only disease state with consistently elevated feedback values was thalassaemia, a disorder known to be associated with excessive ineffective erythropoietic activity.[815][370][258][289][724][854][504][657][232].

It seemed, therefore, that analysis of ferrokinetic data by this method might have functional rather than nosological value. I accordingly examined in detail the clinical records of each individual patient and discovered that in 48 patients with feedback value greater than 0.05, all but 5 had some factor that could be incriminated as a cause of ineffective erythropoietic activity or early random red cell destruction. The details of these patients are set out in Table V-3. As can be seen, a number of the patients had conditions known to be associated with ineffective erythropoietic activity. The majority of the remaining patients admitted to alcohol abuse, many having cirrhosis or siderosis. Hines[386][387] and Chanarin[135] have shown how rapidly ethanol impairs bone marrow folate metabolism, depresses effective erythroid activity, and promotes ineffective erythropoiesis. Twenty-three of the patients studied had RBCUe/RBCUp ratios of less than 0.9 (normal 1.11 ± 0.27) of which nineteen were less than 0.85. The value of this ratio (Chapter IV-5-a) in attempting to analyse ineffective erythropoietic activity has previously been discussed. In this total group of patients (48), only six had $t_{\frac{1}{2}}{}^{51}Cr$ red cell

survival values of less than 22 days (normal mean - 1.SD) showing that haemolysis had not materially contributed to the overall feedback values or the depression of RBCUe values. A further five patients had hypoplastic erythropoiesis as assessed by EI values of less than one (i.e. normal mean - 1.SD) making a total of eleven patients in whom the depressed RBCUe values can be explained by mechanisms other than ineffective erythropoiesis.

It is interesting that some of the patients, despite elevated feedback values, had RBCUe values above RBCUp values. Most of these patients were alcoholics who had been hospitalised and put on haematinic therapy, with consequent improved RBCUe values occurring later in the course of their investigations reflecting a response to withdrawal of a toxic agent and marrow stimulation.

The patients with feedback values of less than 0.05 were also examined in an attempt to assess whether patients with ineffective erythropoiesis or random early red cell destruction were not being detected using this technique. Fifty-one patients were studied. Of these, thirty-two had increased total erythropoietic activity as assessed by an erythropoietic index exceeding 1.50 (mean normal + 1 S.D.)

Eighteen of these thirty-two patients had $t_{\frac{1}{2}}Cr^{51}$ red cell survival values of less than twenty-two days (mean normal 1.S.D.). In these patients, feedback may not have been detected because of a shift of the RBCU curve to the left (see discussion under section IV-5-d), and in fact three of these eighteen patients had E.1. values disproportionately raised relative to the degree of shortening of their red cell survival as measured by $t_{\frac{1}{2}}{}^{51}Cr$ signifying the probable existance of significant I.E.I.T.

Ten of the thirty-two patients remained who had E.1. values above 1.5, whose $t_{\frac{1}{2}}$ ^{51}Cr survival was normal. This E.1. could only be explained as a consequence of increased ineffective erythropoiesis or increased effective erythropoiesis. Two of these ten patients did not have $t_{\frac{1}{2}}$ ^{51}Cr measurements, but in one of these the E.1. was only marginally elevated (1.83) and the haematocrit was raised (54%). The other patient was cirrhotic, had porto systemic encephalopathy, and in addition a low folate level. This patient probably had increased ineffective erythropoiesis that was not detected using this feedback model.

Of the remaining eight patients only three had RBCUe/RBCUp ratios of less than 0.84 (mean normal - 1.S.D.) suggesting that in five of the eight the increased E.1 reflected an increase in effective erythropoietic activity. Two of these patients were polycythaemic and three were iron deficient on treatment with oral iron.

In the remaining three patients the increased E.1. value in combination with the decreased RBCUe/RBCUp ratio probably signified an increase in ineffective erythropoietic activity that was not detected by this feedback model. The low RBCUe values in these three patients (18%, 21% and 8%) may have made interpretation of their RBCU curves more difficult.

Table V-3 Individual patients and their feedback values. Association with disease states or toxins producing ineffective erythropoiesis or random early red cell destruction.

Group	Diagnosis	f	EIT	RBCUe/RBCUp	$t_{\frac{1}{2}}{}^{51}Cr$	Ethanol	Other
Normal	Bot:Ethanol	0.10	0.6257	0.91	28	+	
	Maj:Cirrhosis	0.36	0.3805	0.96	-	+	
	deB:Ethanol	0.19	0.1312	0.93	20	+	Fe↓
	Cor: S.Porph	0.33	0.2483	0.51	34	+	
	Hol: S.Porph	0.14	0.2896	1.35	-	+	
	Len: S.Porph	0.28	0.5330	1.67	-	+	
	Sol: S.Porph	0.06	0.1748	0.98	28	+	
	Thy: S.Porph	0.11	0.2187	1.02	24	+	
	Tan: Diabetes	0.15	0.5579	1.20	-		Nil
Haemolysis							
	Jac: Thall	0.22	3.6793	0.48	15		Thall
	Jac: Thall	0.30	5.1842	0.49	11		Thall
	Lou:Her.Sphero	0.17	1.1019	0.93	19		Hypersplen
	Pre:Her.Ovalo	0.33	1.2565	1.05	22		Nil
	Ngo:G6PD def.	0.27	0.5296	1.53	22	+	
	Gre: AIHA	0.23	0.7404	0.39	22	+	
	Saa:TB spleen	0.17	2.0160	1.09	25		Fe def.
	Bas:Fe def	0.14	0.4271	0.95	17		Fe def.
	Cha: Fe def	0.13	0.6817	0.98	18	+	Fe def.
	Sev:Cirrhosis	0.11	1.5425	0.72	17	+	Folate ↓
	Mar:My.fibrosis	0.20	2.8695	0.35	14	+	
	Kot: ? diGuglielmo's	0.11	8.0064	0.61	15	+	Sidero-blastic anaemia
	Luk:S. Porph	0.20	0.4758	0.91	18	+	

Fe↓ = Iron deficiency.

Thall = Thallassaemia

AIHA = Auto-immune haemolytic anaemia

Table V-3 contd.

Group	Diagnosis	f	EIT	RBCUe/RBCUp	$t_{\frac{1}{2}}$ ^{51}Cr	Ethanol	Other
Hypoplastic							
	duT:Apl.Anaemia	0.34	0.1044	0.50	38	+	Oxymethal-one
	vWy:Ca Breast	0.14	0.7703	1.19	24		BM infil-tration Prednisone
	Ash:CLL	0.27	0.2428	0.85	27	+	BM infil-tration Hypersplen
	Web:P.vera	0.37	0.3438	1.14	29		Cytotoxic therapy
	Vlo:Reiter's Syndrome	0.16	0.3699	0.89	-		Nil
	Koe:Buergers	0.08	0.3057	0.84	30	+	
	Pet:CVA^{COAD}	0.27	0.2853	0.95	-	+	
	Ada:Cirrhosis	0.19	0.3455	0.88	27	+	Malnutri-tion. PAS hyper-sensitivit -Ac.Haem Anaemia
	Eng:S.Porph	0.07	0.1284	0.64	-	+	
	Sco:Cirrhosis	0.44	1.4316	0.82	-	+	Folate↓
Fe Def.	Kna:My.Fibrosis	0.30	0.6800	0.61	27		Fe def.
	Tay:Polycyth.	0.40	0.7238	0.67	34	+	Cytotoxics
	Car:≠skull	0.12	0.2997	0.90	32	+	Chloro-mycetin
	Hoe:SLE	0.07	1.9847	0.53	26	+	AIHA
	Rus:Cirrhosis	0.30	0.3974	1.09	-	+	Folate
	Cha:S.porph	0.35	0.4724	0.89	30	+	Cirrhosis
Myelo-fibrosis	deV:	0.19	0.9835	0.41	30		Nil
	Smi	0.14	0.5376	0.85	26		Nil
Sidero-blastic	Bot:Sidero Achrestic Anaemia	0.69	0.8042	0.40	-		Nil
	Ber: "	0.20	2.5739	8.00	-	+	
Megalo-blastic anaemia	Jan:Intest. Lipoma	0.53	2.2912	0.59	24		B_{12} def.
Others	Ser:Myeloma	0.48	0.5229	0.60	27	+	Cytotoxics

≠ = Fracture

Table V-3 contd.

Group	Diagnosis	f	EIT	RBCUe/RBCUp	$t_{\frac{1}{2}}$ ^{51}Cr	Ethanol	Other
Other	LeG:Good pastures	0.24	0.4620	1.16	36		Nil
	vNi:Ulc. colitis	0.05	1.6259	1.11	15		Blood loss (Stress)

From Table V-3 it can be seen that, in each patient with feedback values exceeding 0.05, there was a good reason to suspect that ineffective erythropoiesis or random red cell destruction was present. In many cases this was not shown by other methods of analysis.

Obviously, if we were able to predict normal RBCUe values on the basis of plasma iron levels and variations of erythropoietic activity, as discussed in appendix B, feedback analysis would be more quantitative. It does seem, however, to give a measure of abnormal erythropoietic activity, in individual patients, that can be roughly quantitated, and used as an index of early erythroblast or erythrocyte destruction. This is basically what I have attempted to obtain, and I suggest that this analytical technique is of some use in its present form. Should we be able to develop the model further, as previously stated, this should be not only an indicator, but also a quantitator of early erythroid destruction. Present methods of assessing ineffective erythropoietic activity are to a large extent unsatisfactory. This model may be of use in the quantitation of this phenomenon.

CHAPTER VI AN EMPIRICAL APPROACH TO THE ANALYSIS OF FERROKINETIC DATA IN NORMAL PATIENTS AND PATIENTS WITH DIFFERENT DISEASE STATES.

VI-a The aim of this chapter is to define empirically the ferrokinetic performance of different disease groups. A total of one hundred and sixty patients have been studied, some of whom had more than one disorder. Those with multiple diagnoses have either been included only in that group corresponding to the major diagnoses, or they have been categorised under more than one disease process. All the ferrokinetic parameters have been statistically compared with those of normal individuals as described in Vl-b.

VI-b Normal subjects.

VI-b-1 Definition:

Subjects in this category were judged, on clinical grounds, to be haematologically normal. This group of patients is not identical to those referred to in Chapter III, as iron deficient patients were not included. Apart from ward patients, a number of ambulant volunteers were studied. The haematological criteria of normality used were those described by Wintrobe,[897] and set out in Table VI-1. Where all of these basic haematological parameters were not available, the patient was accepted as being normal if the haemoglobin, haematocrit, white cell count and peripheral smear were normal. Patients with Symptomatic Porphyria were included as we have previously shown that their ferrokinetic performance is normal.[274]

VI-b-2 The collective results of this group of patients can be seen in Table VI-2. For purposes of analysis, it is obvious that there were inadequate numbers of normal female patients to draw any definite conclusions from our ferrokinetic findings

in this group. For this reason the total group of patients has been used to define statistical criteria for ferrokinetic normality. Only the erythropoietic index and the ratio of experimentally derived to predicted red cell utilisation figures were influenced by the plasma iron concentration.

Table VI-1 Normal Haematological Values.(Wintrobe 897)

		Male	Female
1.	Haemoglobin (Gm.%)	16 ± 2	14 ± 2
2.	Haematocrit (%)	47 ± 5	42 ± 5
3.	Mean Corpuscular Volume (c.u.)	87 ± 5	87 ± 7
4.	Mean Corpuscular Haemoglobin Concentration (%)	34 ± 2	34 ± 2
5.	White Cell Count (cells/mm^3)	7.8 ± 3.0 x 10^3	7.8 ± 3.0 x 10^3
6.	Normal Peripheral smear		
7.	Normal Platelet Counts	150 - 450 x $10^3/mm^3$	

VI-b-2 Results:

1. Blood volume estimation:-

These are expressed on a volume per unit body weight basis (i.e. ml/kg.) That this is not an ideal way of comparing blood volume in different individuals is well known and the problem has been extensively discussed.(16)(291)(417)(827)(712)(676)(439)(634).

For the purpose of a routine laboratory service however, the reporting of blood volumes in relation to body weight has the advantage of being easily understood and interpreted by physicians requesting the estimations, and is the most simple way of reporting the blood volumes comparatively. For these reasons I have chosen to adhere to this convention. My results are tabulated in Table VI-2, and, in general, show reasonable agreement with published values(797)(696)(856)(872)(55)(320)(271)(763)(272)(289)(815)(415)(74). i.e. red cell volume 26.6 ± 5.1 ml/kg. plasma volume 40.9 ± 7.0 ml/kg. total blood volume 67.8 ± 10.3 ml/kg.

VI-b-3 Of interest to me was the relationship between body haematocrit, calculated as $\frac{\text{red cell volume}}{\text{total blood volume}} \times 100$, and the venous haematocrit. This value has been variously published as being 0.864 to 0.96, depending upon the method used to measure the various volumes.(698)(219)(200)(812)(813)(137)(74)(815)(625)(320)(705)(512)(856)(253)(317)(86)(439)(534) This ratio is obviously of some importance in ferrokinetic calculations, as the correction factor for the venous haematocrit in order to reduce it to body haematocrit values is introduced into calculations

of PIT, NEIT and EIT. I have used the correction factor suggested by Cook.[164] Obviously, this correction factor is not absolute, and will vary with the method used in calculating the various volumes, as well as in different disease states, particularly the "big spleen" syndromes where red cell rich blood appears to be sequestered. For routine purposes it would seem reasonable to use a constant correction factor in order to eliminate errors that may be introduced when measuring the various volumes. In fact, this procedure may not be justified, as individual values may vary fairly widely. In my subjects I have found that the mean ratio of body to venous haematocrit is 0.85 $\pm$ 0.09, which agrees reasonably well with published values when plasma volume is measured by the ^{59}Fe transferrin technique.

VI-b-4 2. Plasma Iron, Total Iron Binding Capacity and Percentage Saturation of Transferrin Values in Normal Patients.

The results in my normal group of patients are undoubtedly influenced by the inclusion of patients with symptomatic porphyria. These patients are known to be generally iron overloaded.(844)(491) My mean figures in this group compares reasonably well with published normal results.(794) (663)(662)(503)(315)(87)(77)(691)(690)(702)(729)(783)(756) (122)(502)(500)(378)(607)(673)

The values that I have obtained are:-

Plasma Iron Concentration:-	111.1 ± 40.7 µg%
Total Iron Binding Capacity	277.5 ± 61.3 µg%
Percentage Transferrin Saturation	42.3 ± 21.1%

VI-b-5 3. Ferrokinetic measurements.

Ferrokinetic data from normal individuals are summarised in Table VI-3.

These show a certain amount of variability due to inter alia, variation in plasma iron concentration, intrinsic biological variation, and technical sources of error.

Comparison of my values with published values shows good agreement.(872)(521)(164)(107)(735)(80)(863)(673)

TABLE VI-2 COMPOSITE FERROKINETIC FINDINGS IN NORMAL PATIENTS.

NAME	DIAGNOSIS	Hb G%	PCV %	B.PCV / P.PCV	Bl.Vol ml/kg	RBC Vol. ml/kg	Pl. Vol.	P.I. mg%	% Sat	$t_{\frac{1}{2}}$ PIC (mins)	EI	PIT	NEIT	EIT	RBCUe %	RBCUe / RBCUp	$t_{\frac{1}{2}}$ (days) ^{51}Cr	f
Bot Transient CVA		17.2	51	0.79	64.5	26.1	38.4	114	58	70	1.24	.8644	.2118	.6527	75	0.91	28	0.10
Bot Myoc.Infarct.		17.9	47	0.74	68.8	23.9	44.9	55	24	86	0.72	.3604	.1085	.2519	-	-	-	-
Cra C.V.A		17.6	51	0.93	78.9	37.6	41.3	175	41	130	0.89	.7145	.3251	.3894	-	-	-	-
deV ? Stress Polycyth.		18.0	52	0.85	63.7	28.0	35.7	84	21	60	1.21	.7138	.1506	.5631	-	-	-	-
deV Rec.peptic ulcer		15.3	43	0.77	64.9	21.6	43.3	64	18	46	1.48	.8501	.1354	.7148	-	-	-	-
Isa Myoc. infarct.		16.8	45	0.80	64.0	23.0	41.0	120	-	69	1.39	.8517	.2051	.6466	-	-	-	-
Mag Conval.TB Men.		16.1	46	0.90	65.6	27.1	38.5	163	90	133	0.87	.7096	.3291	.3805	66	0.96	-	0.36
Mar Epilepsy		15.9	48	0.71	60.2	20.4	39.8	60	-	124	0.51	.4518	.1954	.2564	-	-	-	-
Moo COAD		17.6	50	0.86	60.6	26.1	34.5	80	24	79	0.91	.5468	.1512	.3956	-	-	-	-
Nor Polycystic kidneys		16.4	47	0.87	57.2	23.5	33.7	112	-	64	1.40	.9933	.2225	.7708	-	-	-	-
Smi Myoc. infarct.		18.0	50	0.73	55.0	20.0	35.0	101	36	68	1.20	.8009	.1909	.6100	-	-	-	-
Sep COAD		16.5	47	0.78	70.3	25.9	44.9	67	26	83	0.81	.4582	.1331	.3251	-	-	-	-
Sch Normal		18.0	48	0.83	55.0	22.0	33.0	94	31	73	1.10	.7143	.1825	.5318	-	-	-	-
Vyw COAD		17.1	47	0.83	60.7	23.8	36.9	99	41	86	0.97	.6526	.1967	.4560	-	-	-	-
Jan Conval. Fracture		15.2	44	0.98	67.5	29.3	38.2	-	-	66	-	-	-	-	83	-	35	0.00
Fis Conval. Fracture		14.1	42	0.97	66.0	27.0	39.0	-	-	65	-	-	-	-	96	-	27	0.00
Ase Conval. Fracture		15.2	44	0.81	74.0	26.0	48.0	-	-	45	-	-	-	-	72	-	22	0.00
Lou Normal		16.5	47	0.96	72.6	32.5	40.1	172	58	84	1.42	1.1716	.8272	.3445	99	1.46	25	0.00
deB Normal		15.6	43	0.91	73.5	28.8	44.7	62	22	60	1.12	.6245	.4934	.1312	84	0.93	20	0.19
Cor Symp. Porphyric		15.3	46	0.91	59.5	25.0	34.5	123	36	73	1.32	.9719	.7236	.2483	40	0.51	34	0.33
Hol Symp. Porphyric		15.6	44	0.95	85.8	35.8	50.0	139	54	110	0.97	.7521	.4626	.2896	100	1.35	-	0.14
Len Symp. Porphyric		14.7	46	0.94	93.2	40.5	52.7	264	96	130	1.28	1.1713	.6384	.5330	80	1.67	-	0.28
Sol Symp. Porphyric		14.7	47	0.90	56.0	23.7	32.3	88	33	130	0.60	.3842	.2094	.1748	83	0.98	28	0.06
Sam Symp. Porphyric		16.1	44	0.84	68.9	25.4	43.5	123	43	61	1.61	1.2002	.9439	.2562	87	1.13	26	0.00
Thy Symp. Porphyric		18.6	51	0.94	54.0	26.0	28.0	189	63	72	1.69	1.3934	1.0422	.3511	79	1.18	-	0.00
Thy Symp. Porphyric		14.7	44	0.83	90.8	33.0	57.8	105	57	85	1.04	.7352	.5165	.2187	83	1.02	24	0.11
Mal Vestib. Neuronitis		17.3	50	0.76	63.5	24.0	39.5	59	24	62	1.00	.5147	.1115	.4032	-	-	-	-
Hof Normal		15.6	45	0.61	76.3	21.0	55.3	151	69	98	1.14	.9052	.3092	.5960	-	-	-	-
Kho C.V.A.		15.6	45		74.1	26.8	37.3	55	24	51	1.22	.6320	.1128	.5192	99	1.08		-
Tan Diabetes		14.5	40		57.3	20.7	36.6	101	36	82	1.10	.7813	.2234	.5579	96	1.20		0.15
Arm Normal		12.6	38		80.3	31.4	48.9	92	32	62	1.39	.9651	.2094	.7557	90	1.10		-
MEAN				0.85	67.8	26.6	40.6	111.1	42.3	80.9	1.13	.7816	.3415	.4401	83	1.1054	27.0	0.11
S.D				0.09	10.3	5.1	7.0	40.7	21.1	25.7	0.29	.2554	.2653	.1821	15	.2741	4.7	0.22

CVA = Cerebrovascular accident
Myoc. Infarct = Myocardial Infarction
COAD = Chronic obstructive airways disease
Units for PIT, NEIT, EIT = mg/100 ml. whole blood/day.

Table VI-3 Normal Ferrokinetic Values.

Abbreviation		Mean	S.D.	Units
($t_{\frac{1}{2}}$PIC	$t_{\frac{1}{2}}$Plasma Iron Clearance	80.9	25.7	Minutes
(E.I.)	Erythropoietic Index	1.13	0.29	-
(P.I.T.)	Plasma Iron Turnover	0.78	0.2	mg/100 ml.whole blood/day
(NEIT)	Non Erythroid Iron Turnover	0.34	0.27	mg/100 ml. whole blood/day
(EIT)	Erythroid Iron Turnover	0.44	0.18	mg/100 ml. whole blood/day
(RBCUe)	Experimental Red Cell Utilisation	83	15	percent of administered ^{59}Fe
(RBCUe/RBCUp)	Ratio Experimental to Predicted RBCU	1.11	0.27	-

VI-b-6 (a) $t_{\frac{1}{2}}$ Plasma Iron Clearance:- ($t_{\frac{1}{2}}$PIC)

Most authors[872][521][164][107][735][80][863][673] give normal values for this variable ranging from sixty to ninety minutes[872] to seventy-four to one hundred and sixteen minutes.[673]

This range of normal values presumably reflects differences in the iron status of the different population groups used as controls. The values still correlate well with mine i.e. 80.9 ± 25.7 minutes.

VI-b-7 (b) The Erythropoietic Index.

This index as discussed earlier (Chapter III) reflects the effects of total erythropoietic activity, independent of plasma iron concentration, upon the $t_{\frac{1}{2}}$P.I.C. I have previously shown it to be a fairly reliable index of total erythropoietic

function, and the narrow limits of normality found in this study show how useful it's measurement in detecting variations in total erythropoietic activity is.

VI-b-8 (C) The Plasma Iron Turnover (PIT)

This, as previously discussed, measures the sum of erythroid and non-erythroid iron turnover without discrimination. For this reason it would appear not to be as useful as the erythroid iron turnover in describing total erythropoietic activity. My figures agree fairly closely with normal values published by a number of authors.(872)(107)(673)(80)(164).

VI-b-9 (d) Non Erythroid Iron Turnover (NEIT)

Calcualtion of the NEIT has recently been introduced into the ferrokinetic assessment of an individual patient by Cook.(164) As previously described (Chapter III) we have derived similar equations to describe this valuation. It would appear that it is almost totally dependent upon variations in plasma iron concentration, but, as with most biological measurements, there is probably a fair amount of normal individual variation. This is probably greater in different disease states. It also appears reasonable to postulate some deviation from linearity in the relationship between plasma iron concentration and NEIT at extremes of plasma iron concentrations. At extremely low plasma iron concentrations one would, as previously discussed, expect no NEIT. At very high plasma iron concentrations one would expect NEIT to be limited by the rate at which cellular mechanisms handling NEI uptake can occur.

In the range of plasma iron concentrations normally seen, the effect of this variation should be minimal. I have some reservations however about applying this linear relationship to some of the disease states, particularly the aplastic anaemias. As a general measurement however, this function probably approximates reasonably closely the situation found in the body, and because of this, is useful in calculating erythroid iron turnover values.

VI-b-10(e) Erythroid Iron Turnover (EIT)

This appears to be an excellent measure of total erythropoietic activity with a narrow range for normal patients. My values for normal EIT agree fairly well with those of Cook,[164] although the range quoted by him is far smaller than I have found. This presumably reflects better patient selection and suggests that my range of normality is too large.

VI-b-11 (f) The Red Cell Utilisation (RBCUe)

My values for RBCUe agree well with published values[164][872][107][815][443][673][211][74] with a slightly greater range than in most series. Again this presumably reflects differences in patient selection. I believe that the RBCU is, in normal individuals, markedly dependent upon variations in plasma iron concentration, and believe that the ratio of observed RBCU values, when compared with predicted RBCU values (as previously described) eliminates errors due to variable plasma iron levels. The values found are tabulated in Table VI-3.

VI-b-12 4. Red Cell Survival Measurement.

As detailed under methods, I have used a least squares fit for ^{51}Cr data as a function of time, plotted on semilog paper. In this way I have calculated a $t_{\frac{1}{2}}$ ^{51}Cr red cell survival figure for individual patients. Recently, Garby and Mollison[285]

have stressed the variation in reported $t_{\frac{1}{2}}$ ^{51}Cr data using similar methods in different laboratories. Numerous authors[470][753][219][223][222][218][165][407][203][200][198][513][398][561][666][667][825][821][812][813][470][704][443] have postulated methods of correcting ^{51}Cr data for elution in order to measure mean red cell lifespan. My experience with these techniques has been unhappy, and I have decided that the simplest way to report these figures is to use the best semilog least squares fit for the data in order to calculate a $t_{\frac{1}{2}}$ value. While this does not give a direct measure of red cell lifespan, it does give one a comparative measure of red cell longevity, is simple to perform and relatively easy to interpret. I agree that this method is not perfect, but it at least eliminates other potential sources of error from data manipulation. As elution seems to vary in different disease states as well as between normal individuals, it seems easier not to attempt too close an analysis of this relatively inaccurate data. The values obtained in this group of normal patients agree well with other published normal results[470][323][107][152][430][710][513][598][217][753][200][873][872][443][673][198] which vary from twenty-four to forty-two days. In my patients a mean value of 27 days was found, with a standard deviation of 4.7 days.

VI-c Ferrokinetic Data in Patients with Haemolytic Anaemias.

VI-c-1 Selection of patients:-

Two different criteria were used in selecting patients for this study. The first group of patients were selected clinically on the basis of diagnosis being made of haemolytic anaemia with a disease process known to be associated with haemolysis. Included in this group are patients with

thalassaemia, hereditary spherocytosis, auto-immune haemolytic anaemias (AIHA's) (although this group has red cell survival measurements in addition to positive Coombes tests).

The second group of patients was selected on the basis of ^{51}Cr red cell survival data. Patients included in this group were those with $t_{\frac{1}{2}}$ ^{51}Cr red cell survival values of less than twenty-two days (i.e. normal mean - one standard deviation). In a number of these patients, conditions known to be associated with haemolysis were present. In some patients, no obvious cause of haemolysis was found by the clinicians looking after the patient. An attempt has been made to subdivide the patients into various clinical groups. (See Table VI-4).

VI-c-2 Results

These are summarised in Tables VI-4 to VI-12. As a group, these patients showed the features that one would expect in association with increase in total erythropoietic activity. However, the findings varied between different disease groups, and these will be discussed under the individual tests.

VI-c-3 Plasma Iron Concentration

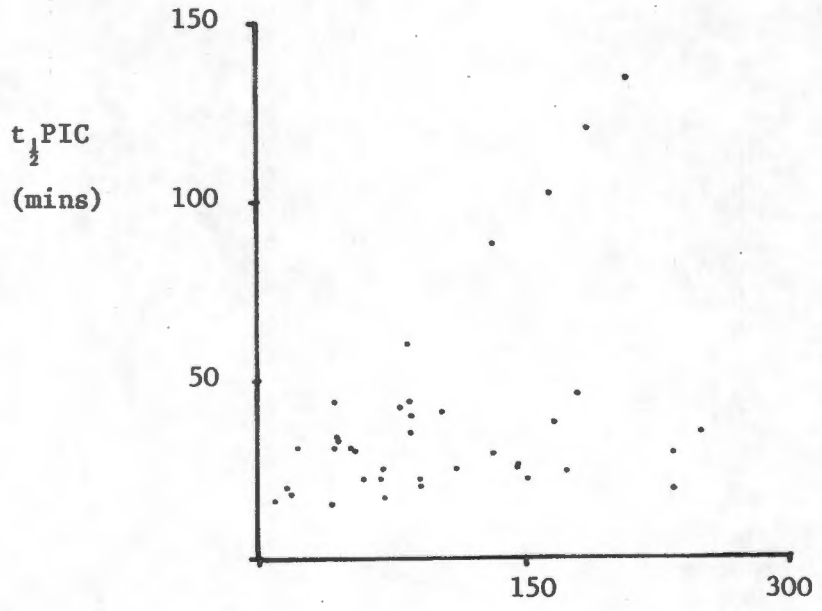
In all the haemolytic patients, taken as a single group, plasma iron concentrations were not significantly raised above the values of normal patients (P = less than .35 Table VI-5). Two groups of haemolysing patients studied by us did, however, have plasma iron levels significantly different to normal values, these being the Thalassaemic patients and patients with iron deficiency associated with haemolysis. The high plasma iron levels in patients with Thalassaemia possibly reflects

their general state of iron overload as a result of multiple previous transfusions and chronically excessive iron absorption secondary to increased total erythropoietic activity.

VI-c-4 $t_{\frac{1}{2}}$ Plasma Iron Clearance Values (See Table VI-6).

Shortening of the $t_{\frac{1}{2}}$ PIC occurred in all states associated with haemolysis, and appeared to reflect the increase in total erythropoietic activity. The most marked shortening of $t_{\frac{1}{2}}$PIC values occurred in the group of patients with iron deficiency. This presumably reflects the avidity of their iron starved marrow for iron rather than an absolute increase in erythropoietic activity, as the EIT values in this group of patients were only slightly above normal values. The fact that this was present, i.e. raised EIT values, shows how rapid is the plasma iron turnover in these patients, as their erythropoietic activity is known to be limited by shortage of precursor iron.(385) The literature uniformly reports shortening of the $t_{\frac{1}{2}}$PIC in conditions associated with haemolysis,(258)(872)(55)(815)(635)(159)(486)(363) this shortening appearing to represent an increase in total erythropoietic activity in these patients. Shortening of the $t_{\frac{1}{2}}$PIC is, however, not specific for haemolytic states, occurring also in conditions associated with relatively normal red cell survival but grossly increased ineffective erythropoietic activity(288)(872)(387)(636)(36)(253)(55)(635)(815)(159)(62)(61)(486)(863) I have found the $t_{\frac{1}{2}}$PIC to be completely independent of plasma iron concentration in this group of patients (see Figure VI-1) with haemolytic anaemias.

Figure VI-1 Relationship between $t_{\frac{1}{2}}$PIC and plasma iron concentrations in diseases associated with haemolysis.



This seems to be the only group of patients in which this relationship is lost. This may reflect the rapid recycling of iron from early erythroblast or erythrocyte destruction which may raise the plasma iron level.(387)(36)(683)(60)

If the effect of ineffective erythropoiesis could be excluded, a relationship between these two parameters would probably be found, i.e. between plasma iron concentrations and $t_{\frac{1}{2}}$ plasma iron clearance.

TABLE VI-4 Ferrokinetic findings in Patients with Haemolytic Anaemias.

Name	Hb	Hct	B PCV / V PCV	PI	$t_{\frac{1}{2}}$PIC	EI	PIT	EIT	RBCUe	RBCUe / RBCUp	$t_{\frac{1}{2}}$ ^{51}Cr	f
1. Thallassaemics.												
Ism	9.4	30	0.96	234	29	6.27	5.8419	5.2490	-		-	-
Ism	6.9	21	0.90	250	35	6.00	5.7629	5.0569	-		-	-
Jac	9.0	32	0.90	146	26	4.77	4.0399	3.6793	32	0.48	15	0.22
Jac	8.8	25	1.10	174	24	6.29	5.6532	5.1842	27	0.49	11	0.30
2. Heriditary Spherocytosis												
Kea	10.6	29	1.00	-	-	-		-	59		10	-
Lou	13.7	36	0.98	85	44	1.91	1.3009	1.1019	77	0.93	19	0.17
Pre	17.6	47	0.73	86	34	2.26	1.4273	1.2565	90	1.05	22	0.33
Sto	11.7	28	0.96	146	25	5.15	4.3356	3.9562	31	0.48	10	0.00
3. G.6.P.D. deficiency.												
Ngo	17.2	45	0.67	187	120	1.08	.9132	.5296	98	1.53	22	0.27
Rob	9.1	28	0.64	80	42	2.05	1.4141	1.2062	97	1.18	17	0.00
4. A.I.H.A's												
Swa	8.0	28	0.57	92	20	4.69	3.3812	3.1422	23	0.29	20	0.00
Ste	10.0	30	0.91	167	38	3.67	3.1486	2.7255	60	1.00	-	0.00
Bor	7.7	18	1.10	86	40	2.42	1.7762	1.5250	93	1.21	10	0.00
Gre	11.7	32	0.91	133	88	1.32	1.0688	.7404	27	0.39	22	0.23
5. Fe deficiencies.												
Saa	10.1	33	0.95	69	22	3.46	2.1814	2.0160	94	1.09	25	0.17
Wil	11.0	33	1.00	22	31	1.54	.4879	.4343	94	0.96	17	0.00
Bas	8.1	23	1.28	9	16	2.53	.4519	.4271	95	0.95	17	0.14
Cha	10.9	36	0.90	19	18	2.52	.7261	.6817	97	0.98	18	0.13
Rix	5.1	16	2.64	16	20	2.31	.6962	.6484	70	0.71	15	0.00
Cad	8.2	23	0.73	42	15	4.21	2.1452	2.0295	81	0.88	20	-
Sev	14.0	44	0.86	71	25	2.85	1.6904	1.5425	63	0.72	17	0.11
vNi	11.7	38	0.75	59	22	3.08	1.7603	1.6259	100	1.11	15	0.05

TABLE VI-4 contd.

Name	Hb	Hct	$\frac{B\ PCV}{V\ PCV}$	PI	$t_{\frac{1}{2}}$PIC	EI	PIT	EIT	RBCUe	$\frac{RBCUe}{RBCUp}$	$t_{\frac{1}{2}}{}^{51}Cr$	f
6. Myeloproliferatives.												
vNi	9.0	26	0.68	152	62	2.18	1.8652	1.4604	41	0.66	22	0.04
Key	12.4	41	0.79	165	102	1.21	1.0075	.6478	51	0.77	17	0.00
Mte	9.6	30	0.91	132	29	4.05	3.2954	2.9610	22	0.32	6	0.00
Rob	12.2	42	0.89	180	46	2.84	2.4010	2.0145	23	0.37	9	-
Mar	9.0	27	0.82	91	22	4.27	3.1089	2.8695	28	0.35	14	0.20
Han	23.0	58	0.77	112	25	3.19	2.0895	1.9066	49	0.58	20	-
Han	8.4	25	1.90	71	17	4.82	3.2737	3.0823	56	0.67	13	0.04
Han	12.4	37	0.82	53	31	2.09	1.1277	1.0053	55	0.60	11	-
Bro	10.4	28	0.90	104	41	2.48	1.8878	1.6175	69	0.91	17	0.00
Wil	9.2	30	1.80	44	34	1.83	.9369	.8254	93	1.01	16	0.00
Koc	8.6	25	1.02	85	60	1.52	1.0762	.8479	29	0.36	9	-
7. Thrombocytopaenias												
Kot	10.3	33	0.64	234	19	9.27	8.5767	8.0064	27	0.61	15	0.11
Dav	10.6	35	0.72	43	31	1.93	.9285	.8264	84	0.90	14	-
8. Others												
Shu	9.6	30	0.90	55	30	2.30	1.3185	1.1792	79	0.89	12	0.00
Jeg	11.4	34	0.91	45	33	1.86	.9371	.8289	92	1.00	19	0.03
Wil	7.3	20	0.89	43	44	1.48	.8048	.6820	54	0.59	17	-
Luk	12.8	38	1.02	209	134	1.14	1.0144	.4758	49	0.91	18	0.20

Table VI-5 Plasma Iron Concentrations compared between normal patients and patients with different haemolytic states.

Group	N	$\bar{x}$	Plasma Iron S.D.	P
1. Normals	28	111.1	40.7	
2. All haemolytics	38	105.0	66.0	< 0.35
3. Thalassaemias	4	201.L	49.2	< .0025
4. Hereditary Spherocytosis	3	105.7	34.9	< .45
5. A.I.H.A's	5	119.5	37.9	< .35
6. Fe Deficiencies	9	38.9	23.6	< .0005
7. Myeloproliferative	12	108.1	45.0	< .45

Table VI-6 $t_{\frac{1}{2}}$PIC values compared between normal patients and patients with different haemolytic states.

Group	N	$\bar{x}$	$t_{\frac{1}{2}}$PIC S.D.	P
1. Normals	31	80.9	25.7	
2. All haemolytics	38	39.3	27.8	< .0005
3. Thalassaemias	4	28.5	4.8	< .0005
4. Hereditary spherocytosis	3	34.3	9.5	< .0005
5. A.I.H.A's	5	46.5	29.1	< .01
6. Fe Deficiency	9	22.2	5.9	< .0005
7. Myeloproliferative	12	42.6	24.5	< .0005

Table VI-7 E.I. values compared between normal patients and patients with different haemolytic states.

Group	Erythropoietic Index			
	N	$\bar{x}$	S.D.	P
1. Normals	28	1.13	0.29	
2. All haemolytics	38	3.13	1.79	< .0005
3. Thalassaemics	4	5.83	0.72	< .0005
4. Hereditary spherocytosis	3	3.11	1.78	< .05
5. A.I.H.A's	4	3.03	1.47	< .01
6. Fe deficiency	8	2.81	0.80	< .0005
7. Myeloproliferative	11	2.77	1.99	< .005

Table VI-8 P.I.T. Values compared between normal patients and patients with different haemolytic states.

Group	Plasma Iron Turnover			
	N	$\bar{x}$	S.D.	P
1. Normals	28	0.7816	0.2554	
2. All haemolytics	38	2.2593	1.8075	<.0005
3. Thalassaemics	4	5.3245	0.8599	<.0005
4. Hereditary spherocytosis	3	2.3546	1.7168	<.10
5. A.I.H.A's	5	2.3437	1.1063	<.0025
6. Fe deficiency	9	0.6581	0.1941	<.10
7. Myeloproliferative	12	2.0063	0.9197	<.0005

Table VI-9 E.I.T. values compared between normal patients and patients with different haemolytic states.

Group	Erythroid Iron Turnover			
	N	$\bar{x}$	S.D.	P
1. Normal	28	0.4401	0.1821	
2. All haemolytics	38	1.9999	1.6902	<.0005
3. Thalassaemics	4	4.7924	0.7463	<.0005
4. Hereditary spherocytosis	3	2.1049	1.6052	<.05
5. A.I.H.A's	5	2.0333	1.1013	<.0025
6. Fe Deficiency	9	0.6079	0.1785	<.01
7. Myeloproliferative	12	1.7489	0.9016	<.0005

Table VI-10 RBCUe values compared between normal patients and patients with and different haemolytic states.

Group	N	$\bar{x}$	RBCU experimental S.D.	P
1. Normal	17	83	15	
2. All haemolytics	37	62.4	27.3	< .0005
3. Thalassaemics	2	29.5	3.5	< .0005
4. Hereditary Spherocytosis	4	64.3	25.6	< .10
5. A.I.H.A.'s	5	50.8	32.7	< .025
6. Fe Deficiency	9	89.4	10.1	< .15
7. Myeloproliferative	12	46.9	21.6	< .0005

Table VI-11 Ratio RBCUe/RBCUp values compared in normal patients and patients and different haemolytic states.

Group	N	$\bar{x}$	Ratio RBCU experimental / RBCU predicted. S.D.	P
1. Normal	14	1.11	0.27	
2. All haemolytics	36	0.78	0.30	<.0005
3. Thalassaemics	2	0.49	0.00	<.0005
4. Hereditary spherocytosis	3	0.82	0.30	<.10
5. A.I.H.A.'s	5	0.72	0.45	<.05
6. Fe Deficiency	9	0.95	0.13	<.05
7. Myeloproliferative	12	0.60	0.24	<.0005

Table VI-12 $t_{\frac{1}{2}}{}^{51}Cr$ red cell survival data compared in normal patients and patients with different haemolytic states.

Group	N	$\bar{x}$	$t_{\frac{1}{2}}{}^{51}Cr$ survival S.D.	P
1. Normal	10	27.0	4.7	
2. All haemolytics	36	16.0	4.5	< .0005
3. Thalassaemics	2	13.0	2.8	< .0005
4. Hereditary Spherocytosis	4	15.2	6.3	< .005
5. A.I.H.A.'s	4	17.3	6.4	< .0125
6. Fe Deficiency	9	17.6	3.3	< .0005
7. Myeloproliferative	12	14.0	5.0	< .0005

VI-c-5 The Erythropoietic Index:

Values for this index and a comparison between normal patients and patients with haemolysis, are shown in Table VI-7. In all groups the erythropoietic index is elevated, reflecting the increase in total erythropoietic activity. In patients with iron deficiency (2.81 $\pm$ 0.80, P = <.0005), I would have expected this value to be smaller on the basis of EIT values (0.6079 $\pm$ 0.1785 P = <.01) and the known restriction imposed on total bone marrow erythropoiesis by limited iron supply. The anomaly is perhaps partially explained by the avidity of the iron starved bone marrow for iron, giving a falsely elevated EI value. On the other hand, total erythropoietic activity and ineffective erythropoietic activity may be raised in iron deficient states[258][164][106][687][688][726][727] and this elevated EI value may reflect this.

In normal patients, the erythropoietic index should, in some way, be related to the haematocrit or to the haemoglobin level. It should increase as the haematocrit or haemoglobin level decreases in an attempt to compensate. If iron supply to the bone marrow remained adequate (i.e. 70µg/100 ml), as well as the other essential haemoglobin building blocks, the relationship between EI and haematocrit should be linear. We have not performed a study of this relationship, as has been done by Hillman[385] but the attempt would be warranted in an effort to detail a normal EI value at various haematocrit levels. Any raising of EI values above this normal response would tend to suggest the presence of ineffective erythropoietic activity which could, perhaps, be reasonably quantitated by

this method. Hillman[385] failed to define this mathematical relationship.

In Figures VI-2 and VI-3, two graphs are presented showing E.I. values plotted against haematocrit values. In the first graph, normal patients as defined in section VI-b, as well as normal patients as defined in Chapter III are included. In the second graph, the results in the haemolytic group of patients are plotted (Figure VI-3).

Figure VI-2 Comparison of Haematocrit and E.I. values in normal patients (see text) from Table VI-2.

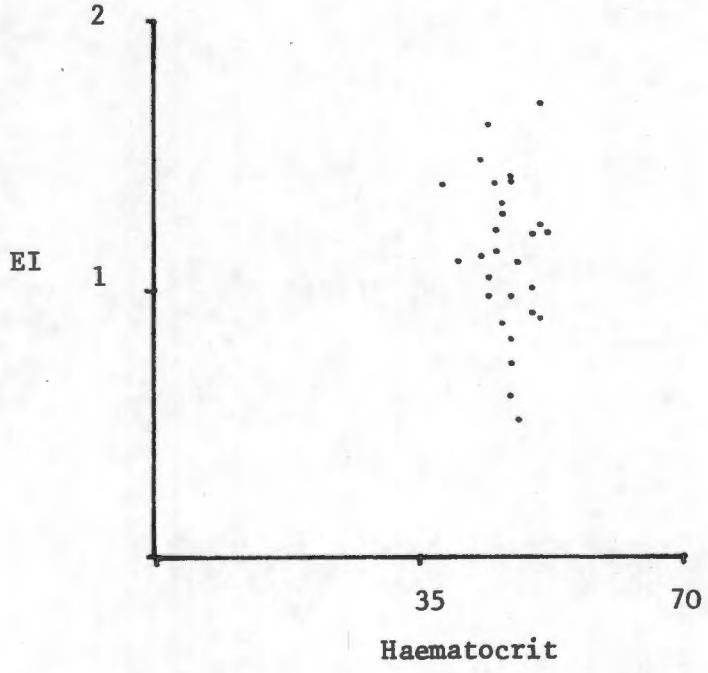
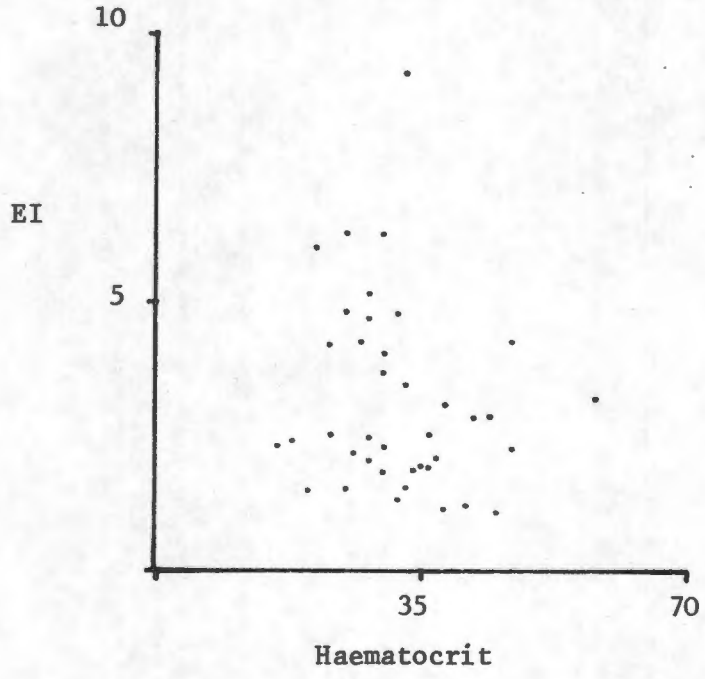


Figure VI-3 Comparison of Haematocrit and E.I. values in patients with different haemolytic disease states (from Table VI-4)



As can be seen, in a number of patients, the E.I. values at similar haematocrit values are much greater than in normal patients. This will reflect not only a shorter red cell survival, but also an increase in ineffective erythropoietic activity. It is of interest that the greatest E.I. values are found in patients with conditions known to be associated with increased ineffective erythropoietic activity i.e. Thallassaemia. Under these circumstances, the E.I. is disproportionately increased relative to the shortening of red cell survival.

VI-c-6 Plasma Iron Turnover and Erythroid Iron Turnover measurements in patients with different haemolytic states.

Table VI-8 and VI-9 detail the statistical comparison of these results with normal values. Both P.I.T. and E.I.T. increase in haemolytic states, although this is least marked in iron deficiency associated haemolysis ($p < .10$ for PIT, $<.01$ for EIT). It seems obvious that gross elevations of EIT values, disproportionate to shortening of red cell lifespan, must be associated with excessive ineffective erythropoiesis. The fairly wide standard deviations in the different disease states probably reflect different severity of the disease process in different individuals. For this reason, one must appreciate the limitations of empirically presented data. The elevated PIT and EIT characterise the haemolytic patients as a whole, but not the patients with iron deficiency associated with haemolysis.

$t_{\frac{1}{2}}$PIC, EI, PIT and EIT have been shown to correlate with total erythropoietic activity, as long as plasma iron supply remains adequate for normal erythropoiesis.(385)(361) Grossly elevated PIT values have been commented on by a number of authors, in association with ineffective erythropoiesis,(387) (36)(636)(253)(815)(289)(80)(232)(852) while most authors find increased PIT values in haemolytic diseases.(289)(683)(80)(232) As with a number of authors(631)(253) I have found all these values to be excellent indicators of total erythropoietic activity but poor indicators of effective erythropoietic activity.

VI-c-7 Experimental red cell utilisation values, and RBCUe/RBCUp ratios in patients with haemolytic anaemias.

Statistical analysis of these measurements in the various haemolytic states studied by me, and a comparison with normal results is shown in Table VI-10 and VI-11. I have previously theorised about the effects of different forms of haemolysis upon the RBCUe values and my theoretical predictions appear to be supported by the results I obtained. In patients with hereditary spherocytic anaemia, where red cell lifespan appears to be finitely reduced, the RBCUe/RBCUp value is 0.82 ± 0.30 ($p = < .10$). In the remaining conditions where ineffective erythropoiesis or random early red cell destruction is present, RBCUe/RBCUp values are markedly depressed ($p < .05$ in all cases). It was also interesting to note that the RBCUe/RBCUp ratio in patients with Fe deficiency related haemolysis was 0.95 ± 0.13 ($p < .05$) being significantly lower than predicted values. This is presumably a reflection of ineffective erythropoietic activity as well as early random red cell destruction in this group of patients.

From this data it would appear unreasonably to assess ineffective erythropoietic activity from RBCUe values alone, as this value reflects the effects of a number of processes. If random red cell destruction has been excluded, however, and the finite red cell lifespan exceeds the duration of the experiment, the ratio of RBCUe/RBCUp appears to give a reasonable indication of the presence and severity of ineffective erythropoietic activity. This ratio takes into account the influence of

plasma iron concentrations upon the RBCUe, but not the influence of variable erythropoiesis.

Most authors have found RBCUe values decreased in haemolytic anaemias,[872][55][815][289][211][284] some stating that plateau values are reached earlier in association with haemolysis,[872][211][212] and have found a decrease in RBCUe values in association with ineffective erythropoiesis.[387][36][636][253][386][443][211] Some feel that the RBCUe value alone is a good indicator of the severity of the process.[211][253] The delay in RBCUe plateau levels associated with ineffective erythropoiesis has also previously been commented upon[211][212] as has the decrease in RBCUe values with increasing plasma iron concentrations.[211][187][576][62][252][284][914][212] It appears that RBCUe values are poor indicators of effective erythropoiesis in the presence of random red cell haemolysis.[212]

In conclusion, there appears to be no way of differentiating the effects of ineffective erythropoiesis, effective erythropoiesis and random red cell destruction upon RBCUe values, particularly if all processes are operating simultaneously.

VI-c-8 $t_{\frac{1}{2}}$ ^{51}Cr red cell survival data in patients with haemolytic anaemia.

Statistical analysis of the data and comparison with normal patients is seen in Table VI-12. As previously discussed, there are limitations imposed by us on the usefulness of this technique for measuring mean red cell lifespan. By this

method of analysis, no differentiation of random and finite shortening of red cell lifespan can be made. I do not dispute that, with more care, this differentiation can be made. All our patients had shortened red cell survivals (as measured by $t_{\frac{1}{2}}{}^{51}Cr$ data) when compared to normal patients. The success with which haemolysis was detected, can be assessed from Table VI-12. It is obvious that this data bears no true relationship to mean red cell lifespan, being only semi-quantitative; moreover, it appears that this method will probably only detect haemolysis once red cell lifespan has become significantly decreased.

VI-d Ferrokinetic data in Patients with Hypoplastic anaemias.

VI-d-1 Selection of Patients.

Two broad groups of patients were selected for this study. The first group (Group A) consisted of patients who had a true hypoplastic anaemia, diagnosed clinically, morphologically and by other haematological criteria. A number of these patients were on anabolic steroids when studied, and two at least appeared to be in a clinical remission when studied.

The second group of patients (Group B) included those who had ferrokinetic criteria for qualifying, or who had a presumptive diagnosis of hypoplastic anaemia made that was not confirmed on further investigation. It appears inevitable that some patients, who are otherwise haematologically reasonably normal, will present with hypoplastic ferrokinetic findings. I have attempted to analyse these

"false positives" for hypoplasis. Ferrokinetic criteria for inclusion in this group include EI values of < 0.84 (i.e. mean normal - 1 SD) or EIT values of < 0.2580 (mean normal - 1 SD) or PIT values of < 0.5262 (mean normal - 1 SD). An attempt has been made to show cause why these patients should not be labelled as hypoplastic.

VI-d-2 Results.

These are tabulated in Tables VI-16 to Table VI-24. Comparison of the true hypoplastic with the ferrokinetically hypoplastic group is shown in Table VI-25. Theoretically, this group of patients should show prolonged $t_{\frac{1}{2}}$PIC, normal or low PIT, low EIT and EI, normal red cell survival and low RBCUe values, all reflecting depressed bone marrow function.

Table VI-15 Comparison of differences of ferrokinetic data between true hypoplastic anaemia patients and ferrokinetically found hypoplastic anaemias (see text).

	True Hypoplastics (A)		Ferrokinetic Hypoplastics (B)		
	$\bar{x}$	SD	$\bar{x}$	SD	P
Plasma Iron	202.4	74.4	89.4	50.2	< .0005
$t_{\frac{1}{2}}$PIC	221.8	111.5	99.4	52.6	< .0025
EI	0.73	0.24	0.99	0.36	< .01
PIT	0.6204	0.1535	0.5173	0.3110	< .15
EIT	0.1657	0.2087	0.3331	0.2462	< .05
RBCUe	17.7	8.9	74.4	20.1	< .0005
RBCUe/ RBCUp	0.41	0.18	0.88	0.14	< .0005
$t_{\frac{1}{2}}{}^{51}$Cr	29.8	9.0	28.4	4.0	< .35

Table VI-16 Ferrokinetic findings on patients with Hypoplastic Anaemias

Aplastics	Hb	Hct	B.PCV / V.PCV	PI	$t_{\frac{1}{2}}$PIC	EI	PIT	EIT	RBCUe	RBCUe / RBCUp	$t_{\frac{1}{2}}{}^{51}$Cr	f
All	10.6	35	-	250	270	0.67	0.6278	0.0345	25	0.60	-	-
Bai(1)	9.4	27	0.96	160	150	0.93	0.8017	0.3808	26	0.43	29	0.00
(2)	14.1	41	0.97	72	62	1.18	0.7233	0.5663	-	-	26	-
Bur	-	24	-	-	300	-	-	-	-	-	24	-
Coo	12.0	35	0.98	153	180	0.70	0.5802	0.2147	-	-	-	-
duT	11.5	31	0.84	284	249	0.85	0.8149	0.1044	15	0.50	38	0.34
Das	11.4	32	0.90	170	311	0.45	0.3857	0.0000	6	0.10	45	0.07
Fra	11.2	36	-	230	273	0.62	0.5365	0.0251	25	0.52	19	-
Lam	13.2	37	0.90	274	126	1.52	1.4344	0.8018	54	1.42	23	0.00
Pep	11.8	34	0.90	294	433	0.49	0.4664	0.0000	9	0.30	40	0.00
vWy	11.2	34	1.10	137	86	1.35	1.0998	0.7703	82	1.19	24	0.14
Hypoplastics												
Sol	14.7	47	0.87	88	130	0.60	0.3842	0.2094	83	0.98	28	0.06
Lou	18.0	52	0.75	124	193	0.47	0.2583	0.0838	-	-	-	-
Hol	17.6	46	0.82	123	183	0.53	0.3885	0.1402	-	-	-	-
Ash	8.5	35	0.99	64	110	0.66	0.3995	0.2428	75	0.85	27	0.27
Ver	21.2	64	0.60	101	99	0.71	0.4195	0.2741	-	-	-	-
Bot	17.9	47	0.74	55	86	0.72	0.3604	0.2519	-	-	-	-
Web	16.2	52	0.75	88	91	0.82	0.5044	0.3438	99	1.14	29	0.37
Vlo	12.7	41	0.94	36	50	1.08	0.4484	0.3699	85	0.89	-	0.16
Sep	16.5	47	0.98	67	83	0.81	0.4582	0.3251	-		-	-
Koe	20.0	56	0.81	97	100	0.75	0.4703	0.3057	72	0.84	30	0.08
Sch	11.0	36	0.85	21	34	1.37	0.4131	0.3639	98	1.00	36	0.00
OCo	13.1	35	1.12	243	210	0.85	0.7845	0.2079	40	0.91	-	-
Pet	16.4	42	0.90	29	51	0.98	0.3475	0.2853	92	0.95	-	0.27
Ada	5.7	16	0.85	39	72	0.88	0.4619	0.3455	80	0.88	27	0.19
Hoo	9.9	29	0.85	16	29	1.54	0.4045	0.3635	92	0.93	22	0.14
Loz	13.2	36	1.05	9	35	1.13	0.1740	0.1529	88	0.87	-	0.00
Joh	12.9	37	0.90	138	120	0.95	0.7554	0.4381	37	0.52	25	0.04
Eng	11.5	32	0.98	100	188	0.51	0.3753	0.1284	50	0.64	-	0.07
Sco	9.9	27	0.64	153	63	2.13	1.8340	1.4316	51	0.82	-	0.44
Mag	16.1	46	0.90	163	133	0.87	0.7096	0.3805	66	0.96	-	0.36
Hal	8.0	28	0.89	32	57	0.97	0.4161	0.3329	-	-	-	-
Rob	16.8	50	0.80	75	90	0.77	0.4500	0.3083	-	-	-	-
Cra	17.6	51	0.93	175	130	0.89	0.7145	0.3894	-	-	-	-
Pet	19.0	60	0.74	147	113	0.81	0.5852	0.3537	-	-	-	-

Table VI - 17 Comparison of plasma iron concentrations in normal and aplastic patients.

Group	Plasma Iron			
	N	$\bar{x}$	SD	P
1. Normal	28	111.1	40.7	
2. Aplastics (A)	10	202.4	74.4	< .0005
3. Hypoplastics (B)	24	89.4	50.2	< .05

Table VI - 18 Comparison of $t_{\frac{1}{2}}$PIC values in normal and aplastic patients

Group	$t_{\frac{1}{2}}$ PIC			
	N	$\bar{x}$	SD	P
1. Normal	31	80.9	25.7	
2. Aplastics (A)	11	221.8	111.5	< .0005
3. Hypoplastics (B)	24	99.4	52.6	< .10

Table VI - 19 Comparison of erythropoietic Indices in normal and aplastic patients.

Group	EI			
	N	$\bar{x}$	SD	P
1. Normal	28	1.13	0.29	
2. Aplastics (a)	8	0.73	0.24	< .0005
(b)	10	0.88	0.36	< .05
3. Hypoplastics (B)	24	0.91	0.36	< .01

Table VI - 20 Comparison of PIT values in normal and aplastic patients.

Group	PIT			
	N	$\bar{x}$	SD	P
1. Normal	28	0.7816	0.2554	
2. Aplastics (A)	10	0.6204	0.1535	< .025
3. Hypoplastics (B)	24	0.5173	0.3110	< .0025

Table VI - 21 Comparison of EIT values in normal and aplastic patients.

Group	EIT			
	N	$\bar{x}$	SD	P
1. Normal	28	0.4401	0.1821	
2. Aplastics (A)	10	0.1657	0.2087	< .0005
3. Hypoplastics (B)	24	0.3331	0.2462	< .05

Table VI - 22 Comparison of RBCUe values in normal and aplastic patients.

Group	RBCUe			
	N	$\bar{x}$	SD	P
1. Normal	17	83	15	
2. Aplastics (A)	6	18	8.9	< .0005
3. Hypoplastics (B)	15	74	20.1	< .10

Table VI - 23 Comparison of the ratio RBCUe : RBCUp in normal and aplastic patients.

Group	Ratio $\frac{\text{RBCUe}}{\text{RBCUp}}$			
	N	$\bar{x}$	SD	P
1. Normal	14	1.11	0.27	
2. Aplastics (A)	6	0.41	0.18	< .0005
3. Hypoplastics (B)	15	0.88	0.14	< .0005

Table VI - 24 Comparison of $t_{\frac{1}{2}}{}^{51}Cr$ red cell survival in normal and aplastic patients.

	$t_{\frac{1}{2}}{}^{51}Cr$			
	N	$\bar{x}$	SD	P
1. Normal	10	27	4.7	
2. Aplastics (A)	9	30	9.0	< .25
3. Hypoplastics (B)	8	28	4.0	< .30

VI-d-3 Plasma Iron Levels (Table VI - 17)

In the true aplastic group the average plasma iron concentration was significantly elevated ($P < .0005$) whereas in Group (B) patients, it was significantly depressed. A number of Group (B) patients had markedly depressed plasma iron levels on the basis of inflammation, infection or iron deficiency, known to be associated with low plasma iron concentrations as well as impaired erythropoiesis (see later under specific disease groups). The increased plasma iron levels in the patients wit h true aplastic anaemia were probably due to a large extent, to transfusional siderosis. There may also have been a contribution from normal iron absorption in the presence of depressed erythropoietic activity.

VI-d-4 $t_{\frac{1}{2}}$PIC and EI values (Table VI-18 and VI -19)

The $t_{\frac{1}{2}}$PIC should be prolonged in these patients because of inactive erythropoiesis. We have previously shown in (Chapter III) that the initial rate constant of the plasma iron clearance curve reflects mainly erythropoietic activity. Where erythroid iron removal is less, non-erythroid iron uptake should be greater than normal. I have previously suggested that NEIT does not differ in different disease states, being dependent upon plasma iron concentration only (VI-b-9) The rate of non-erythroid iron uptake should therefore, have a minimal effect upon the $t_{\frac{1}{2}}$PIC. Under these conditions, the erythropoietic index should be an excellent indicator of total erythropoietic activity and will be a valuable guide as to the severity of the hypoplasia. The $t_{\frac{1}{2}}$PIC has been found to be an excellent

guide of depressed bone marrow activity by a number of workers,[300][812][848][735][872][486][900][863][284][914][55] particularly in sequential studies. A number of authors however, including myself, have shown how $t_{\frac{1}{2}}$PIC values become prolonged with increased plasma iron values[80][384][62][61][735][863][284] and because of this, the use of the erythropoietic index as a guide to erythroid activity would eliminate errors from this variable as well as achieving a semi-quantitative assessment of total erythropoietic activity relative to the normal. The EI in this situation appears to be an excellent indicator of depressed bone marrow function (see Table VI - 19). In the truly aplastic patients, the EI was markedly depressed (Group A in Table VI - 19) ($P < .0005$). In the second group of patients, the $t_{\frac{1}{2}}$PIC was not as markedly prolonged as in the first group of patients, probably because the plasma iron values were not elevated to the same degree. EI values were, however, depressed. As can be seen in Table VI - 16, a number of these patients had reasons for bone marrow depression i.e. bone marrow infiltration, recent excessive ethanol ingestion, cytotoxic therapy, inflammatory states, malnutrition and chronic renal failure. A small number of patients, however, had no obvious cause, and these presumably reflect a temporary or permanent state of bone marrow hypofunction. In Table VI - 19, I have underlined the patients, who by ferrokinetic crit eria, are probably not totally hypoplastic although at least one ferrokinetic parameter suggests that they are. Where a result falls into the normal range (i.e. mean normal $\pm$ SD), it is suggested that depression of any other parameter may be produced by some other process.

Hypoplastic values are :-

Erythropoietic index	0.84
Plasma Iron Turnover	0.5262 units mg/100 ml.whole blood/day (in absence of Fe def.)
Erythroid Iron Turnover	0.2580 units mg/100 ml.whole blood/day (in absence of Fe def.)

It is of interest that two patients referred with a bone marrow morphologically diagnostic of aplasia associated with cirrhosis had, on ferrokinetic investigation, normal or increased erythropoietic activity. (Johnson & Scott).

VI-d-5 Plasma and Erythroid Iron Turnover Values.

In a number of aplastic patients, the PIT was within the normal range (see Table VI-16). Average values were however, depressed in both groups (see Table VI-20). ($P < .025$ and $< .0025$ respectively) tending to be more depressed in the second group of patients where plasma iron values were lower and, hence, the contribution of non-erythroid iron turnover was smaller. In my group of patients, the PIT appeared to be of some use in detecting bone marrow hypoplasia, although EIT values were undoubtedly more accurate. (see Table VI-21) ($P < .0005$ and $<.05$ respectively).

It is interesting that the Group B patients have less significantly depressed EIT values than the Group A patients. A number of authors have found normal or slightly raised PIT values in association with bone marrow hypoplasia.[289] [735][80][232] I have expressed some reservations about NEIT calculations at extremes of plasma iron concentrations (VI-b-9), and believe that in some of the patients included in the second group of patients, we have probably over-estimated NEIT to give a low value for EIT (i.e. OCo, Cra,) both these patients having normal EI values. Presumably

the same has occurred with the aplastic group of patients, although one suspects that the contribution of NEIT to PIT values may be slightly greater in this group of patients.

It would appear, taken overall, that the measurement of the EIT is a useful procedure, in association with EI values, for the detection of hypofunction of the bone marrow. There is obviously a need for further investigation to define non-erythroid iron kinetics at the extremes of plasma iron concentration. This has not been attempted in the present work.

Patients with extremely low plasma iron levels may be unable to raise their EIT values above those in the hypoplastic range, but the absence of hypoplasia in these patients would be detected by a raised EI value. Should the EI value not be raised, one may postulate an inadequate bone marrow erythroid response in these patients, and some other factor should be looked for.

VI-d-6 RBCUe and RBCUe/RBCUp

As can be seen from Tables VI-22 and VI-23, the patients who were truly hypoplastic had markedly depressed RBCUe values, as well as depressed RBCUe/RBCUp ratios. (Lamb and van Wyk have been excluded from this analysis as, by clinical and other criteria, they appeared to be in remission). It seems obvious that where less erythroid activity is present, less iron will be needed for haemoglobin formation. Less obvious to me is the fact that less label

will be incorporated into this haemoglobin, because plasma iron clearance will be markedly prolonged where hypoplasia is present, and the percentage of the plasma iron turnover going to non-erythroid iron uptake has been postulated as being a constant fraction of the plasma iron turnover. The only way to explain this discrepancy is to postulate that NEIT is indeed greater in patients with bone marrow hypoplasia, as ineffective erythropoiesis and random red cell destruction, which could lower RBCUe values, have not been shown to be present in this group of patients.

To a certain extent, the EI and RBCUe/RBCUp values in these patients should have been similar, as ineffective erythropoiesis and random red cell destruction were not present - i.e. depression of RBCUe would reflect depression of EI.

Comparison of these figures in individual hypoplastic patients does indeed show a fairly good correlation except in some patients on anabolic steroid therapy (see Table VI-26).

Table VI-26.

Patient	EI	RBCUe/RBCUp	
All	0.67	0.60	
Bai	0.93	0.43	oxymethalone
duT	0.85	0.50	"
Das	0.45	0.10	"
Fra	0.62	0.52	
Lam	1.52	1.42	"
Pep	0.49	0.30	"
VWy	1.35	1.19	prednisone

This may reflect the presence in patients Bai, dul', Das, of a certain amount of ineffective erythropoietic activity, or random early red cell destruction. The EI and the RBCUe/RBCUp correlate well in all patients where random red cell destruction or ineffective erythropoietic activity was absent. A comparison of this data, may therefore, be of some use in detecting the presence of these defects.

My results, in the two groups of hypoplastic patients, for RBCUe and RBCUe/RBCUp values, appear to be markedly different. In the first group of patients, both were markedly depressed (see Table VI-22 and VI-23) - ($P < .0005$) whereas in the second group of patients (Group B) RBCUe values, while being depressed, were not as greatly effected ($P < .10$). This appears to reflect mainly the effect of different plasma iron concentrations, although the ratio of RBCUe/RBCUp in the second group of patients was not as greatly depressed as in the first group. It may be that the superior RBCU of this second group of patients represented a temporary depressant effect of a disease process or toxin on the bone marrow which, while still active on the first day of study, became less active with treatment during the course of the study. This is supported by the generally larger RBCUe/RBCUp values, when compared to EI values in this group of patients (see Table VI-27).

Table VI-27

Patient	EI	RBCUe/RBCUp	Toxin
Sol	0.60	0.98	Ethanol
Ash	0.66	0.85	CLL
Web	0.82	0.14	P32
Vlo	1.08	0.89	Reiters
Koe	0.75	0.84	Buergers
Sch	1.37	1.00	Reiters
OCo	0.85	0.91	Haemochrom
Pet	0.98	0.95	Chloro
Ada	0.88	0.88	Ethanol
Hoo	1.54	0.93	PAS sensitivity Haem. crisis
Loz	1.13	0.87	Ethanol Cirrhosis
Joh	0.95	0.52	Cirrhosis
Eng	0.51	0.64	Ethanol
Maz	0.87	0.96	T.B.Mening.

In a large number of these patients RBCUe/RBCUp values exceeded EI values. At low plasma iron levels, where RBCUp will be 100%, RBCUe cannot go above this figure, so that EI values of 1.0 at low plasma iron levels cannot possibly be matched by RBCUe/RBCUp figures of equal magnitude.

Where RBCUe/RBCUp values exceed EI values, I suggest that temporary bone marrow suppression may have been present that had disappeared during the course of the experiment. If correct, observation of this phenomenon might help in separating temporary hypoplastic states from permanent ones.

VI-d-7 Red Cell survival data in patients with hypoplastic anaemias.

These are detailed in Table VI-24. As can be seen, neither group of patients had $t_{\frac{1}{2}}$ ^{51}Cr survival values that were significantly different to those for normal patients.

VI-e Ferrokinetic Parameters in patients with Polycythaemia.

VI-e-1 Selection of Patients.

All patients included in this study had haemoglobin values exceeding eighteen grams/100 ml. in males, sixteen grams/100 ml. in females, and haematocrit values above 52% in males and above 47% in females. These values were chosen as exceeding normal figures (mean + one standard deviation).(897)

It was found that the patients could be subdivided into four groups.

Group 1.

This consisted of patients with proven polycythaemia vera who may have been treated or not, but were referred for blood volume estimations. These patients had previously been diagnosed as having Polycythaemia vera by conventional methods. This group is unfortunately rather heterogenous because both treated and non treated patients have been included. The number of non treated patients was however too small for adequate analysis.

Group 2.

These patients satisfied the haemoglobin and haematocrit criteria, but on blood volume measurements had plasma volumes of less than 33 ml/kg (i.e. normal mean minus one standard deviation).

Group 3.

These patients satisfied the haemoglobin and haematocrit criteria, and had red cell volumes exceeding 35 ml/kg (i.e. normal mean plus one standard deviation).

Group 4.

These patients satisfied the haemoglobin and haematocrit criteria but, on blood volume meansurements were found to have volumes within the normal range (i.e. mean normal $\pm$ 1 standard deviation).

These groupings were selected for convenience of analysis.

VI-e-2 Results

These may be seen in Tables VI-28 through to Table VI-38. As can be seen, most of these patients had only one day studies so that RBCUe values could not be obtained. The few that were followed were so small in number that analysis of their results is probably meaningless. Many of the patients referred with low plasma volumes were on diuretic therapy, and many of the patients had unfortunately been venesected prior to referral.

VI-e-3 Blood volume studies.

(a) Total blood volume:-

In only one group of patients, was total blood volume significantly elevated (see Table VI-29), this being Group (3) patients ($P < .05$). In most of these patients, the increased total blood volume was due to increase in red cell mass. The majority of these patients suffered from chronic obstructive airways disease (COAD), although one patient had cyanotic congenital heart disease (tetralogy of Fallot) and one had polycythaemia secondary to a hepatoma. The diagnosis of COAD was established by full pulmonary function studies. It is of interest to me that, of the many patients seen at this hospital with obstructive airways disease (often of an incapacitating severity), so few present with polycythaemia despite markedly depressed arterial oxygen concentrations. One wonders whether these polycythaemic patients may not have either abnormally sensitive bone marrows to a normal

erythropoietin stimulus, or true polycythaemia vera in addition to COAD. They may, of course, produce excessive erythropoietin in response to a relatively minor drive. It seems of interest to study these patients further, at a later stage, in an attempt to assess exactly why they become polycythaemic, where patients with equally severe lung disease do not.

(b) Red cell volumes.

These were significantly elevated in all but the fourth group of patients (see Table VI-30) ($P < .005$, $< .05$, $< .0005$, $< .20$ respectively), who satisfied haemoglobin and haematocrit criteria, and yet had normal volumes. In none of these patients was I able to convince myself that peripheral haemoconcentration was present, none had splenomegaly and the cause seems obscure. In an attempt to study this problem further, the ratio of body to venous haematocrit has been studied in these patients, and compared with my normal results. The comparison is seen in Table VI-32. It can be seen that this ratio was significantly depressed in group (4) patients only, being normal or slightly increased in the other groups of patients. As commented upon in the section on normal patients, a variety of ratio values have been published for normal patients, varying from 0.86 to 0.96, with 0.92 being the most generally accepted figure. Groups (1), (2) and (3) are within acceptable limits for this value, but group (4) patients have a ratio of 0.76 ± 0.06 which is significantly

lower than normal.

I am not certain why this should be so. The most obvious possible cause is experimental error, the potential for which undoubtedly exists when blood volumes are measured by the techniques that I have used. It seems unlikely that experimental error could have been operating in so large a group of patients. I have studied the ratio in all the patients investigated (see Table VI-39), and only in group (4) patients was a consistent lowering of the ratio found. The other conclusion that may be drawn is that this group of patients have excessive peripheral haemoconcentration when compared to normal patients. I am not sure that I can resolve this problem without analysis of haematocrit values from central vessels. This has unfortunately not been done, but remains a project for further study.

(c) Plasma volume measurements.

Two groups of patients (group (2) and (4)) had plasma volumes that varied significantly from the normal range ($P < .0005$ and $<.005$ respectively). In the case of group (4) patients, the amount of variation was minimal, although present. In group (2) patients, selected because of reduced plasma volumes, a number of aetiological factors were present. A number of these patients were heavy smokers, and this may have interfered with antidiuretic hormone activity.

Some of the patients were, in addition, upon diuretic therapy for cor pulmonale or pulmonary oedema. These patients as a group had significantly elevated red cell volumes ($P < .05$) so that they may be masked polycythaemiavera patients. This possibility cannot be further studied in these patients as repeat estimations of therapy are difficult to get. It may, however, be of interest to study further the influence of smoking on the plasma volume.

TABLE VI-28

(1) Treated and Non-Treated Proven Polycythaemiavera Patients.

Name	Hb	B.PCV / V.PCV	Hct	Bl.Vol ml/kg	RBC Vol ml/kg	Pl.Vol ml/kg	PI	$t_{\frac{1}{2}}$PIC	EI	PIT	EIT
Gre	18.1	0.76	54	60	24.6	35.4	267	139	1.09	.9694	.4991
Hay	19.3	0.77	56	54.5	23.4	31.1	150	104	0.94	.6992	.4447
Lyn	15.9	0.84	51	92.9	39.6	53.3	29	29	1.65	.5373	.4831
Luc	20.3	0.83	60	73.8	36.6	37.2	40	30	1.67	.5953	.5326
Sch	17.6	0.86	52	69.0	31.0	38.0	207	122	1.05	.8850	.5071
Swe	19.1	0.89	59	83.5	49.9	39.6	58	23	2.50	1.1529	1.0601
Ser	18.2	0.67	51	60.6	20.6	40.0	186	72	1.67	1.3712	1.0257
San	20.4	0.81	57	68.8	31.8	37.0	236	114	1.16	.9846	.5917
Tim	20.7	0.88	60	67.1	35.4	31.7	217	114	1.04	.8528	.5125
Vig	20.6	0.73	51	61.2	22.7	38.5	40	27	1.96	.7893	.7150
Wic	16.2	0.73	48	68.9	24.1	44.8	86	41	1.86	1.1857	1.0177
Vig	18.9	1.04	53	63.5	35.0	28.5	280	115	1.38	1.2476	.7454
Lon	19.9	0.77	54	65.0	27.0	38.0	135	46	2.03	1.4704	1.2326
Lon	22.8	0.91	65	81.4	48.0	33.4	198	64	1.62	1.2379	.9593
Lon	22.6	0.88	67	88.1	52.0	36.1	282	52	2.47	2.0803	1.7017
Row	19.3	1.01	35	68.5	24.1	44.4	59	20	3.46	1.9901	1.8501
Jaf	22.0	0.77	68	89.6	47.1	42.5	243	51	2.23	1.7945	1.4760
Jou	16.2	0.93	42	84.4	33.1	51.3	89	49	1.67	1.1145	.9234
Pet	19.0	0.74	60	65.6	29.0	36.6	147	113	0.81	.5852	.3537
Row	20.4	1.04	60	75.2	46.7	28.5	26	15	2.97	.7765	.7358
Web	16.2	0.75	52	54.1	21.2	32.9	88	91	0.82	.5044	.3438

(2) Patients referred with Hb greater than 18 (male) greater than 16 (female). PCV greater than 52 (male) greater than 47 (female) Plasma volume less than 33 ml/kg ($\bar{x}$ - 1 SD)

Name	Hb	B.PCV / V.PCV	Hct	Bl.Vol ml/kg	RBC Vol ml/kg	Pl.Vol ml/kg	PI	$t_{\frac{1}{2}}$PIC	EI	PIT	EIT
Vis	20.9	0.93	70	65.4	42.5	22.9	72	27	2.10	.9672	.8775
Thy	18.6	0.94	51	53.3	25.5	27.8	189	72	1.69	1.3934	1.0422
Sch	18.3	0.83	48	55.0	22.0	33.0	94	73	1.09	.7143	.5318
deB	19.6	0.85	61	63.8	33.2	30.6	104	48	1.54	.9587	.7990
Gal	21.2	0.95	63	81.4	48.7	32.7	190	41	2.53	1.9482	1.6686
Pev	18.2	0.99	66	54.5	35.7	18.8	123	73	1.04	.6593	.4917

Table VI-28 contd.

(3) Patients referred with Hb greater than 18G% (male) or 16G% (female); PCV greater than 52 (male) 47 (female); with red cell volume greater than 35 ml/kg ($\bar{x}$ + 1 SD)

Name	Hb	B.PCV / V.PCV	Hct	Bl.Vol ml/kg	RBC Vol ml/kg	Pl.Vol ml/kg	PI	$t_{\frac{1}{2}}$ PIC	EI	PIT	EIT
Vis	20.9	0.93	70	65.4	42.5	22.9	72	27	2.10	.9672	.8775
Mar	20.4	0.85	60	87.5	44.4	43.1	101	52	1.42	.8786	.7204
vdV	18.8	1.05	57	116.0	69.7	46.3	74	36	1.81	.9669	.8437
Gal	21.2	0.95	63	81.4	48.7	32.7	190	41	2.53	1.9482	1.6686
Pev	18.2	0.99	664	54.5	35.7	18.8	123	73	1.05	.6593	.4917
Mat	20.4	0.72	62	104.0	46.6	57.4	65	32	1.83	.8672	.7695

(4) Patients referred with Hb greater than 18G% (male) 16G% (female) PCV greater than 52 (male) 47 (female) with normal blood volumes

Name	Hb	B.PCV / V.PCV	Hct	Bl.Vol ml/kg	RBC Vol ml/kg	Pl.Vol ml/kg	PI	$t_{\frac{1}{2}}$ PIC	EI	PIT	EIT
Koe	20.0	0.81	56	64.6	29.4	35.2	97	100	0.75	.4703	.3057
Lou	22.0	0.75	65	65.6	31.9	33.7	124	193	0.40	.2583	.0838
Smi	18.9	0.73	50	55.0	20.0	35.0	101	68	1.20	.8009	.6100
Afr	20.4	0.82	58	65.0	31.0	34.0	123	81	1.04	.7056	.5048
Ver	21.2	0.59	64	63.2	23.8	39.4	101	99	0.71	.4195	.2741
McN	20.3	0.76	62	65.7	31.1	34.6	204	78	1.42	1.1236	.8168
Hey	20.3	0.77	64	70.2	34.8	35.4	64	28	2.05	.9399	.8478
Mar	19.2	0.78	56	67.9	29.6	38.3	59	30	1.98	.9631	.8630
Car	20.0	0.72	59	72.3	30.5	41.8	215	105	1.14	.9362	.5921
Sch	17.5	0.76	51	66.8	26.0	40.8	101	57	1.42	.9405	.7529
Ada	17.1	0.75	50	56.8	21.3	35.5	64	38	1.70	.9095	.7885
deV	18.1	0.83	53	63.7	28.0	35.7	84	60	1.20	.7138	.5631

Table VI-29 Blood volumes in Normal/Polycythaemic Patients

	N	$\bar{x}$	SD	P
Normal	31	67.8	10.3	
Polycythaemia (1)	21	71.2	11.5	< .15
(2)	6	62.2	10.7	< .15
(3)	6	84.8	23.0	< .05
(4)	12	64.7	4.9	< .10

Table VI-30. Red cell volumes in Normal and Polycythaemic Patients

	N	$\bar{x}$	SD	P
Normal	31	26.6	5.1	
Polycythaemia (1)	21	33.2	9.8	< .005
(2)	6	34.6	10.1	< .05
(3)	6	47.9	11.6	< .0005
(4)	12	28.1	4.5	< .20

Table VI-31 Plasma Volumes in Normal and Polycythaemic Patients

	N	$\bar{x}$	SD	P
Normal	31	40.9	7.0	
Polycythaemia (1)	21	38.0	6.5	< .10
(2)	6	27.6	5.7	< .0005
(3)	6	36.9	14.8	< .30
(4)	12	36.6	2.7	< .005

Table VI-32 B.PCV / V.PCV Ratios in normal and Polycythaemic patients.

		N	$\bar{x}$	SD	P
Normal		28	0.85	0.09	
Polycythaemia	(1)	21	0.84	0.11	< .40
	(2)	6	0.92	0.06	< .025
	(3)	6	0.92	0.12	< .10
	(4)	12	0.76	0.06	< .0005

Table VI-33. Plasma Iron concentrations in normal and Polycythaemic patients

		N	$\bar{x}$	SD	P
Normal		28	111.1	40.7	
Polycythaemia	(1)	21	145.9	88.9	< .10
	(2)	6	128.7	49.9	< .25
	(3)	6	104.2	47.4	< .40
	(4)	12	111.4	50.6	< .495

Table VI-34. $t_{\frac{1}{2}}$PIC values in normal and Polycythaemic Patients

		N	$\bar{x}$	SD	P
Normal		31	80.9	25.7	
Polycythaemia	(1)	21	68.1	40.1	< .15
	(2)	6	55.7	19.8	< .01
	(3)	6	43.5	16.8	< .0005
	(4)	12	78.1	44.9	< .45

Table VI-35 Erythropoietic Index in Normal and Polycythaemic Patients

	N	$\bar{x}$	SD	P
Normal	28	1.13	0.29	
Polycythaemia (1)	21	1.72	0.71	< .0005
(2)	6	1.67	0.58	< .025
(3)	6	1.79	0.52	< .0025
(4)	12	1.25	0.50	< .475

Table VI-36 Plasma Iron Turnover values in normal and polycythaemic patients

	N	$\bar{x}$	SD	P
Normal	28	0.7816	0.2554	
Polycythaemia (1)	21	1.0869	0.4574	< .005
(2)	6	1.1069	0.4870	< .10
(3)	6	1.0479	0.4552	< .10
(4)	12	0.7651	0.2611	< .45

Table VI-37 Erythroid Iron Turnover values in normal and Polycythaemic Patients

	N	$\bar{x}$	SD	P
Normal	28	0.4401	0.1821	
Polycythaemia (1)	21	0.8434	0.4330	< .0005
(2)	6	0.9018	0.4299	< .01
(3)	6	0.8952	0.4026	< .01
(4)	12	0.5836	0.2528	< .05

Table VI-38 Comparison of the statistical difference of ferrokinetic parameters in different types of polycythaemia compared with true polycythaemia vera.

P values (i.e. probability of significance of the difference) have been entered.

(2) (3) (4) as defined in text (VI-e-1)

	GROUP		
PARAMETER	(2)	(3)	(4)
Blood volume	< .05	< .10	< .025
RBC volume	< .40	< .005	< .05
Plasma volume	< .0005	< .45	< .20
B.PCV/V.PCV	< .025	< .10	< .01
PI	< .30	< .10	< .10
$t_{\frac{1}{2}}$PIC	< .20	< .025	< .30
EI	< .45	< .40	< .025
PIT	< .475	< .45	< .01
EIT	< .40	< .40	< .025

Table VI-39 Comparison of body PCV/venous PCV ratios in different disease groups.

GROUP	N	$\bar{x}$	SD	P
Normal	28	0.85	0.09	
Aplastics	8	0.94	0.08	< .40
Hypoplastics	24	0.86	0.12	< .10
Fe deficiency				
(a)	11	0.93	0.16	< .45
(b)	3	0.87	0.15	< .05
(c)	31	0.97	0.35	< .15
(d)	17	0.93	0.28	< .05
Myelofibrosis	15	1.03	0.38	< .10
Reticuloses	11	1.01	0.37	< .35
Infections	13	0.87	0.14	< .30
Inflammations	9	0.89	0.21	< .40
Cirrhosis	11	0.86	0.11	< .30
Megaloblastic Anaemia	8	0.87	0.07	< .20
Symptomatic Porphyrics	12	0.88	0.10	< .35
Neoplasms	9	0.87	0.14	< .40
Chronic Renal Failure	3	0.94	0.06	< .025
Polycythaemics				
(1)	21	0.84	0.11	< .40
(2)	6	0.92	0.06	< .025
(3)	6	0.92	0.12	< .10
(4)	12	0.76	0.06	< .0005
Thallassaemia	4	0.96	0.09	< .021
Heriditary spherocytosis	4	0.92	0.13	< .45
Auto-immune Haemolytic Anaemia	5	0.87	0.22	< .25
Fe deficiency and haemolysis	9	0.90	0.19	< .10
Myeloproliferative	12	1.03	0.42	< .005

VI-e-4 Plasma Iron Values

These are statistically presented in Table VI-33. In none of the groups was plasma iron concentration significantly different to normal values. A number of individual patients were iron deficient as a result of repeated venesection, but this was not mirrored in the overall results of the groups as a whole.

VI-e-5 $t_{\frac{1}{2}}$PIC and EI values (see Tables VI-34 and VI-35)

The $t_{\frac{1}{2}}$PIC was significantly elevated in groups (2) and (3), reflecting their increase in erythropoietic activity to maintain a larger than normal circulating red cell mass. EI values were significantly elevated in groups (1), (2) and (3) where red cell mass was also significantly elevated. This shows the value of this parameter in detecting increased erythropoietic activity. Of interest are the normal $t_{\frac{1}{2}}$PIC and EI values in group (4) patients, tending to support the thesis that their red cell volumes were not elevated, and that their peripheral haematocrit represented a difference in distribution in red cell rich blood.

VI-e-6 PIT and EIT values. (see Tables VI-36 and VI-37)

PIT values were significantly elevated only in group (1) patients, this probably reflecting their slightly greater plasma iron values. EIT values, however, show significant elevation in groups (1), (2) and (3), where erythropoietic activity must be increased to maintain an increased red cell mass. Once again, group (4) patients have normal

values for this parameter, suggesting that blood volume estimations were experimentally sound.

VI-e-7 Thus, all the indices previously postulated as indicating increased total erythropoietic activity have shown good correlation in practice, with situations where red cell mass is increased and erythropoiesis must be increased. The best of these indices undoubtedly appears to be the erythropoietic index.

VI-f Ferrokinetic Parameters in Patients with Iron Deficiency, and in disease processes associated with low plasma iron levels.

VI-f-1 Patient selection.

Four groups of patients have been selected for discussion in this section.

Group (1) Classical Iron Deficiency Anaemias:-

The criteria for inclusion of patients into this group are:-

a) Haemoglobin less than 12 G%

b) Microcytosis and hypochromia on peripheral smear examination.

c) Plasma iron concentrations of less than 30µgm%, with total iron binding capacities exceeding 300µgm%.

A number of these patients had associated disease processes which were thought not to have interfered with their ferrokinetic performance.

Group (2) Iron deficiency by the above criteria unassociated with anaemia. A number of these patients were obtained, particularly amongst patients with polycythaemia treated by venesection.

Group (3) Patients with low plasma iron levels (<70µgm%) behaving ferrokinetically in the same way as patients with classical iron deficiency anaemia. These patients have been included under a separate group, as they often had low TIBC values or a disease process that may have interfered with their ferrokinetic performance.

Group (4) Patients with low plasma iron levels with suboptimal ferrokinetic performance. In this group, as in group (3), a plasma iron level of <70µg% was selected, as Hillman has shown this level of plasma iron to be necessary for normal erythroid function.(384)

The ferrokinetic value chosen in order to decide whether a patient was ferrokinetically suboptimal or not was an erythropoietic index of less than 1.45. Where the erythropoietic index exceeded 1.45, the patient was put into the appropriately responding group. This figure was chosen arbitrarily, but was decided upon because it represented the upper limit of normal EI values. Patients with iron deficiency have, under normal circumstances, bone marrows that are avid for iron, and these patients would therefore, have rapid $t_{\frac{1}{2}}$PIC values, more rapid than predicted in normal patients (Chapter III).

VI-f-2 Results

These may be seen in Table VI-40 through to Table VI-49. A level of plasma iron of less than 30μg% has been chosen for my group (1) patients as this level, in association with a TIBC level of >300 g%, virtually establishes the diagnosis of classical iron deficiency anaemia.

VI-f-3 Plasma Iron Levels. (See Table VI-41)

The values in the different groups was, not surprisingly, significantly depressed. Many of the group (3) patients, in fact, had classical iron deficiency and were only excluded from groups (1) or (2) because their plasma iron levels were >30 but <70μg%. In patients of groups (3) or (4), the low plasma iron levels were usually associated with blood loss. In a number of patients, this could not be demonstrated and, in eleven patients, the causative agent appeared to be inflammation or infection. Six patients had low plasma iron levels in association with neoplasms.

VI-f-4 $t_{\frac{1}{2}}$PIC and EI values (Tables VI-42 and VI-43)

As can be seen from the relevant tables (VI-42 and VI-43), $t_{\frac{1}{2}}$PIC was significantly depressed below normal in all patients. To some extent, this reflected the decrease in plasma iron concentration, as the EI values were, in groups (2) and (4), not as significantly depressed. Moreover, group (4) patients, had EI values within the normal range, in sharp contrast to the patients in the other groups. The greatly elevated EI values in the responsive iron deficient situation may be related to a number of factors.

(i) the extreme avidity of the bone marrow for iron.

(ii) an increase in total erythropoietic activity to compensate for

a) increased ineffective erythropoietic activity. (258) (164) (106) (687) (688) (766) (727)

b) decreased or shortened red cell survival. (198) (246) (292) (411) (687) (833)

c) the presence of anaemia with presumable increase of erythropoietin production.

The $t_{\frac{1}{2}}$PIC in the patients with iron deficiency was even more rapid than that of patients with haemolytic anaemias (see Table VI-6), although in most cases EI values were not as greatly elevated (Table VI-7). The extremely rapid $t_{\frac{1}{2}}$PIC values probably indicated the influence of low plasma iron levels as well as of increased erythropoietic activity. The variation due to changes in plasma iron level are removed by using the erythropoietic index, which appears to reflect increased total erythropoietic activity in these patients. Almost certainly, these patients were not producing nearly as much haemoglobin as the EI values suggest, because they remained anaemic until iron therapy was given. A major portion of their erythropoietic activity must, therefore, have been ineffective.

Group (4) patients were specifically chosen because they stood out as a separate group when EI values were examined. In this group were seven patients suffering from acute

infective processes and, in two of these, ethanol was a major cause for their acute infection (aspiration lung abscesses). Two of the patients had chronic renal failure, two had blood dyscrasias with bone marrow involvement, and two of the patients were unconscious, on catheter drainage of the bladder, and were demonstrated to have urinary tract infections. In the remaining patients, the only associated factor that I could find that may have been associated with depressed bone marrow function was ethanol ingestion which was usually associated with a poor diet.

A clear message emerges from this study. If, on ferrokinetic investigation, a patient is found who has plasma iron levels of <70µg%, and has an EI value of <1.45, another associated disease process causing bone marrow suppression must be actively looked for and excluded. These findings confirm much of the previously described ferrokinetic findings in iron deficient subjects.(62)(61)(284)(675)(385)

VI-f-5 PIT and EIT values (see Tables VI-44 and VI-45)

Theoretically, because of restricted bone marrow iron supply, one would expect PIT and EIT values to be depressed. The values obtained were, however, normal or increased in all groups except group (4), whilst PIT values tended to be normal ($P < .45, < .20, < .005$). EIT values tended to be raised ($P < .005, < .025, < .0025$) in groups (1) to (3) respectively. Values for PIT and EIT were significantly depressed in group (4) ($P < .0005, < .10$). This generalisation

certainly does not apply to all individual patients within the various groups. and where plasma iron levels were excessively low, this was mirrored by 'hypoplastic' PIT and EIT values. Had iron been freely available to these patients, it is almost certain that PIT and EIT values would have been far higher, as described by Hillman.(385) This finding of normal or increased PIT values in iron deficiency has been previously reported by a number of workers,(679)(232)(258) although at least one worker has found generally depressed values in this condition.(80) Presumably ineffective erythropoietic activity accounts for a major fraction of EIT and PIT as many of these patients remain anaemic.

VI-f-6 RBCUe and RBCUe/RBCUp values. (Tables VI-46 and VI-47)

A number of workers have shown that ineffective erythropoietic activity is greater than normal in iron deficient states.(258)(164)(106)(687)(688)(726)(727) This would explain my findings in patients with iron deficiency, where the ratio RBCUe/RBCUp is significantly depressed in all groups. Most authors state that RBCUe values should be 100% in conditions associated with iron deficiency.(211)(576)(62)(212)(164)(258) We have not found this to be the case, and this presumably reflects the early removal of iron label from the red cells by either random haemolysis or ineffective erythropoiesis. It is interesting that of all the groups, group (4) patients have the highest RBCUe and RBCUe/RBCUp values. This

presumably reflects the withdrawal of a temporarily depressant factor from the patient while in hospital and on treatment, as previously discussed with the second group of hypoplastic patients.

VI-f-7 $t_{\frac{1}{2}}{}^{51}Cr$ Red Cell Survival Values. (see Table VI-48)

I have found, as previously discussed (VI-c), significant shortening of red cell survival in patients with severe iron deficiency. This may represent the effects of blood loss, but independent shortening of red cell lifespan in this situation has been found by a number of workers,[198][246][292][411][833][687] the defect being thought to be an intrinsic cellular abnormality. Group (4) patients, whose low plasma iron levels were associated with depressant disease processes, had normal red cell survival, as measured by $t_{\frac{1}{2}}{}^{51}Cr$ data.

VI-f-8 Comparison of the results in the different iron deficient groups.

The results of the different groups have been compared with group (1) used as a standard. The comparison is presented in Table VI-49. As can be seen, group (2) patients behave identically to group (1) patients. Group (3) patients, because higher plasma iron levels have been accepted, behave identically to group (1) patients except under circumstances where increase in plasma iron level will increase the value of an individual ferrokinetic parameter (PI, $t_{\frac{1}{2}}$PIC, PIT, EIT). Group (4) patients are totally different in their ferrokinetic behaviour to

group (1) patients, this difference reflecting their selection on the basis of depressed bone marrow function.

Table VI-40 Ferrokinetic findings in patients with low serum Iron values.

(1) = Classical Iron Deficiency (Hb <12, PI <35, TIBC >300, %Sat <10)

(2) = Iron Deficiency as above unassociated with anaemia

(3) = Low Plasma Iron (<70) with EI > 1.45

(4) = Low Plasma Iron (<70) with EI < 1.45

	Patient	Hct	B.PCV	PI	$t_{\frac{1}{2}}$PIC	EI	PIT	EIT	RBCUe	$\frac{RBCUe}{RBCUp}$	$t_{\frac{1}{2}}{}^{51}Cr$	f
(1)	Arm	33	0.96	10	22	1.84	.3165	.2922	81	.80	-	-
	Bas	23	1.28	9	16	2.53	.4519	.4271	95	.94	17	.00
	Cha	36	0.90	19	18	2.52	.7261	.6817	97	.98	18	.14
	Dav	35	0.72	43	31	1.93	.9285	.8264	84	.90	14	.13
	Bur	37	1.13	23	16	2.97	.9365	.8834	-	-	-	-
	Hol	24	-	37	22	2.70	1.2987	1.1978	-	-	-	-
	deV	34	0.84	32	27	1.98	.8236	.7466	-	-	-	-
	Ans	38	0.90	32	30	1.75	.7031	.6303	100	1.04	33	.03
	Wil	33	1.00	22	31	1.54	.4879	.4343	94	.96	17	.00
	Kin	33	0.72	32	14	3.84	1.6386	1.5606	-	-	-	-
	Rix	16	0.88	16	20	2.31	.6962	.6484	70	.71	15	.00
	Kna	35	0.91	17	16	2.74	.7204	.6800	60	.61	27	.30
(2)	Row	60	1.04	26	15	2.97	.7765	.7358	-	-	-	-
	Lyn	51	0.84	29	29	1.65	.5373	.4831	-	-	-	-
	Tay	54	0.74	26	17	2.69	.7696	.7238	66	.67	43	.40
(3)	Saa	33	0.95	69	22	3.46	2.1814	2.0160	94	1.09	26	.17
	Car	38	1.05	53	35	1.84	.4204	.2997	82	.90	32	.12
	Hey	64	0.77	64	28	2.05	.9399	.8478	85	.90	26	.00
	All	23	0.98	38	17	3.55	1.7623	1.6574	86	.92	-	.00
	Eat	39	1.13	44	28	2.10	1.0076	.9089	63	.68	-	-
	Swe	59	0.90	58	23	2.50	1.1529	1.0601	-	-	-	-
	Hoo	29	0.85	16	29	1.54	.4045	.3635	92	.93	22	.14
	Sil	30	0.90	55	30	2.30	1.3185	1.1792	79	.89	12	.00
	Mar	56	0.78	59	30	1.98	.9631	.8630	-	-	-	-
	Pla	56	0.90	5	25	1.46	.0970	.0885	-	-	-	-
	Vig	51	0.73	40	27	1.96	.7893	.7150	-	-	-	-
	Luc	60	0.83	40	30	1.66	.5953	.5326	-	-	-	-

Table VI-40 contd.

	Patient	Hct	B.PCV / V.PCV	PI	$t_{\frac{1}{2}}$PIC	EI	PIT	EIT	RBCUe	RBCUe / RBCUp	$t_{\frac{1}{2}}^{51}$Cr	f
(3)	Cad	23	1.01	42	15	4.21	2.1452	2.0295	81	.88	20	-
	Jeg	34	0.91	45	33	1.86	.9371	.8289	92	1.00	19	.03
	Bei	25	0.67	39	40	1.51	.7508	.6456	-	-	-	-
	Wil	20	2.27	43	44	1.48	.8048	.6820	54	0.59	17	-
	Han	37	.87	53	31	2.09	1.1277	1.0053	55	.60	11	-
	vNi	38	.75	59	22	3.08	1.7603	1.6259	100	1.11	15	.05
	Row	35	1.01	59	20	3.46	1.9901	1.8501	-	-	-	-
	Fer	35	.85	46	33	1.86	.9596	.8505	21	.23	38	.00
	vdM	31	.83	25	22	2.27	.8197	.7572	60	.62	21	.00
	Wil	30	1.88	44	34	1.83	.9369	.8254	93	1.01	16	.00
	Sch	26	.94	33	31	1.81	.8151	.7273	89	.95	33	.00
	vdM	32	.83	70	25	3.09	2.0160	1.8431	87	1.01	28	.20
	Mab	62	.72	65	32	1.83	.8672	.7695	-	-	-	-
	Hoe	40	1.34	65	19	3.69	2.1285	1.9847	47	.53	26	.07
	Ada	50	.75	64	38	1.70	.9095	.7885	-	-	-	-
	deV	43	.77	64	46	1.48	.8501	.7148	-	-	-	-
(4)	Vlo	41	.94	36	50	1.08	.4484	.3699	85	.89	-	.16
	Rus	36	.96	49	64	.98	.5121	.3974	100	1.09	-	.30
	Mau	31	.94	44	52	1.19	.6048	.4948	100	1.09	-	.00
	Kho	45	.93	55	51	1.22	.6320	.5192	100	1.09	-	-
	Ada	16	.85	39	72	.88	.4619	.3455	80	.88	27	.00
	Loz	36	1.05	9	35	1.13	.1740	.1529	88	.87	-	.00
	Ash	35	.99	64	110	.66	.3955	.2428	75	.85	27	.27
	Pax	29	.94	41	54	1.12	.5619	.4567	73	.78	27	.00
	Mal	50	.75	59	62	1.00	.5147	.4032	-	-	-	-
	Cha	37	.71	51	57	1.12	.5902	.4724	81	.89	35	.35
	Bot	47	.74	55	86	.72	.3604	.2519	-	-	-	-
	deB	43	.69	62	60	1.12	.6245	.4934	84	.93	20	.19
	Sep	47	.78	67	83	.81	.4582	.3251	-	-	-	-
	Sal	23	1.94	70	71	1.16	.7806	.5874	96	1.16	24	.00
	Hal	28	.89	32	57	.97	.4161	.3329	-	-	-	-
	Pet	42	.90	29	51	.98	.3475	.2853	92	.95	-	.27
	Sch	36	.85	21	34	1.37	.4131	.3639	98	1.00	36	.00

Table VI-41 Plasma Iron Values in normal and Iron Deficiency Patients.

GROUP	Plasma Iron			
	N	$\bar{x}$	σ	P
Normal	28	111.1	40.7	
Fe deficiency				
(1)	12	24.3	10.8	< .0005
(2)	3	27.0	1.7	< .0005
(3)	28	48.5	16.0	< .0005
(4)	17	46.0	17.0	< .0005

Table VI-42 $t_{\frac{1}{2}}$PIC values in normal and Iron deficient patients

GROUP	$t_{\frac{1}{2}}$PIC			
	N	$\bar{x}$	σ	P
Normal	31	80.9	25.7	
Fe deficiency				
(1)	12	21.9	6.3	< .0005
(2)	3	20.3	7.6	< .0005
(3)	28	28.9	7.6	< .0005
(4)	17	61.7	18.7	< .0025

Table VI-43 Erythropoietic Indices in normal and Iron deficient patients

GROUP	Erythropoietic Index			
	N	$\bar{x}$	σ	P
Normal	28	1.13	0.29	
Fe deficiency				
(1)	12	2.39	0.65	< .0005
(2)	3	2.44	0.70	< .0025
(3)	28	2.27	0.79	< .0005
(4)	17	1.03	0.18	< .10

Table VI-44 Plasma Iron Turnover values in Normal and Iron deficient patients

GROUP	Plasma Iron Turnover			
	N	$\bar{x}$	σ	P
Normal	28	.7816	.2554	
Fe deficient				
(1)	12	.8107	.3656	< .45
(2)	3	.6945	.1362	< .20
(3)	28	1.1232	.5713	< .005
(4)	17	.4880	.1398	< .0005

Table VI-45 Erythroid Iron Turnover values in normal and Iron Deficiency patients

GROUP	Erythroid Iron Turnover			
	N	$\bar{x}$	σ	P
Normal	28	.4401	.1821	
Fe deficiency				
(1)	12	.7507	.3476	< .0005
(2)	3	.6476	.1426	< .025
(3)	28	1.0164	.5459	< .0025
(4)	17	.3820	.1135	< .10

Table VI-46 Red cell utilisation in normal and iron deficient patients

GROUP	RBCUe			
	N	$\bar{x}$	σ	P
Normals	17	83	15	
Fe deficiency				
(1)	9	85.1	14.2	< .40
(2)	-	-	-	-
(3)	18	75.6	20.9	< .15
(4)	13	88.6	9.7	< .15

Table VI-47 Ratio RBCUe/RBCUp values in normal and Iron deficient patients

GROUP	RBCUe/RBCUp			
	N	$\bar{x}$	σ	P
Normal	14	1.11	0.27	
Fe deficiency				
(1)	9	.87	0.15	< .01
(2)	-	-	-	-
(3)	18	.83	0.23	< .0025
(4)	13	.96	0.11	< .05

Table VI-48 $t_{\frac{1}{2}}{}^{51}Cr$ Red Cell Survival values in normal and iron deficient patients.

GROUP	$t_{\frac{1}{2}}{}^{51}Cr$			
	N	$\bar{x}$	σ	P
Normal	10	27	4.7	
Fe deficiency				
(1)	8	20.1	7.1	< .025
(2)	-	-	-	-
(3)	16	22.6	9.3	< .10
(4)	7	28.0	5.7	< .40

Table VI-49 Comparison of differences in various parameters, of different groups of Fe deficient patients (results expressed in terms of significance of the differences), compared with group (1) patients (i.e classical Fe. deficiency anaemia)

	GROUP		
	(2)	(3)	(4)
PI	< .25	< .0005	< .0005
$t_{\frac{1}{2}}$PIC	< .40	< .0025	< .0005
EI	< .475	< .30	< .0005
PIT	< .20	< .025	< .0005
EIT	< .25	< .05	< .0025
RBCUe	-	< .10	< .30
RBCUe/RBCUp	-	< .30	< .10
$t_{\frac{1}{2}}{}^{51}$Cr	-	< .25	< .025

VI-g Ferrokinetic studies in Patients with Neoplastic Disease.

VI-g-1 Patients were selected for this study who were shown histologically to have some form of neoplasm, either benign or malignant. In this group of patients, a number of disease processes were often operative so that this study does not characterise ferrokinetic performance due to neoplastic processes alone. A number of the patients studied were on anti-metabolites or receiving radiotherapy, and a number had associated folate deficiency.

VI-g-2 The results in this group of patients may be seen in Table VI-50. In this group of patients, plasma iron, $t_{\frac{1}{2}}$PIC, and $t_{\frac{1}{2}}{}^{51}Cr$ values did not show any significant deviation from normal results. EI, PIT and EIT values tended to be raised, whereas RBCUe and RBCUe/RBCUp values were significantly depressed. From these results, it would appear that erythropoietic activity in these patients was increased, although much of this increase appeared to be ineffective. This may have been due to the effects of 'conditioned folate deficiency' by a growing tumour or a result of cytotoxic therapy.

VI-g-3 As described in the literature, the anaemia associated with neoplastic disease is usually associated with decreased erythropoietic activity and increased red cell destruction, (47) (39) (149) (151) (150) (197) (733) (583) (414) (413) a finding

not found in this group of patients. In addition, impaired release of catabolised haemoglobin iron from reticulo-endothelial sites has been postulated,[361][460] with plasma iron levels being reduced.[330] Other factors may aggravate the anaemia i.e. blood loss, bone marrow infiltration, conditioned deficiency of Vitamin B_{12} or folate, haemolysis and microangiopathy.[392][269][677][514]

Table VI-50 Ferrokinetic values, and their statistical difference from normal in patients with Neoplastic Diseases.

Name	Neoplasms	Hct	B.PCV / V.PCV	PI	$t_{\frac{1}{2}}$PIC	EI	PIT	EIT	RBCUe	RBCUe / RBCUp	$t_{\frac{1}{2}}^{51}$Cr	f
	a) Benign											
deV	Phaeo	43	0.77	64	46	1.48	.8501	.7148	-	-	-	-
Eat	Phaeo	39	1.13	44	28	2.10	1.0076	.9089	-	-	-	-
Jan	B_{12}def. Lipomatosis	24	0.90	96	29	3.43	2.5530	2.2912	45	0.59	24	0.53
	b) Malignant											
Mab	Hepatoma	62	0.87	65	32	1.83	.8672	.7695	-	-	-	-
Sil	Hepatoma	30	0.90	55	30	2.30	1.3185	1.1792	79	0.89	12	0.00
Sen	Myeloma	37	0.90	217	140	1.14	1.0238	.5229	31	0.60	27	0.48
Tay	Hyper-nephroma Folate ↓	54	0.74	26	17	2.69	.7696	.7238	66	0.67	43	0.40
vdM	Ca.uterus + R/T Retro-peritoneal	31	0.83	25	22	2.27	.8197	.7572	60	0.62	21	0.00
vRo	Adeno Ca.+R/T	32	1.18	193	148	1.03	.9201	.4435	-	-	-	-
vWy	Ca.breast + R/T	34	0.69	137	86	1.35	1.0998	.7703	82	1.19	24	0.14
Ret	Ca.caecum +5-FU+R/T	54	0.95	331	158	1.14	1.0542	.4712	-	-	-	-
Pau	Ca.Oesoph.	45	0.78	114	60	1.54	1.1134	.8796	-	-	-	-
Normal	$\bar{x}$			111.1	80.9	1.13	.7816	.4401	83	1.11	27	
	σ			40.7	25.7	0.29	.2554	.1821	15	0.27	4.7	
	N			28	31	28	28	28	17	14	10	
Benign	$\bar{x}$			68.0	34.3	2.34	1.4702	1.3050	-	-	-	-
	σ			26.2	10.1	1.00	.9410	.8596	-	-	-	-
	N					3						
	P					<.475						
Malignant												
	$\bar{x}$			129.2	77.0	1.70	.9985	.7241	63.6	0.79	25.4	
	σ			102.4	57.9	0.60	.1722	.2291	20.4	0.25	11.3	
	N			9	9	9	9	9	6	6	6	
	P			<.35	<.45	<.005	<.005	<.0025	<.025	<.01	<.40	

VI-h **Ferrokinetic studies in patients with infectious diseases.**

VI-h-1 All patients included in this study were studied while on appropriate chemotherapy for their infective process.

VI-h-2 The results that I have obtained in this group of patients are detailed in Table VI-51. Characteristic of the group was:-

No significant difference from normal in PI, $t_{\frac{1}{2}}$PIC, PIT, RBCUe or $t_{\frac{1}{2}}{}^{51}$Cr.

A significant increase in EI ($P < .05$) and EIT ($P < .05$).

A significant decrease in RBCUe/RBCUp ($P < .05$)

Once again, there appears to be increased erythropoietic activity, with a major portion of this being ineffective as evidenced by a decreased RBCUe/RBCUp ratio in the presence of normal $t_{\frac{1}{2}}{}^{51}$Cr red cell survival. Two of the patients included in this study had evidence of folate deficiency, one patient had an associated auto-immune haemolytic anaemia, and seven of the patients were known to be alcoholics. These complicating factors undoubtedly influenced the results, so that I cannot claim to have demonstrated any ferrokinetic pattern characteristic of infection alone.

VI-h-3 Plasma iron levels tend to be decreased in the presence of infection,[129] [130] [673] [698] [796] [273] [735] [252] despite adequate body iron stores.[29] Impaired red cell

production appears to be the main cause of the anaemia associated with infection.[113][273] This may, in part, be due to impaired reticulo-endothelial release of catabolised haemoglobin iron, due either to increased reticulo-endothelial avidity for iron or impaired release mechanisms.[113][273] The bone marrow, as emerges from my results, appears to be capable of normal utilisation of transferrin iron. A moderate haemolytic element has also been described in the anaemia of infection,[100] this usually being of minor significance although it may assume major importance.[433][110][579][148][742][538] Limitation of erythroid activity may be conditioned by lowered plasma iron levels, but there also appears to be inadequate production of erythropoietin.[129] Haurani et al have also shown that gastrointestinal absorption of iron is depressed in the anaemia of infection.[363][362]

Table VI-51 Ferrokinetic values, and their statistical difference from normal in patients with Infectious Diseases.

Infections Name	Hct	B.PCV / V.PCV	PI	$t_{\frac{1}{2}}$PIC	EI	PIT	EIT	RBCUe	RBCUe / RBCUp	$t_{\frac{1}{2}}$ 51 Cr	f
All	23	.98	38	17	3.55	1.7623	1.6574	86	0.92	-	.00
Afr	58	.82	123	81	1.04	.7056	.5048	-	-	-	-
Car	38	1.05	53	35	1.84	.4204	.2997	82	0.90	32	.12
Car	59	.72	215	105	1.14	.9362	.5921	-	-	-	-
Hoo	29	.85	16	29	1.54	.4045	.3635	92	0.93	22	.14
Joh	37	-	138	120	0.95	.7554	.4381	37	0.52	25	.04
Lob	29	.90	139	90	1.37	1.1324	.7757	-	-	-	-
Loz	36	1.05	9	35	1.13	.1740	.1529	88	0.87	-	.00
Maj	46	.90	163	133	0.87	.7096	.3805	66	0.96	-	.36
Ngo	45	.70	187	120	1.08	.9132	.5296	98	1.53	22	.27
Pax	29	.94	41	54	1.12	.5619	.4567	73	0.78	27	.00
Rus	36	.96	49	64	0.98	.5121	.3974	100	1.09	12	.30
Swa	28	.57	92	20	4.69	3.3812	3.1422	23	0.29	21	.00
Ste	30	.91	167	38	3.67	3.1486	2.7255	60	1.00	-	.00
Normal $\bar{x}$			111.1	80.9	1.13	.7816	.4401	83	1.11	27	
σ			40.7	25.7	0.29	.2554	.1821	15	0.27	4.7	
N			28	31	28	28	28	17	14	10	
Infection											
$\bar{x}$			102.1	67.2	1.78	1.1084	.8869	73.2	0.89	23	
σ			68.1	40.5	1.24	.9910	.9395	24.9	0.31	6.2	
N			14	14	14	14	14	11	11	7	
P			<.35	<.15	<.05	<.15	<.05	<.15	<.05	<.10	

VI-i Ferrokinetic Performance in patients with inflammatory and/or auto-immune disturbances.

VI-i-1 The patients selected for this study all had non-infectious (as far as could be established) inflammatory or immune disturbances. Patients with auto-immune haemolytic anaemias were, however, specifically excluded from this study.

VI-i-2 The results obtained on study of these patients, may be seen in Table VI-52. Values showing minimal deviation from normal are the PIT, EIT, RBCUe, RBCUe/RBCUp, $t_{\frac{1}{2}}{}^{51}Cr$ (P for all > 0.05). Plasma iron was significantly depressed ($P < .05$), and $t_{\frac{1}{2}}$PIC values were significantly shorter than normal ($P < .0125$). EI values were moderately but significantly raised ($P < .05$). These results suggest a suboptimal bone marrow response to a low plasma iron level.

VI-i-3 A review of the literature shows that depression of plasma iron levels is common in association with inflammation, (111)(439)(440)(643)(873)(810)(734)(221)(273) although body iron stores appear to be adequate. The defect appears to be reticulo-endothelial retention of catabolised haemoglobin iron.(363)(656) Defective gastro-intestinal iron absorption has been described in association with inflammation.(722)(810)(849)(73) Measurements of red

cell survival are usually normal.[65][221][512][716][873] The anaemia seems to be one due to relative rather than absolute bone marrow suppression, perhaps aggravated by reduced plasma iron supply.

Table VI-52 Ferrokinetic values, and then statistical difference from normal, in patients with inflammatory and auto-immune diseases.

Inflammations and Immune disorders Name		Hct	B.PCV / V.PCV	PI	$t_{\frac{1}{2}}$PIC	EI	PIT	EIT	RBCUe	RBCUe / RBCUp	$t_{\frac{1}{2}}$ 51 Cr	f
Bow	Chr.Pancreatitis	40	.86	177	94	1.40	1.1913	.7998	80	1.27	24	.00
Hoe	SLE	40	1.34	65	19	3.69	2.1285	1.9847	47	.53	26	.07
Koe	Buergers	56	.81	97	100	.75	.4703	.3057	72	.84	30	.08
leG	Good pastures	57	.57	102	77	.99	.6325	.4620	100	1.16	36	.24
Sch	Reiters	36	.85	21	34	1.37	.4131	.3639	98	1.00	36	.00
Vlo	Reiters	41	.94	36	50	1.08	.4484	.3699	85	.89	-	.16
vNi	Ulcerative Colitis	38	.75	59	22	3.08	1.7603	1.6259	100	1.11	15	.05
Arm	Rheumatoid Arthritis Fe.def.	33	.96	10	22	1.84	.3165	.2922	81	.80	-	.00
"	"	38	.92	92	62	1.39	.9651	.7557	90	1.10	-	-
Normal	$\bar{x}$			111.1	80.9	1.13	.7816	.4401	83	1.11	27	
	σ			40.7	25.7	0.29	.2554	.1821	15	.27	4.7	
	N			28	31	28	28	28	17	14	10	
Inflammation												
	$\bar{x}$			75.1	53.3	1.73	.9251	.7733	83.7	.97	27.8	
	σ			48.5	31.6	1.00	.6491	.6198	16.9	.23	8.0	
	N			9	9	9	9	9	9	9	6	
	P			<.05	<.0125	<.05	<.30	<.10	<.475	<.10	<.45	

VI-j Ferrokinetic studies in patients with cirrhosis.

VI-j-1 Patients selected for this group were those whose clinical summaries contained mention of histologically diagnosed cirrhosis, or abnormal liver function studies with a clinical diagnosis of cirrhosis having been made.

VI-j-2 My findings in this group of patients are detailed in Table VI-53. PI, $t_{\frac{1}{2}}$PIC, RBCUe and $t_{\frac{1}{2}}^{51}$Cr red cell survival did not deviate significantly from normal.

VI-j-3 EI, PIT and EIT values were all significantly increased ($P < .025$, $< .05$, $< .01$ respectively) while the ratio RBCUe/RBCUp was mildly depressed ($P < .0005$). These findings suggest the presence of a mild degree of ineffective erythropoiesis in this group of patients, related probably to disturbed folate levels and excessive ethanol consumption. Two patients had evidence of haemolysis, one having a severe iron deficiency anaemia and the other being a patient with porto-systemic encephalopathy. In both patients, blood loss was probably a contributory factor.

VI-j-4 The anaemia associated with liver disease appears to have certain common characteristics independent of the nature of the disease process affecting the liver. Other abnormalities frequently co-exist, particularly blood loss, iron deficiency, folate deficiency and acquired haemolytic disease.(194)(374)(403)(429)(431)(466)(471)(478)(582)(516)(738)(772)(785)(868) The bone marrow is frequently

hyperplastic with macronormoblastic features.[56][437][582][648] Although liver B_{12} levels are usually decreased, serum levels are frequently elevated above normal,[377][699][711][799][31][506][349][823][505] due to release of B_{12} from damaged hepatocytes. Liver folate concentration is also decreased[31][506] and folate can easily be displaced from the liver.[144] In most reports, plasma iron levels are reported as normal or mildly elevated except in the presence of an acute liver insult.[672] TIBC levels are often depressed, and % saturation levels increased.

VI-j-5 Increased gastrointestinal iron absorption, often in the presence of excessive body iron stores, has been demonstrated by a number of workers.[157][194][891] The plasma volume of these patients tends to be increased[164][229][343][517] especially in the presence of portal hypertension, and this may give rise to a 'dilutional' anaemia. Erythropoietic activity is usually increased. A number of workers have demonstrated a decrease in red cell survival,[15][133][137b][250][343][429][442][451][466][680] the defect being extracorpuscular. A number of different causes for this extracorpuscular defect have been described, although corpuscular defects have also been described as in Zieve's Syndrome.[148][915][461][619][35b][70][880][166][204][308][554][780][790][412] The abnormalities at an extracorpuscular level that have been described include G6PD deficiency, the development of a positive Coombe's test,

and haemolysis caused by viral hepatitis. In some patients, haemolysis appears to be the dominant haematological defect.

VI-j-6 Reported ferrokinetic studies tend to show increased total erythropoietic activity with increase in both effective and ineffective components.(145)(157)(466)(289)(343) The increase is thought, however, to be suboptimal

VI-j-7 The anaemia of liver disease is frequently complicated by the presence of other variables i.e. blood loss, malnutrition, iron deficiency, folate deficiency, vitamin deficiency, infection, alcohol etc. Alcohol seems to play a major role in the anaemia of liver disease, usually by interference with folate metabolism (decreased folate intake, decreased folate absorption, increased folate requirements, decreased hepatic avidity for folate and depletion of liver enzymes required for folate metabolism plus increased urinary excretion).(91)(194)(374)(386)(438)(465)(471)(788)(348)(127b)(580)(711) These factors may all combine to produce severe folate deficiency with megaloblastic bone marrow changes and concomitant changes in ferrokinetic performance. Alcohol also appears to exert a direct toxic effect upon erythropoiesis,(438)(571)(228)(386)(401)(818)(49)(865)

VI-j-8 Anaemia associated with liver disease presents a complex pathogenetic problem, and my findings probably reflect the effects of folate deficiency rather than any other factor.

Table VI-53 Ferrokinetic values, and then statistical difference from normal, in patients with cirrhosis.

Cirrhosis Name	Hct	B.PCV / V.PCV	PI	$t_{\frac{1}{2}}$ PIC	EI	PIT	EIT	RBCUe	RBCUe / RBCUp	$t_{\frac{1}{2}}$ 51 Cr	f
All	23	.98	38	17	3.55	1.7623	1.6574	86	.92	-	.00
Ada	16	.85	39	72	.88	.4619	.3455	80	.88	27	.19
Cha	36	.90	19	18	2.52	.7261	.6817	97	.98	18	.13
Cha	37	.71	51	57	1.12	.5902	.4724	81	.89	35	.35
Cra	51	.93	175	130	.89	.7145	.3984	-	-	-	-
Gre	32	.91	133	88	1.32	1.0688	.7404	27	.39	23	.23
Joh	37	.90	138	120	.95	.7554	.4381	37	.52	25	.04
Rus	36	.96	49	64	.98	.5121	.3974	100	1.09	12	.30
Sco	27	.60	153	63	2.13	1.8340	1.4316	51	.82	22	.44
Sev	44	.90	71	25	2.85	1.6904	1.5425	63	.72	17	.11
vdM	32	.83	70	25	3.09	2.0160	1.8431	87	1.01	28	.20
Normal	$\bar{x}$		111.1	80.9	1.13	.7816	.4401	83	1.11	27	
	σ		40.7	25.7	0.29	.2554	.1821	15	.27	4.7	
	N		28	31	28	28	28	17	14	10	
Cirrhosis	$\bar{x}$		85.1	61.7	1.84	1.1029	.9044	70.9	.82	23	
	σ		54.2	39.4	1.01	.5988	.5865	25.2	.22	6.8	
	N		11	11	11	11	11	10	10	9	
	P		<.10	<.10	<.025	<.05	<.01	<.10	<.005	<.10	

VI-k Ferrokinetic parameters in chronic renal disease.

VI-k-1 Only three patients with renal disease severe enough to warrant repeated dialysis were studied. In these patients the results are summarised in Table VI-54. As a group these patients had EI and EIT values that were not significantly different from normal values, but had significantly depressed PI, $t_{\frac{1}{2}}$PIC (reflecting the depressed PI level) and PIT values (again reflecting low PI values). In the two patients in which RBCUe values were obtained, these were both maximum and within the normal RBCUe/RBCUp range. Red cell survival was normal in the one patient in which it was measured. All of the patients were anaemic, indicating that bone marrow erythroid activity, while normal in absolute terms, was depressed in relation to the haematocrit levels.

VI-k-2 Reviewing the literature, it would appear that a number of factors are operative in the anaemia of renal failure. Inappropriately low levels of erythropoietin have been found,[7] and a haemolytic element to the anaemia has been described.[800] Some degree of marrow failure is invariably present,[20] the degree of depression usually being related to the amount of urea retention present[95][732] and the degree of glomerular filtration rate depression. Bleeding disorders also contribute to the anaemia.[131][230][523] Variable PI levels are reported, although TIBC levels are usually depressed.[523] Iron absorption appears to be normal.[862][7][221] Red cell survival studies have usually

demonstrated some degree of haemolysis,[457][483][233][137b][196][443][523][701][771][800][851] this being related to the severity of the renal failure and thought to be due to extracorpuscular abnormalities. Studies of red cells have demonstrated a number of abnormalities in red cell metabolism.[196][410][516][613][815][855][879][892][293][294] Bone marrow erythroid activity is suppressed, this being due to impaired or decreased production of erythropoietin,[7][102][103][282][281][545][626] although relative bone marrow hyporesponsiveness to erythropoietin has been described.[89][485][549][845] Ferrokinetic studies show depressed PIT values[7][103][221][826][483][484][536][770][443][457][523][545][701] and depressed RBCUe values. This latter abnormality has not been demonstrated in my patients, but it should be noted that they were on repeated dialysis treatment which has been shown by a number of authors to improve ferrokinetic performance.[58][128][221][984][545] On the other hand dialysis has been shown to remove folate and iron from the body, producing deficiencies of both of these essential substrates.[353][221]

Table VI-54 Ferrokinetic values, and then statistical difference from normal, in patients with chronic renal failure.

Chronic Renal Disease Name	Hct	B.PCV / V.PCV	PI	$t_{\frac{1}{2}}$PIC	EI	PIT	EIT	RBCUe	RBCUe / RBCUp	$t_{\frac{1}{2}}$ 51 Cr	f
Hel	28	.89	32	57	.97	.4161	.3329	-	-	-	-
LeG	37	1.00	102	77	1.21	.6325	.4620	100	1.16	36	.24
Mau	31	.94	44	52	1.19	.6048	.4948	100	1.09	-	.00
Normal	$\bar{x}$		111.1	80.9	1.13	.1816	.4401	83	1.11	27	
	σ		40.7	25.7	0.29	.2554	.1821	15	.27	4.7	
	N		28	31	28	28	28	17	14	10	
Chronic Renal Disease											
	$\bar{x}$		59.3	62	1.12	.5511	.4299	-	-	-	-
	σ		37.4	13.2	0.13	.1178	.0856	-	-	-	-
	N		3	3	3	3	3	-	-	-	-
	P		<.025	<.025	<.475	<.005	<.45	-	-	-	-

VI-7 Ferrokinetic observations in Megaloblastic Anaemias.

VI-7-1 All patients in this group had megaloblastic bone marrow change. They all had low serum B_{12} or folate levels or showed haematological response to these agents. In addition, all showed macrocytosis on examination of the peripheral blood. Many of the patients were started on specific therapy shortly after initiation of their ferrokinetic investigations. The results of study on this group of patients are shown in Table VI-55.

VI-7-2 As can be seen from the results, grossly increased erythropoietic activity was present in the group as a whole, as manifested by shortened $t_{\frac{1}{2}}$PIC values ($P < .0005$), increased erythropoietic indices ($P < .0005$), increased plasma iron turnover and erythroid iron turnover ($P < .0005$ for both). This increased erythropoietic activity appears to be largely ineffective as judged by depressed RBCUe and RBCUe/RBCUp values ($P < .05$ and $< .005$ respectively). Red cell survival in the group as a whole was also significantly shortened ($P < .025$). As a group, my patients demonstrated significantly depressed PI values ($P < .01$).

VI-7-3 The coincidence of iron deficiency with megaloblastic anaemia has frequently been observed[64][117][180][290] although the reverse has also been noticed.[119][180][573] Bone marrow morphology has demonstrated increased total erythropoiesis, and increased bone marrow volume has likewise

been noticed.[178][519][709][449][596] Morphological features of maturation arrest, nuclear cytoplasmic dissociation, megaloblastic erythropoiesis, as well as myelocyte and megakaryocyte abnormalities have been described. The abnormality of erythroid maturation has been confirmed by bone marrow culture,[889] which has showed an inability of megaloblasts to complete DNA synthesis and enter mitosis during maturation. All changes respond rapidly to treatment with the deficient factor i.e. B_{12} or folate.[184][383]

VI-7- 4 Many workers have shown the presence of ineffective erythropoiesis and haemolysis in megaloblastic anaemias.[524][526][350][754][258] A combined intrinsic and extrinsic haemolytic defect is present that responds rapidly to treatment with the deficient factor (i.e. B_{12} or folate). Iron absorption in untreated megaloblastic anaemias is normal or increased[72] and PI levels are usually increased in association with an increase in body iron stores. In the untreated state ferrokinetic investigation shows markedly increased erythropoietic activity (decreased $t_{\frac{1}{2}}$PIC, increased PIT) that is largely ineffective (decreased RBCUe), these parameters usually returning to normal with treatment.[253][383][622] All these changes occur in both B_{12} and folate deficiency.[373][375][376]

Table VI-55 Ferrokinetic findings in patients with Megaloblastic Erythropoiesis and comparison of these results with those of normal patients.

Megaloblastic Anaemias

Name	Hct	$\frac{B.PCV}{V.PCV}$	PI	$t_{\frac{1}{2}}$PIC	EI	PIT	EIT	RBCUe	$\frac{RBCUe}{RBCUp}$	$t_{\frac{1}{2}}$ ^{51}Cr	f
All	23	.98	38	17	3.55	1.7623	1.6574	86	.92	-	.00
Cad	23	.73	42	15	4.21	2.1452	2.0295	81	.88	20	-
Cup	30	.90	113	53	1.99	1.5436	1.2573	82	1.11	22	.32
Jan	24	.90	96	29	3.43	2.5530	2.2912	45	.59	24	.53
Mte	30	.91	132	29	4.05	3.2954	2.9610	22	.32	6	.00
Sev	44	.90	71	25	2.85	1.6904	1.5425	63	.72	17	.11
vdM	32	.83	70	25	3.09	2.0160	1.8431	87	1.01	28	.20
vdM	31	.83	25	22	2.27	.8197	.7572	60	.62	21	.00
Normal $\bar{x}$			111.1	80.9	1.13	.7816	.4401	83	1.11	27	
σ			40.7	25.7	0.29	.2554	.1821	15	.27	4.7	
N			28	31	28	28	28	17	14	10	
Megaloblastic Anaemia											
$\bar{x}$			73.4	26.9	3.18	1.9782	1.7924	65.8	.77	19.7	
σ			38.0	11.7	.79	.7324	.6667	23.1	.26	6.9	
N			8	8	8	8	8	8	8	7	
P			<.01	<.0005	<.0005	<.0005	<.0005	<.05	<.005	<.025	

VI-m Ferrokinetic studies in patients with symptomatic porphyria.

VI-m-1 In this disease process, iron overload is common,[844][491] alcohol abuse is almost invariable[768][206] and analysis of the liquor consumed by these patients has shown it to have a high iron content.[744] I have investigated a number of patients proven to have this condition, the results of this study being shown in Table VI-56. This information has been previously published.[274]

VI-m-2 The patients were studied under metabolic ward conditions, and most had been without ethanol for four or five days prior to study. As a group, these patients showed normal red cell survival values, but had significantly raised PI ($P < .025$), elevated $t_{\frac{1}{2}}$PIC ($P < .20$) with normal EI values ($P < .1$) suggesting that the elevated $t_{\frac{1}{2}}$PIC values were due to their greater PI concentrations. For the same reason PIT was significantly elevated ($P < .01$) although this was confined to only a few patients in the group who also tended to have elevated EI values. These individuals may have had folate deficiency conditioned by ethanolic excess, as described in cirrhotic patients (see VI-j). RBCUe values were depressed, but once again this appeared to reflect the effect of increased PI values, as RBCUe/RBCUp was not significantly different from normal values.

VI-m-3 In effect, these patients, studied while not drinking ethanol, had normal haematological and ferrokinetic performance.

Table VI-56 Ferrokinetic findings in patients with symptomatic porphyria and comparison of these results with those of normal patients

Symptomatic Porphyrics Name	Hct	B.PCV / V.PCV	PI	$t_{\frac{1}{2}}$PIC	EI	PIT	EIT	RBCUe	RBCUe / RBCUp	$t_{\frac{1}{2}}$ 51 Cr	f
Cha	37	.71	51	57	1.12	.5902	.4724	81	.81	35	.35
Cor	46	.91	123	73	1.32	.9719	.7236	40	.51	34	.33
Eng	32	.98	100	188	.51	.3753	.1284	50	.64	-	.07
Hol	44	.95	139	110	.97	.7521	.4626	100	1.35	-	.14
Lou	45	-	186	53	2.44	2.0450	1.6635	-	-	-	-
Luk	38	1.02	209	134	1.14	1.0144	.5387	49	.91	18	.20
Len	46	.94	264	130	1.28	1.1713	.6384	80	1.67	-	.28
Sol	47	.90	88	130	.60	.3842	.2094	83	.98	28	.06
Sam	44	.84	123	61	1.61	1.2002	.9439	87	1.13	26	.00
Thy	51	.94	189	72	1.69	1.3934	1.0422	79	1.18	-	.00
Thy	44	.83	105	85	1.04	.7352	.5165	83	1.02	23	.11
vNi	26	.68	152	62	2.18	1.8652	1.4604	41	.66	23	.04
vNi	38	.84	212	57	2.71	2.4404	1.9578	18	.34	14	.00
Normal x			111.1	80.9	1.13	.7816	.4401	83	1.11	27	
σ			40.7	25.7	0.29	.2554	.1821	15	.27	4.7	
N			28	31	28	28	28	17	14	10	
Symptomatic Porphyrics											
x			149.3	93.2	1.43	1.1491	.8275	65.9	0.93	25.1	
σ			59.8	41.6	0.67	.6403	.5622	25.1	0.37	7.3	
N			13	13	13	13	13	12	12	8	
P			<.025	<.20	<.10	<.005	<.01	<.025	<.10	<.30	

VI-n Ferrokinetic observations in patients with myelofibrosis.

VI-n-1 Patients selected for this study were those diagnosed as having agnogenic myelofibrosis by the clinician looking after them, usually in consultation with the haematology service of the hospital. The results of study in this group of patients are summarised in Table VI-57. As can be seen, PI levels were normal, but the remaining parameters all differed significantly from normal. Erythropoietic activity tended to be greatly increased with shortened $t_{\frac{1}{2}}$PIC values ($P < .0005$), greatly elevated EI values ($P < .0005$) and greatly elevated values for PIT and EIT ($P < .0005$ for each). RBCUe and RBCUe/RBCUp were greatly depressed ($P < .0005$ for both), while red cell survival was significantly shortened.

VI-n-2 The anaemia of myelofibrosis, as seen in these patients, appears to represent a defect in effective erythropoiesis rather than, as the name implies, inadequate total erythropoiesis. The apparently excessive erythropoietic activity appeared to be largely ineffective, although a haemolytic element, probably on the basis of hypersplenism, was probably also operative. Other workers have reported ferrokinetic evidence of increased erythropoietic activity, (631a)(863) and haemolysis (631a)(130b) and ineffective erythropoietic activity.(902) Our results appear to agree with these observations, presenting a fairly typical ferrokinetic picture when the clinical and other haematological features are considered.

Table VI-57 Ferrokinetic findings in patients with myelofibrosis and comparison of these results with those of normal patients.

Myelofibrosis Name	Hct	B.PCV / V.PCV	PI	$t_{\frac{1}{2}}$ PIC	EI	PIT	EIT	RBCUe	RBCUe / RBCUp	$t_{\frac{1}{2}}$ 51 Cr	f
deV	44	0.73	196	84	1.62	1.3931	.9835	25	0.41	30	0.19
Fer	35	0.85	46	33	1.86	.9596	.8505	21	0.23	38	0.00
Gol	44	0.99	180	34	3.75	3.1511	2.7761	10	0.15	27	0.00
Han 1961	58	0.77	112	25	3.19	2.0895	1.9066	49	0.58	20	-
Han 1970	25	1.90	71	17	4.82	3.2737	3.0823	56	0.67	13	0.04
Han 1970	17	1.21	128	24	5.35	4.5949	4.2170	-	-	-	-
Han 1971	27	0.90	150	29	4.57	3.8347	3.4401	-	-	-	-
Han 1971 (AML)	37	0.82	53	31	2.09	1.1277	1.0053	55	0.60	11	-
Hun	36	0.96	169	63	2.11	1.7941	1.3985	22	0.35	-	-
Kna	35	0.89	17	16	2.77	.7204	.6800	60	0.61	27	0.30
Lif	29	1.16	81	77	1.12	.7713	.5634	65	0.79	27	0.00
Mte	30	0.91	132	29	4.05	3.2954	2.9610	22	0.32	6	0.00
Mar	27	0.82	91	22	4.27	3.1089	2.8695	28	0.35	14	0.20
Rou	-	-	-	67	-	-	-	7	-	16	-
Smi	37	0.67	89	79	1.08	.7431	.5376	70	0.85	26	0.14
Wil	30	1.88	44	34	1.83	.9369	.8254	93	1.01	16	0.00
Normal $\bar{x}$			111.1	80.9	1.13	0.7816	0.4401	83	1.11	27	
σ			40.7	25.7	0.29	0.2554	0.1821	15	0.27	4.7	
N			28	31	28	28	28	17	14	10	
Myelofibrosis											
$\bar{x}$			103.8	41.5	2.97	2.1196	1.8731	44.3	0.53	20.8	
σ			57.6	23.7	1.42	1.3055	1.2317	24.7	0.26	9.1	
N			16	16	15	15	15	14	13	13	
P			<.35	<.0005	<.0005	<.0005	<.0005	<.0005	<.0005	<.025	

VI-n-3 During the course of my work, I had occasion to study one patient repeatedly. He had previously had ferrokinetic studies while in Johannesburg, and the progression of his disease is of interest in that it showed the classical progression of polycythaemia to myelofibrosis terminating in acute monomyeloblastic anaemia. This patient, a Mr. Han, first consulted a doctor in 1961 when he was found to have a haemoglobin of 23.5G% and a white cell count of 18600. In 1962, a bone marrow aspiration was performed with difficulty, but the morphology of this was reportedly normal. Bone biopsy was subsequently performed and showed fibrous hypocellular bone marrow tissue. Ferrokinetics studies (see Table VI-57) showed increased total erythropoiesis, largely ineffective, with considerable splenic myeloid metaplasia. Myelofibrosis was diagnosed, and over the next seven years he remained reasonably well, on no treatment, with a leuko-erythroblastic peripheral blood picture.

VI-n-4 In 1970 he consulted a doctor again because of a palpable abdominal mass. Splenomegaly and mild hepatomegaly were found on examination. Bone marrow aspiration failed and his second lot of ferrokinetic investigations were performed. This showed considerable pooling of red cells in the spleen, with a B.PCV/V.PCV ratio of nearly 2. Total erythropoiesis was markedly increased, this being largely ineffective, and external counting again showed splenic erythropoiesis. Red cell survival was shortened.

VI-n-5 In an attempt to shrink the spleen, the patient was started on myeleran therapy, but this was stopped because of significant depression of the haematocrit and lack of effect on the spleen. Repeated ferrokinetics at this stage, still showed active erythropoiesis but the blood volume had decreased considerably and splenic pooling was no longer present. It was concluded that haemolysis was a dominant feature, and the patient was started on prednisone, oxymethalone, pyridoxine and folate, as well as being transfused. On the prednisone, he developed diabetes, the dose of prednisone was decreased, and as a consequence of this, his haematocrit dropped sharply. The prednisone was once again increased, the diabetes being controlled with oral therapy. He was then referred to Prof. Bothwell for his opinion concerning the advisability of splenectomy. This was recommended on the grounds of increasing transfusion requirements, and was performed in May of 1971. Ferrokinetics at this stage still showed grossly elevated erythropoiesis.

VI-n-6 The spleen was removed and weighed 4.3 kg. Histology showed marked splenic myeloid metaplasia. The post operative period was complicated by thrombocytopaenia and anaemia, requiring platelet packs and whole blood transfusion, but after one month the patient was discharged on methenolone, small doses of prednisone, folate and pyridoxine. The haemoglobin and haematocrit were well maintained for approximately two weeks, when the patient was again admitted with a massive gastro-intestinal bleed that was shown to come from two large benign gastric ulcers. He was resuscitated, and during the convalescent

period, repeat ferrokinetic study showed a similar pattern to previous tests. At this stage, AML was diagnosed on bone marrow examination and the patient died shortly thereafter.

VI-n-7 This patient's ferrokinetic features are consistent with the diagnosis of myelofibrosis, no deficiency of precursor material being demonstrated at any stage. This is in contrast to patient Smi., whose ferrokinetic performance was nearly normal, as were those of Lif. Progress of the disease, as seen in these patients, appears to be associated with progressively increasing ineffective erythropoiesis as emphasised in the literature.

VI-o <u>Ferrokinetic studies in patients with Reticulo-endothelial tumours (Reticuloses)</u>.

VI-o-1 This group included patients with leukaemia and lymphosarcoma. The diagnosis in all patients was made histologically. The ferrokinetic findings are presented in Table VI-58. As a group, these patients showed significantly increased total erythropoietic activity, (increased EI, decreased $t_{\frac{1}{2}}$PIC, increased PIT, increased EIT) that was largely ineffective (decreased RBCUe, decreased RBCUe/RBCUp). Red cell lifespan was also markedly depressed. Most of the patients were, at the time of study, receiving cytotoxic therapy, and many appeared to be folate deficient as judged by elevated MCV values.

This may have contributed to their ferrokinetic performance. One patient had markedly depressed erythropoiesis.

Decreased red cell survival, ineffective erythropoiesis, and variable total erythropoietic activity have all been reported in association with the reticuloses.[55][27b][631]

Table VI-58 Ferrokinetic findings in patients with Reticuloses or Leukaemia and comparison of these results with those of normal patients.

Name	Hct	B.PCV / V.PCV	PI	$t_{\frac{1}{2}}$ PIC	EI	PIT	EIT	RBCUe	RBCUe / RBCUp	$t_{\frac{1}{2}}$ 51 Cr	f
Reticuloses											
Ash	35	0.99	64	110	0.66	.3955	.2428	75	0.85	27	0.27
Bro	28	0.94	104	41	2.48	1.8878	1.6175	69	0.91	17	0.00
Key	41	0.79	165	102	1.21	1.0075	.6478	51	0.77	17	0.00
Kin	33	0.87	32	14	3.84	1.6386	1.5606	-	-	-	-
Mah	14	-	-	20	-	-	-	20	-	-	-
Rob	42	1.49	180	46	2.84	2.4010	2.0145	23	0.37	9	-
Sal	23	1.94	70	71	1.16	.7806	.5874	96	1.16	24	0.00
Siz	29	0.91	225	85	2.09	1.9408	1.3634	8	0.18	-	0.00
vNi	26	0.68	152	62	2.18	1.8652	1.4604	41	0.66	23	0.04
vNi	38	0.84	212	57	2.71	2.4404	1.9578	18	0.34	24	0.00
Wal	25	0.84	95	28	3.50	2.5940	2.3379	18	0.23	28	0.00
Han	37	0.82	53	31	2.09	1.1277	1.0053	55	0.60	11	-
Normal x			111.1	80.9	1.13	.7816	.4401	83	1.11	27	
σ			40.7	25.7	0.29	.2554	.1821	15	0.27	4.7	
N			28	31	28	28	28	17	14	10	
Reticuloses											
x			122.9	55.6	2.25	1.6436	1.3450	43.1	0.61	20	
σ			66.9	31.5	0.98	0.7268	.6584	28.5	0.32	6.8	
N			15	15	11	15	15	14	10	9	
P			<.30	<.005	<.0005	<.0005	<.0005	<.0005	<.0005	<.0125	

VI-p Ferrokinetic studies in Sidero-Achrestic Anaemias.

VI-p-1 No attempt has been made to study this disease in depth, as only two patients were studied. The subject has been excellently reviewed in a recent issue of the British Journal of Haematology,(597b) where mention is made of grossly elevated erythropoietic activity, the majority of this being ineffective. This was confirmed in my patients. The interest in my one patient lies in her response to iron removal by intramuscular desferrioxamine, after all other therapy appeared not to have helped her. The ferrokinetic findings, before and after desferrioxamine are shown in Table VI-5ϕ. This patient first presented in 1960 with bilateral axillary hydro-adenitis that was treated with 600r of local irradiation. In 1961, she presented with vague abdominal pain, general symptoms and fever. She was extensively investigated for her obscure pyrexia, investigations including laparotomy. The only positive finding was an anaemia (Hb 9.5G%, PCV 30), a raised PI with raised B_{12} and folate levels; peripheral smear showed two cell populations, one microcytic hypochromic, the other normochromic and anisocytotic. Bone marrow showed erythroid hyperplasia with megaloblastic features as well as features of dyshaemopoiesis, ringed sideroblasts and increased bone marrow iron stores. Ferrokinetics at this stage showed increased, largely ineffective, erythropoiesis. A definitive diagnosis of

sidero-achrestic anaemia was made, for which no obvious cause was present. She was treated with pyridoxine and ascorbic acid with no response until her ferrokinetic investigation in 1970. On the basis of the reported mitachondrial toxicity of excessive mitachondrial iron deposits[318][59][170b][597b] and the suggestion that chelating agents may be of use, as well as the report by Crosby showing response to vigorous phlebotomy, it was decided to attempt to deplete her iron stores with intramuscular injection of desferroxamine (500 mg. daily). Over the course of one month her haemoglobin rose by 1.5G%, and two months later it had risen to 11.5G%. During this period her PI fell from 253µg% to 71µg%. The improvement in her ferrokinetic performance is detailed in Table VI-58. She has apparently relapsed since stopping desferrioxamine therapy.

Table VI-59 Ferrokinetic findings in patients with sidero-achrestic anaemias and comparison of these results with those of normal patients.

	Reticuloses Name	Hct	B.PCV / V.PCV	PI	$t_{\frac{1}{2}}$ PIC	EI	PIT	EIT	RBCUe	RBCUe / RBCUp	$t_{\frac{1}{2}}$ ^{51}Cr	f
1965	Bot				38		2.8700		48		26	
1970	Bot(1)	33	-	253	124	1.51	1.4209	.8042	16	0.40	-	0.69
1971	Bot(2)	34	-	75	30	6.13	1.7180	1.5376	64		-	-
	Ber	18	-	377	86	3.99	3.6749	2.5739	8		-	0.20

APPENDIX A

METHODS

METHODS

Informed consent was obtained from all patients studied. This necessitated a brief description of the nature of radioactivity and the experimental protocol to the patient. Two patients refused study. All experiments started between 8.30 and 10.30 a.m.

A-1 LABELLING TECHNIQUES

a) A total of 35 ml. of blood was taken from the patient at the start of the experiment. Of this, 5.0 ml. was placed into a polycarbonate tube containing 2 mg. of powdered ethylene diamine tetra acetic acid (EDTA) for haematological study, 10 ml. was placed into an iron free tube for serum iron determinations and 20 ml. was put into a sterile McConkey bottle containing 5 ml. of acid citrate dextrose solution (ACD).

b) ^{59}Fe was obtained as a sterile solution of ferric citrate in isotonic saline from Amersham, U.K. This solution contained 100 mg/ml. of sodium citrate and 6 mg/ml of sodium chloride. The specific activity of the iron varied between 5 and 10 microcuries per microgram of iron. The amount of iron used for labelling approximately 15 ml. of plasma varied between one and two micrograms.

c) ^{51}Cr was obtained as sodium chromate in isotonic saline from Amersham, U.K. This had a specific activity

of 125 to 250 microcuries per microgram of chromium. The amount of radioactive chromate used for labelling varied between one and two micrograms, the recommended limit being 10 micrograms/ml. of packed red cells.(1)

d) The doses of isotope used were usually 10 microcuries of ^{59}Fe and 100 microcuries of ^{51}Cr. These doses are recommended by the South African Atomic Energy Board, and deliver a total or whole body radiation dosage of approximately 500 milli Rads to the patient,(2) this being well within the maximum recommended dose of 5 rads/year. In children the dose given was decreased in proportion to body weight.

e) In a few patients known to have high serum iron concentrations with high transferrin iron saturation, Australia-antigen-free donor plasma was labelled and injected.

f) Blood was prepared for labelling as follows:- The 20 ml. of patient's blood plus 5 ml. of ACD was gently inverted two or three times to insure adequate mixing. The McConkey Bottle was then centrifuged for 10 minutes at 5000 revolutions per minute (rpm). The supernatant plasma was aspirated and transferred to a second sterile McConkey bottle. The remaining red cells were resuspended in a volume of sterile isotonic saline equal to the volume of aspirated plasma.

g) To the plasma, 10 microcuries of ^{59}Fe as ferric citrate was added. The mixture was gently rotated and then incubated for 30 minutes at 37°C. After this period all but 1.5 ml. of the labelled plasma was drawn up into a sterile disposable syringe, the syringe plus plasma was then weighed prior to injection.

h) An ^{59}Fe standard solution was then prepared. The McConkey bottle plus residual labelled plasma was first weighed. One ml. of the labelled plasma was then pipetted out of the McConkey bottle and transferred to a clean 100 ml. volumetric flask. This was then made up to 100 ml. with distilled water. The flask was stoppered. The McConkey bottle and any residual plasma was weighed, the difference in weights prior to and after the removal of 1.0 ml. of plasma being the weight of 1.0 ml of the labelled plasma.

i) 100 microcuries of ^{51}Cr as sodium chromate was added to the resuspended red cells in the original McConkey bottle. The mixture was gently agitated for approximately one minute and was then incubated at room temperature for 30 minutes. At the end of this period, the mixture was centrifuged at 5000 rpm. for five minutes, the supernatant solution was aspirated, and fresh sterile normal saline was added. This washing procedure was repeated twice. The washed labelled red cells were then resuspended in an approximately equal volume of sterile normal saline. All but 1.5 ml of the red cell mixture was then drawn up into a sterile disposable syringe. The syringe

containing labelled red cells was then weighed.

j) A ^{51}Cr standard solution was prepared in the same way as the ^{59}Fe standard solution (A-1-h).

k) Four 4.0 ml. and four 2.0 ml. samples of ^{59}Fe standard were pipetted into clean uncontaminated disposable counting tubes for later use as a counting standard. The same procedure was carried out on the ^{51}Cr standard. The remaining standard solution was stoppered for use as standards for external counting.

A-2 HAEMATOLOGICAL MEASUREMENTS.

These were performed as detailed by Dacie.[3]

a) Haemoglobin concentration was measured by the cyanmethaemoglobin method, and gave a reproducibility of $\pm$ 0.5 G%.

b) Haematocrit volumes were measured by the micro-haematocrit method using a Clay Adams microhaematocrit centrifuge and spinning samples for 5 minutes each. The haematocrit was read off in a Clay Adams micro-haematocrit reader. The reproducibility of this method was $\pm$ 0.5 volumes %.

c) White cell count, platelet count, mean cell volume and mean cell haemoglobin concentration were measured as described by Dacie.[3] The peripheral smear was

examined by trained haematology technicians who reported upon red cell morphology, white cell morphology and differential white cell counts.

e) Other haematological parameters were assessed in these patients by the clinician referring the patient for isotope investigation. At the end of my period of laboratory work, the hospital records of all patients studied were obtained and summarised for subsequent analysis.

A-3 INJECTION OF PATIENTS AND SAMPLE COLLECTION

a) Injection of labelled plasma and red cells:-
Prior to injection of the labelled solutions, the weight of the syringe and contents was recorded. A tourniquet was then applied to the patient's arm, a suitable large vein was identified, and venepuncture performed.
Once the needle was adequately placed in the vein, the syringe containing the labelled red cells was emptied, followed by the syringe containing the labelled plasma. The time of injecting the plasma was recorded immediately. The two empty syringes were then weighed, the weights recorded and the syringes discarded.

b) Sample collection:
Whole blood samples were taken at fifteen minute intervals for the first hour. These, and subsequent samples were

injected into clean test tubes containing five drops of heparin made up to a concentration of 5000 units per ml. with normal saline. The tubes were stoppered, mixed and identified with the patient's name, the time at which the sample had been collected, and the date. Samples were taken by repeated venepuncture, 10 ml. being taken on each occasion. In patients who were being studied for prolonged plasma iron clearance, repeated samples were taken as follows:

(i) Every 15 minutes for the first hour

(ii) Every 30 minutes for the next two hours.

(iii) Every 60 minutes for the next six hours.

(iv) At six hourly intervals for the next 24 hours

(v) Twice daily for the next three days

(vi) Daily thereafter until the end of the 14th day.

In patients not having prolonged plasma iron clearance studies, samples were taken daily for the first week, and on alternate days for the second week of the experiment.

A-4 PREPARATION OF SAMPLES FOR COUNTING

a) The first four samples were handled as follows:

(i) 2.0 ml of whole blood was pipetted into clean new disposable counting tubes.

(ii) the remaining blood was centrifuged

(iii) 2.0 ml. of supernatant plasma was pipetted into clean new disposable counting tubes. Where enough plasma was available, this was performed in duplicate.

(iv) the remaining red cells were washed in normal saline and stored until the end of the experiment.

All samples were stoppered.

b) Subsequent samples were handled as follows:-

(i) the sample was centrifuged

(ii) 4 ml. of supernatant plasma was pipetted into clean disposable counting tubes which were then stoppered.

(iii) the remaining red cells were washed in normal saline and retained as packed cells until the end of the experiment.

c) At the termination of the experiment, the patient had usually been venesected of between 140 and 200 ml. of blood, 10 ml. samples being taken with each venepuncture.

d) At the end of the experiment, haemoglobin solutions were prepared from the packed red cells. This was done by adding distilled water to the packed cells, stoppering the tubes and mixing until all the cells had been lysed.

The solutions prepared were made up to an approximate volume of 10 ml. Each sample was then analysed for its haemoglobin concentration using the cyanmethaemoglobin method. Two ml. of each haemoglobin solution was then pipetted into clean disposable counting tubes, this usually being done in duplicate.

e) At the time of counting, each patient had the following samples counted:

4 x 4.0 ml ^{59}Fe standard solutions

4 x 4.0 ml ^{51}Cr standard solutions

4 x 2.0 ml ^{59}Fe standard solutions

4 x 2.0 ml ^{51}Cr standard solutions

4 x 2.0 ml whole blood solutions

4 x 2.0 ml plasma solutions

(this may have been done in duplicate).

Subsequent daily 4.0 ml plasma solutions and 2.0 ml. haemoglobin solutions (usually in duplicate) - usually twelve days samples being counted (24 to 36 tubes) i.e. a total of between 48 and 60 samples for each patient.

f) Background count rates were obtained on plain clean disposable counting tubes which were inserted at every tenth space to insure that change of background count rate did not occur during the time of the experiment.

A-5 SAMPLE COUNTING TECHNIQUES

a) Counting Equipment and Settings:

A dual channel, 100 sample capacity, Packard Automatic Gamma Spectrometer with a lead shielded three inch sodium iodide crystal, was used for sample counting. To this was attached an automatic printer which printed out, on each sample, the sample number, duration of counting, and the counts recorded during the counting interval in each channel.

The counter was housed in a "counting room" where no radioactivity other than that contained in the samples being counted was allowed. The isotope spectra for ^{59}Fe and ^{51}Cr were plotted on a spectrum scanner and settings for counting these two isotopes were planned so that no ^{51}Cr radioactivity was detectable in the upper channel while the upper photopeak of ^{59}Fe was measured in this channel.

The "upper window" used for counting ^{59}Fe was 490 to 750 kev. In this window setting, ^{51}Cr activity was not detectable. The "window" setting for ^{51}Cr was 200 to 380 kev. At this setting, counts due to both ^{51}Cr and ^{59}Fe radioactive decay were detectable.

b) Sample Counting:

Each patient's sampleswere counted in a simple "run" at the termination of his study. For reasons of convenience and because of pressure upon the machine for counting time, each sample was counted for a maximum of forty minutes. During this period 10,000 counts were usually recorded, giving a standard deviation of $\pm$ 1% using the equation

$$SD = \frac{100}{\sqrt{NT}}$$

where SD = standard deviation

N = count rate in cpm.

T = time of counting

as suggested by Veall and Vetter.[6] Background count rates were 60 cpm in the lower channel and 40 cpm in the upper window.

c) Separation of ^{51}Cr and ^{59}Fe counts in each sample. ^{59}Fe and ^{51}Cr standards were counted in each run. The background count was subtracted from the sample count in each channel. Because of the window settings, no ^{51}Cr radioactivity was detected in the upper window setting, whereas the lower channel counts recorded were usually a mixture of ^{51}Cr and ^{59}Fe counts. It was possible to calculate the number of ^{59}Fe counts in the lower channel by a knowledge of the ratio of ^{59}Fe standard counts in the upper and lower channels. In a mixed sample, the ^{59}Fe counts (n) in the lower channel would be:

$$n = \frac{^{59}\text{Fe Standard counts in lower window}}{^{59}\text{Fe Standard counts in upper window}} \times \text{Sample counts in upper window}$$

Counts due to ^{51}Cr in the lower channel could then be calculated as the difference between total recorded counts in the lower channel and counts in the lower channel due to ^{59}Fe as calculated above.

A-6 CALCULATION OF TOTAL BLOOD VOLUME

This was calculated by the isotope dilution principle using ^{51}Chromium (^{51}Cr) labelled red cells. The total amount of radioactivity injected, in counts per minute ($Cpm_{(inj)}$), as ^{51}Cr labelled red cells was calculated as follows:

$$^{51}Cr \text{ .} Cpm_{(inj)} = \frac{W_1}{W_2} \times Cpm_{(s)} \times \text{dilution factor}_{(s)}$$

Where W_1 = the weight of labelled red cells injected (Gms)

W_2 = the weight of 1.0 ml of labelled red cells (Gms)

$Cpm_{(s)}$ = the radioactivity contained in 2.0 ml. of the standard solution of diluted red cells (Cpm).

$\text{Dilution factor}_{(s)}$ = the volume in which 1.0 ml. of the labelled cells had been diluted in the preparation of the standard solution.

The total blood volume (TBV $_{(^{51}Cr)}$) was then calculated as follows:

$$TBV_{(^{51}Cr)(ml)} = \frac{^{51}Cr.\ Cpm_{(inj)}}{Cpm_{(bl)}}$$

where $Cpm_{(bl)}$ = the amount of ^{51}Cr radioactivity contained in a 2.0 ml. sample of whole blood.

The same volume of blood and standard was counted, obviating any need to correct for volume. The $Cpm_{(bl)}$ value was obtained from the mean ^{51}Cr radioactivity (Cpm) contained in four samples of whole blood taken at fifteen minute intervals after injection of the ^{51}Cr labelled red cells.

The patient was weighed (kg), and the blood volume was divided by the patients weight to give a recording of blood volume in ml/kg.

A-7 CALCULATION OF PLASMA VOLUME

This was performed using ^{59}Fe labelled transferrin as the isotope marker, and the isotope dilution principle as outlined in A-6.

Four blood samples were taken from the patient at fifteen minute intervals after the injection of the ^{59}Fe labelled transferrin in plasma (^{59}Fe plasma).
Two ml. samples of plasma were prepared from each sample of whole blood as described in A-4. These samples were then counted, where possible in duplicate. The radioactivity of each sample (Cpm) was plotted as a function of time on semilogarithmic paper with time being the independent variable. A regression equation describing the best

semilogarithmic least squares fit for these values was then calculated, and the correlation coefficient (r) of this line was determined. Where (r) values were less than 0.95 it was assumed that either free (unbound) ^{59}Fe had been injected or that equilibration of labelled transferrin between the plasma and an extravascular transferrin pool had occurred. This data was, therefore, discarded in calculating plasma volumes. In this group of patients (less than 5% of all patients tested) plasma volume was indirectly calculated from the $TBV_{(^{51}Cr)}$ as follows:

$$PV_{(^{51}Cr)} = TBV_{(^{51}Cr)} \times \left[\frac{100 - (Hct \times 0.92)}{100}\right]$$

where $PV_{(^{51}Cr)}$ = Indirectly calculated plasma volume

Hct = Patient's haematocrit (volumes %)

0.92 = A correction factor to bring the peripheral Hct. to the value of the body haematocrit

i.e. Body Hct = Venous Hct x 0.92

This correction factor of 0.92 is recommended by Cook[8] and used in all his ferrokinetic equations. As a large proportion of my work has been based upon his calculations, it seems prudent to use the same correction factor.

Where the correlation coefficient exceeded 0.95 (more than 95% of patients studied), plasma volume was calculated using the ^{59}Fe plasma method.

The theoretical value of radioactivity per unit volume present at the time of injection (t_o) was calculated from the regression equation. The plasma volume was then calculated as follows:

$$PV_{(^{59}Fe)}(ml/kg) = \frac{Cpm_{(inj)}\ ^{59}Fe}{Cpm_{(pl)}} \times \frac{1}{Wt.}$$

Where $PV_{(^{59}Fe)}$ (ml/kg) = total plasma volume in ml.

$Cpm_{(inj)}\ ^{59}Fe$ = total amount of ^{59}Fe radioactivity injected (Cpm)

$Cpm_{(pl)}$ = the amount of ^{59}Fe radioactivity present in a 2.0 ml. sample of plasma at (t_o).

Wt = Patient's weight in kgs.

$Cpm_{(inj)}\ ^{59}Fe$ was calculated as follows:

$$Cpm_{(inj)}\ ^{59}Fe = \frac{W_1}{W_2} = Cpm_{(s)} \times d.f.$$

Where W_1 = weight of ^{59}Fe labelled plasma injected (Gm)

W_2 = weight of 1.0 ml of labelled plasma (Gm)

$Cpm_{(s)}$ = the amount of radioactivity (Cpm) in a 2.0 ml sample of the ^{59}Fe standard solution.

d.f. = the volume in which 1.0 ml. of labelled plasma had been diluted in preparing the standard.

A-8 CALCULATION OF RED CELL VOLUME

This was calculated as the difference between the total blood volume and the plasma volume.

A-9 CALCULATION OF TRUE BODY HAEMATOCRIT.

This was calculated as follows:-

$$Hct_{(B)}(\%) = \frac{\text{Red cell volume (ml)}}{\text{Total blood volume (ml)}} \times 100$$

A-10 CALCULATION OF CORRECTION FACTOR TO CORRECT VENOUS HAEMATOCRIT TO BODY HAEMATOCRIT VALUES.

This was calculated as the ratio of body haematocrit to venous haematocrit

i.e. Correction factor = $\frac{\text{Body haematocrit}}{\text{Venous haematocrit}}$

A-11 CALCULATION OF THE $t_{\frac{1}{2}}$ PLASMA IRON CLEARANCE ($t_{\frac{1}{2}}$PIC)

This was calculated from the regression equation described in A-7 which described the best least squares fit between the logarithmic values of plasma radioactivity/unit volume of plasma, and time.

$t_{\frac{1}{2}}$PIC was calculated as follows:

$$t_{\frac{1}{2}}PIC = \frac{Log_e\ 2}{\text{Slope of regression line}}$$

$t_{\frac{1}{2}}$PIC values were expressed in minutes.

A-12 CALCULATION OF PLASMA IRON TURNOVER (PIT)

This was calculated by the method of Bothwell[7]. This method excludes the potential error of inaccurate plasma

volume estimations, and has the added advantage that data from patients with differing plasma volumes or weights can be compared. Results from different laboratories may also be compared. The method of calculation is as follows:

$$PIT = \frac{PI}{t_{\frac{1}{2}}PIC} \times \left[\frac{100 - (Hct \times 0.92)}{100}\right] \text{mg/100 ml.whole blood/day}$$

Where PI = Plasma Iron Concentration in µg%

Hct = Haematocrit

0.92 = A correction factor to convert venous haematocrit values to body haematocrit values.

A-13 CALCULATION OF NON ERYTHROID IRON TURNOVER (NEIT)

This was calculated by the method of Cook.[8]

i.e.

$$NEIT = 0.0035 \times PI \times \left[\frac{100 - (Hct \times 0.92)}{100}\right] \text{mg/100 ml. whole blood/day}$$

A-14 CALCULATION OF ERYTHROID IRON TURNOVER (EIT)

This was calculated as the difference between the PIT and the NEIT.

A-15 CALCULATION OF THE ERYTHROPOIETIC INDEX

The rationale and development of this index has been detailed in my thesis (IV-3-o). The erythropoietic index (EI) is the ratio of predicted normal $t_{\frac{1}{2}}PIC$ values at the same serum iron concentration as the patient to the patient's actual $t_{\frac{1}{2}}PIC$.

i.e.

$$EI = \frac{\text{predicted } t_{\frac{1}{2}}PIC}{\text{Experimental } t_{\frac{1}{2}}PIC}$$

The predicted $t_{\frac{1}{2}}PIC$ is calculated by the following equation:

$$t_{\frac{1}{2}}PIC \text{ (Pred)} = (0.87 \times PI \times PCT) + 34.67$$

where PCT = plasmatocrit = $\frac{100 - (Hct \times 0.92)}{100}$

A-16 CALCULATION OF RED BLOOD CELL UTILISATION (RBCUe)

This value measures the percentage of injected ^{59}Fe radioactivity getting into the haemoglobin of circulating red cells. It was important, to me, because of the model that I have generated, to attempt an accurate measurement of this value. My method of calculating RBCU values is as follows:

$$RBCU\ (\%) = \frac{^{59}Fe \text{ Cpm (rbc's)}}{^{59}Fe \text{ Cpm (inj)}} \times 100 \qquad (1)$$

where ^{59}Fe Cpm in rbc's is the amount of ^{59}Fe radioactivity in circulating red cells. This value ^{59}Fe Cpm.(rbc's) has been derived as follows:

At the end of each patient's experiment, the packed red cells were resuspended in distilled water. These packed cells had previously been separated from the plasma, washed in saline and stored at $4^{o}C$. Each days samples were accurately labelled. Resuspending the cells in

distilled water resulted in cell lysis and liberation of free haemoglobin. On each sample thus prepared, a haemoglobin estimation was performed as previously described. (A.1.b) Two ml. aliquots of haemoglobin solution were then pipetted into counting tubes, and counted. From this, the specific activity of the haemoglobin could be calculated.

$$Cpm_{(^{59}Fe)} \text{ /Gm. Hb.} = \frac{Cpm_{(s)}}{\text{Hb. conc.}_{(s)}} \qquad (2)$$

where Hb = haemoglobin

$\text{Hb. conc.}_{(s)}$ = haemoglobin concentration/100 ml. in sample being counted

$Cpm_{(s)}$ = total ^{59}Fe radioactivity counted in the sample.

The total amount of circulating haemoglobin (Hb_{circ}) could be calculated as follows:

$$\text{Hb.}_{(circ)} = \frac{Hb_{(pt)}}{100} \times TBV_{(^{51}Cr)} \qquad (3)$$

where $Hb_{(pt)}$ = the haemoglobin concentration of the patient during the period of observation (Gm/100 ml. whole blood)

The total amount of circulating red cell ^{59}Fe radioactivity could then be calculated as:

$$^{59}Fe\ \ Cpm(rbc's) = \frac{Cpm_{(s)}}{Hb.Conc._{(s)}} \times Hb_{(circ)}$$

$\therefore$ from (3)

$$= \frac{Cpm_{(s)}}{Hb.\ conc._{(s)}} \times \frac{Hb_{(pt)}}{100} \times TBV(^{51}Cr)$$

$$= \frac{Cpm_{(s)}}{Hb_{(s)}} \times Hb_{(pt)} \times TBV(^{51}Cr)$$

$$= Cpm_{(s)} \times \frac{Hb_{(pt)}}{Hb_{(s)}} \times TBV(^{51}Cr) \qquad (4)$$

$^{59}Fe\ Cpm_{(inj)}$ is calculated as previously described (A-7) where all the samples and the standard are counted on the same "run". RBCU can then be calculated as detailed in A-16 (1).

Using this method to calculate RBCU, I have managed to obtain very reproducible values on same day samples counted in duplicate, and very little day to day variation in RBCU values once the plateau phase of the RBCU curve has been reached ($\pm$ 2%).

A-17 CALCULATION OF PREDICTED NORMAL RBCU (RBCUp)

This calculation has been derived as stated in the text of my thesis. (IV-3-o) and is calculated by the equation

$$RBCU\ predicted\ (\%) = 103.5096 - (.3633 \times PI \times PCT)$$

A-18 CALCULATION OF $t_{\frac{1}{2}}{}^{51}$CHROMIUM RED CELL SURVIVAL ($t_{\frac{1}{2}}{}^{51}Cr$)

For reasons stated in the text (VI-f-7), I have elected to calculate $t_{\frac{1}{2}}{}^{51}Cr$ rather than to correct this data so as to arrive at a value for mean cell survival. This decision was taken before the recommendations of the standardisation committee were made known, and the majority of patients were likewise studied before this committee's recommendations appeared in print.

The two ml. samples of haemolysed red cells used for counting ^{59}Fe haemoglobin specific activity were used to calculate ^{51}Cr specific activity. The method by which the ^{51}Cr specific activity of the haemoglobin solution was calculated was:

$$^{51}Cr \text{ Specific Activity} = \frac{Cpm_{(^{51}Cr)} \text{ of sample}}{Hb_{(s)}}$$

where $Hb_{(s)}$ = the haemoglobin concentration of the sample.

^{51}Cr Haemoglobin specific activity was plotted against time on semilogarithmic paper. A semilogarithmic least squares regression line was calculated and a regression equation obtained. The $t_{\frac{1}{2}}{}^{51}Cr$ was calculated using this regression equation i.e.

$$t_{\frac{1}{2}}{}^{51}Cr = \log_{(e)} 2/\text{slope of regression equation.}$$

A-19 EXTERNAL COUNTING

This was performed on a number of patients. An attempt was made to obtain "true organ counts" daily. True organ counts in this context may be defined as the counts over an organ due solely to ^{59}Fe sequestered in that organ, to the exclusion of counts due to ^{59}Fe coming from the organ caused by perfusion of the organ by labelled blood or plasma.

(i) Counting technique:

Patients having external counting performed upon themselves were injected while lying upon a stretcher under the crystal of a scanner (Picker Nuclear Dual Probe Magna Scanner). This machine had a five inch sodium iodide crystal, and was connected to a Picker Nuclear Digital counter (scaler/timer). The crystal was mounted over the patient at the site where counting was to be performed. Four sites were counted routinely - viz - over the sacrum, the spleen, the liver and the heart. These spots were marked with ink and counted repeatedly on serial days of the experiment. Points chosen to count these organs, when palpable enlargement was not present, were chosen anatomically. The heart count was obtained over the fourth left intercostal space, approximately one inch to the left of centre. The spleen count was obtained over

the line joining the ninth costal cartilage and the mid axillary line. The liver count rate was obtained over the line joining the ninth costal cartilage and anterior axillary line on the patient's right side. The sacral counts were obtained over the point joining the posterior iliac crests, in the midline. These points were marked and accurately localised using a light mounted in the head of the counting crystal. The height at which the crystal was situated above the patient was recorded on the first day of counting, and this height was used throughout the period of observation.

A focusing collimeter with a five inch focal point was used for counting. Settings for counting were determined on the basis that the patient had both ^{51}Cr and ^{59}Fe activity in his body. Initially, two window settings were used, the higher window being selected so as to exclude any counts above background when a ^{51}Cr standard was being counted. This window was set at 400 to 1000 Kev. The lower window was set to include ^{59}Fe and ^{51}Cr counts, the setting being 200 to 380 kev. The counts due to ^{51}Cr and ^{59}Fe were determined using the method detailed in A-5-(iii). It was found that count rates due to ^{51}Cr were disappointingly low using this method, and the data obtained was highly suspect. Eventually it was decided to discard this ^{51}Cr data and to count ^{59}Fe alone. ^{59}Fe counts were always high and the accuracy of the count rate was $\pm$ 1%. These counts were very reproducible and good

curves were obtained using ^{59}Fe data alone.

(ii) Calculation of organ counts.

A series of calculations was required to correct the gross count rate obtained on different days to a count rate corrected for variations in machine efficiency and for change in count rates due to isotope decay. This correction was performed by counting a standard ^{59}Fe solution daily. The steps in the correction procedure may be detailed as follows:

a) count patient and obtain gross count rate on $day_{(t)}$

b) subtract background count rate on $day_{(t)}$ from this value. Gross count rate - BG count rate

= Organ count on $day_{(t)}$

(hereafter called O_t)

c) Count standard ^{59}Fe solution on day_o.
Subtract background count rate on day 0.
(hereafter called S_o)

d) Count standard ^{59}Fe solution on day (t).
Subtract background count rate on day (t).

This gives the ^{59}Fe standard count rate on day (t) (hereafter called S_t).

e) Gross organ count rate on $day_{(t)}$ may then be corrected for isotope decay and for day to day variations in machine counting efficiency by the equation:

$$\text{Corrected } O_{(t)} = O_{(t)} \times \frac{S_{(o)}}{S_{(t)}}$$

The counts of each organ, corrected in this way, could then be plotted as a function of time. This yields a line showing the gross day to day variation in organ counts. These counts may be corrected to show changes in specific organ counts as follows:

If I assume that at $t_{(o)}$, the time of injection of isotope, all the counts obtained over an organ are due to perfusion of that organ by blood or plasma containing isotope, the count rate due to uptake of that isotope by the organ itself will be zero.

The ratio of organ counts to counts obtained over the heart will, at t_o, reflect the ratio of blood perfusing the organ to blood perfusing the heart.

$\therefore$ At t_o, Organ counts due to blood perfusing the organ will be:-

$$\frac{O_o}{H_o} \times H_o = \text{Zero}$$

where O_o = organ counts at t_o

H_o = heart counts at t_o

If we assume that all counts obtained over the heart are due to blood perfusing this area, then count rates over

the organ being counted due to perfusion by blood can be calculated

i.e.

$$B_{(t)} = \frac{O_{(o)}}{H_{(o)}} \times H_{(t)}$$

where $B_{(t)}$ = counts over organ due to perfusing blood at time (t)

$H_{(t)}$ = counts over heart at time (t)

This value can then be subtracted from the corrected organ counts at time (t) to give a measure of "true" organ counts at time (t), i.e. counts in the organ due to active or passive isotope uptake

i.e.

$$TO_{(t)} = O_{(t)} - B_{(t)}$$

$$= O_{(t)} - \left[\frac{O_{(o)}}{H_{(o)}} \times H_{(t)} \right]$$

where $TO_{(t)}$ = true organ counts at time (t).

This value ($TO_{(t)}$) may then be plotted as a function of time, resulting in a graph that will represent the uptake or loss of isotope by the organ as a function of time, independent of counts due to blood perfusing it.

This procedure has been followed in order to obtain "true organ counts" over liver, spleen and sacral marrow.

In some patients it was not possible to obtain counts at $t_{(o)}$. This obviously influenced the ratio (Oo/Ho)

considerably, particularly in organs such as the marrow where ^{59}Fe uptake occurs very rapidly. It was reasoned that all ^{59}Fe sequestered in the erythroid marrow would be released by day 14, i.e. the period at which the experiment in each individual was terminated. As a result, I decided that, for the marrow, the ratio of O_{14}/H_{14} would probably more accurately reflect the ratio of marrow to heart blood perfusion, where O_{14} and H_{14} are the corrected organ counts on the 14th day of the experiment, respectively. In the other organs, i.e. the liver and spleen, I have assumed iron uptake not to be as rapid, and have used the Oo/Ho ratio. In all patients, it was possible to perform the initial count within thirty minutes of injection.

Using this corrected method of obtaining organ specific count rates, it was possible to obtain more specific data about the distribution of isotope within the body. The values were plotted as a function of time. A positive slope of the graph means active uptake of isotope by the organ, a negative slope meaning active loss of isotope from the organ. In the liver area, positive slopes usually meant iron storage, and usually occurred in individuals with high serum iron concentrations. Biphasic curves over the liver almost invariably occurred where hepatic myeloid metaplasia was present. Positive slopes over the spleen occurred in splenic stasis syndromes and where splenic red cell destruction was present. Biphasic slopes over the spleen were taken as evidence of splenic myeloid metaplasia. No effort was made to quantitate these curves, the count rates being dependent upon injected

doses of radioactivity and body mass. However, the shape of the curves often gave valuable information, particularly with regard to hypersplenism and myeloid metaplasia, and were vastly superior to data obtained with ^{51}Chromium. These curves also showed more effectively, the presence of iron storage, myeloid metaplasia and splenic sequestration.

A-20 WHOLE BODY COUNTING

Unfortunately, the whole body counter used by the Department of Diagnostic Isotope services at Groote Schuur Hospital, arrived towards the end of my period in the department. The model used was a Nuclear Enterprises Whole Body Counter based upon the shadow shield principle. The machine initially had a single 3" sodium iodide crystal, and was linked to a Nuclear Enterprises scaler and rate meter. In the context of this thesis, I used the machine only to study loss of radioactive ^{59}Fe from the body during the course of the experiment. This averaged 3%, reflecting sampling from the patient.

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APPENDIX B

DEVELOPMENT OF A MODEL FOR DESCRIBING BODY ERYTHROPOIESIS

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INTRODUCTION

The development of this model depended almost entirely upon the ingenuity of Mr. Paul Haddingham, a graduate of the University of Cape Town, who was, at the time, working in the Department of Chemical Engineering at the University. No apology is made for utilising his skill, as it seemed important to me to attempt the development of a model to describe the function of the bone marrow as an organ intermediary between the plasma and red cell compartments, in an attempt to mathematically unite these compartments. To a large extent, this has been done.

MODEL DESCRIPTION

Erythropoietic activity can be conveniently reduced to four physiologically defined concurrent processes for purposes of modelling iron kinetics.

1-a Uptake of transferrin bound iron from the plasma by maturing erythroblasts.

1-b Maturation of erythroblasts.

1-c Release of matured erythroblasts into the circulation as erythrocytes.

1-d Destruction of red cells or erythroblasts at the termination of their lifespan.

Each of these physiological processes can be mathematically described so that an overall model of erythropoietic activity can be generated. Iron kinetics can be described in terms of four compartments (see Figure I).

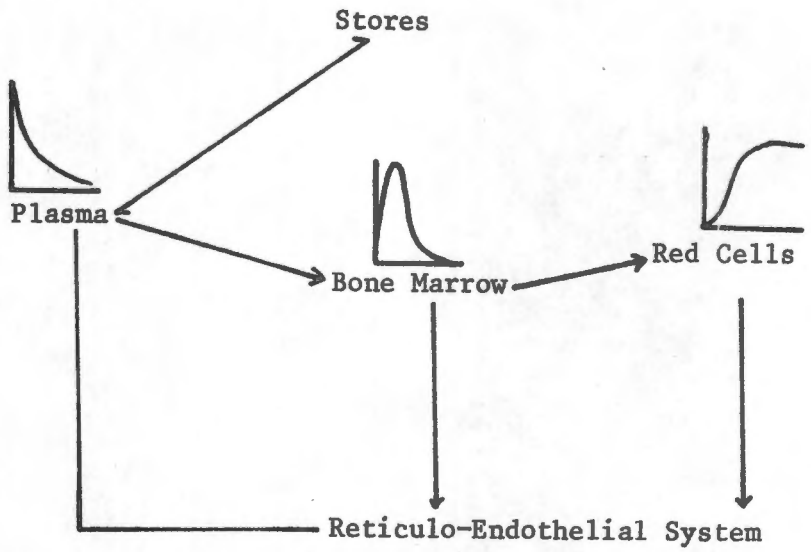
2-a Plasma containing labile, transferrin bound iron.

2-b Bone marrow - the site of erythroblast maturation and haemoglobinisation.

2-c The circulating red cell.

2-d Parenchymal iron stores.

Figure I. Model diagram of the different compartments in the body concerned with iron kinetics, showing the typical changes, in time, of their radioactive iron concentration.



For the purposes of this model, the reticulo-endothelial system (RES) is seen as a rapidly turning over iron pool, acting as a transfer station between

(1) catabolised haem from bone marrow and red cells and

(2) the plasma.

The model developed describes the short term relationships between these compartments, and tends to ignore long term feedback of iron from red cells dying outside the period of the experiment. The model does, however, account for any feedback of iron that may occur from this source during the experimental period (which is two weeks). The amount of feedback of iron from these combined sites (i.e. intramedullary and early extramedullary haem catabolism) is quantitated in an attempt to describe the erythropoietic process.

Physiology of radioactive iron kinetics.

The aspect of the model has been largely covered in the review section of this thesis.

3-a The plasma compartment:-

After injection of ^{59}Fe labelled transferrin into this pool, radioactivity is cleared in a way that may be described by a number of exponential decays. The first exponential decay tends to zero within twenty four hours of injection. ^{59}Fe is taken up by both the maturing erythroblast and non erythroid parenchymal iron stores, this uptake being

a first order process indicated by the first exponential of the plasma iron clearance curve. The other exponentials of the plasma iron clearance curve appear to arise as a result of feedback of label from other sites into the plasma, i.e. lymphatics, ineffective erythropoiesis, storage sites.[164] The rate constants of the first order processes to the bone marrow and stores can be determined from the initial exponential of the plasma iron clearance curve. This will be mathematically shown (4-1).

3-b The bone marrow compartment:

In the bone marrow (BM) there are erythroblasts at all stages of maturation. The iron uptake of an individual erythroblast has been shown to be dependent upon the state of maturation of the erythroblast (section III-b-5 of review). One can attempt to define a function, the probability of iron uptake by the maturing erythroblast, in relation to its maturity. Red cell production involves mitotic division of a single erythroblast precursor. The initial erythroblast precursor, plus its mitotic derivatives, can be looked upon as a single 'unit' in the bone marrow. The iron uptake by this unit will be the sum of the iron uptake of its members. As a generalisation, all the bone marrow units, under stable conditions, will have the same amount of iron uptake. One can attempt to define the probability of iron uptake by this unit as a function of time, and, based upon this probability curve, a

mathematical model describing BM haemoglobin production may be derived.

There is a lot of evidence to suggest that the maturation period of erythroblasts is approximately 6.5 days (see section III-b-3 of review section) and that this maturation period remains fairly constant in different disease states. We have attempted to define, by external counting methods, an accurate measure of this maturation period.

Nineteen patients thought to have relatively normal erythropoiesis were used in this study. Shortly after injection of ^{59}Fe labelled transferrin, they were externally counted over the sacral bone marrow, as detailed under methods. Bone marrow curves were calculated for each patient, the results being compared and analysed statistically. Count rates were normalised as a percentage of the maximal count rate obtained. The results can be seen in Table 1 and Figure II. By extending the initial slope of the bone marrow clearance curve to the x axis, we obtained an average erythroblast maturation period of 6.7 ± 0.7 days in these nineteen patients. The remaining area under the curve was thought to represent uptake by erythroblasts of ^{59}Fe from feedback sites. For convenience, we have assumed an erythroblast maturation period of 6.5 days for purposes of this model as this agrees well with our data and that calculated by other workers (see section III-b-3). This curve, in effect, defines the delay between injection of

labelled transferrin into the plasma, and its clearance from the bone marrow, and characterises the amount of clearance of label from the bone marrow with the passage of time.

Figure II Composite Bone Marrow Clearance Curve following injection of radioactive Iron bound to plasma transferrin. Results of a study of nineteen patients summarised statistically (mean ± 1 SD).

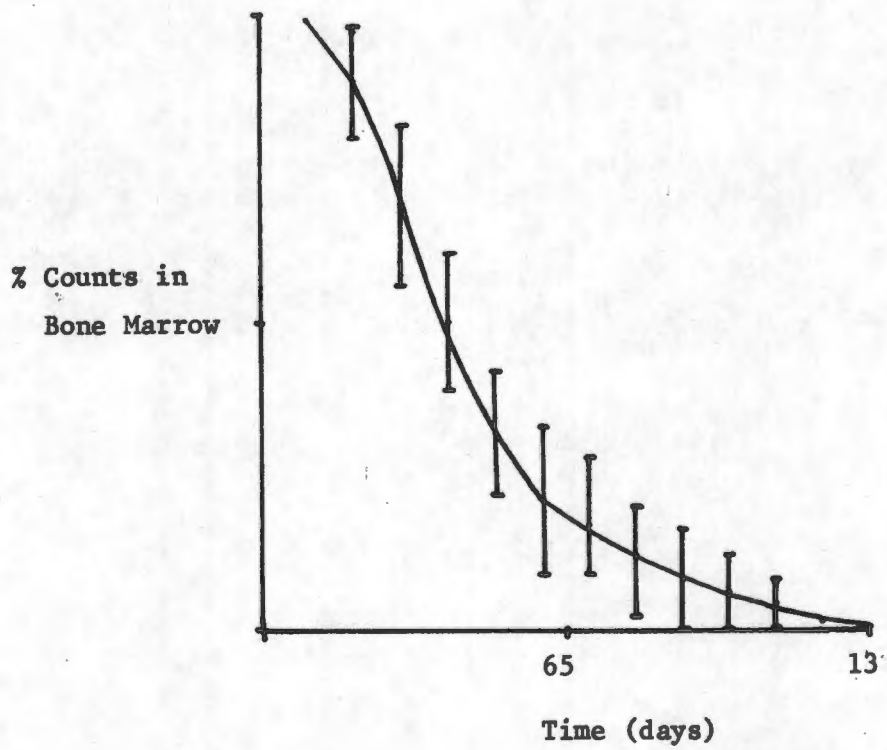


Table I. Experimental Results in Nineteen Patients. Standardised Sacral Bone Marrow Curves (see text).

Patient	Day													
	0	1	2	3	4	5	6	7	8	9	10	11	12	13
Vlo	63	100	92	74	60	25	18	12	9	4	10	0	0	0
Mar	42	100	-	81	-	-	-	-	-	0	0	0	0	0
Bai	72	100	-	-	-	-	-	-	-	0	0	0	0	0
Rus	74	100	94	78	63	50	38	24	19	11	9	5	3	0
Eng	20	87	100	-	-	29	3	-	0	0	0	0	0	0
Thy	91	100	93	62	39	22	14	9	6	5	4	3	2	0
Len	36	100	86	86	53	44	24	18	19	9	6	1	0	0
Luk	12	100	74	65	56	34	41	34	26	19	13	3	0	0
Smi	25	100	95	82	68	-	-	-	-	-	-	7	3	0
Jac	14	100	91	66	48	35	26	18	13	10	6	3	0	0
All	46	100	96	50	30	17	7	4	3	0	0	2	0	0
Bot	14	100	96	65	38	23	9	2	0	0	0	0	0	0
Hol	69	100	89	64	46	24	12	9	3	0	0	0	0	0
Bot	61	100	85	53	44	32	28	26	23	20	17	14	0	0
Wal	100	93	75	55	42	28	21	14	13	11	8	4	3	0
Nqw	78	100	90	75	57	46	40	36	31	25	21	16	-	3
Gog	0	99	100	88	60	45	35	28	19	13	8	5	0	0
Ada	39	100	89	84	58	33	19	11	14	13	13	6	4	0
Bas	100	92	69	52	35	22	10	6	3	0	0	0	0	0
$\bar{x}$		99	89	69	50	32	21	17	12	8	6	4	1	0
σ		35	8.8	12.5	11.1	10	12.2	10.7	9.8	7.9	6.7	4.5	0.3	0.6
N	19	19	17	17	16	16	16	15	17	18	17	19	17	17

3-c The Circulating Red Cell Compartment.

Mature labelled red cells are released from the bone marrow into the general circulation. The percentage of injected dose released as a function of time, is described by the RBCU curve. This in turn depends upon the amount of iron label taken up by the maturing erythroblasts, and the state of maturation of those erythroblasts. Thus, iron label released between t_o and t_{24} hours will reflect the amount of iron label taken up by the most mature erythroblasts (i.e. between 5.5 and 6.5 days old). The amount of iron released between t_{24} and t_{48} hours will reflect the amount of iron taken up by erythroblasts 4.5 - 5.5 days old, and so on. Only terminally will the RBCU curve be influenced by feedback iron, as erythroblasts not originally present when the initial bolus of radioactive iron was injected, become labelled by recycled label.

As previously discussed, the RBCU curve will also be influenced by variable erythropoietic activity (both effective and ineffective) as well as changes in red cell survival (whether finite in which case $\bar{l}$ must be < 14 days, or random) (see Section IV-5). In practise, the two factors most influencing the RBCU curve will be ineffective erythropoiesis and random red cell destruction. Both of these processes will tend to slur the RBCU curve, as well as cause a decrease in its maximum value.

Extramedullary haemolysis will, however, shift the curve to the left, whereas ineffective erythropoiesis will shift it to the right. From the point of view of feedback iron label from these sources, earlier feedback will occur with ineffective erythropoiesis.

4 Mathematical Derivations:

4-1 Iron transport from plasma to bone marrow and parenchymal iron stores.

As described in section 3-a, iron uptake by BM and parenchymal iron stores are first order processes.

i.e. $$\frac{dN_{BM}}{dt} = k_1 N_p \qquad (1)$$

where N_{BM}, N_s and N_p are the amounts (in Cpm) of radio iron in the bone marrow, parenchymal iron stores and plasma respectively.

$$\frac{dN_s}{d_t} = k_2 N_p \qquad (2)$$

k_1 and k_2 are the rate constants for transport of iron from the plasma to the BM and parenchymal iron stores respectively.

Now $$\frac{dN_p}{d_t} = -\left(\frac{d N_{BM}}{d_t} + \frac{d N_s}{d_t}\right)$$

$$= -(k_1 N_p + k_2 N_p)$$

$$\therefore \quad N_{p(t)} = e^{-(k_1+k_2)t}$$

By substituting $e^{-(k_1+k_2)t}$ into equation (1) and solving by integration

$$\frac{dN_{BM}}{dt} = k_1 . e^{-(k_1+k_2)t} - \frac{k_1}{k_1+k_2} . e^{-(k_1+k_2)t} + \frac{k_1}{k_1+k_2}$$

$$\therefore \quad N_{BM(t)} = \frac{k_1}{k_1+k_2} \left[1 - e^{-(k_1+k_2)t} \right]$$

where $\frac{k_1}{k_1 + k_2}$ = constant of integration

where $k_1 + k_2 = \lambda_1$ = the decay constant of the first exponential of the PIC curve.

$$N_{BM_t \to \infty} = \frac{k_1}{k_1+k_2} = \frac{k_1}{\lambda_1}$$

After approximately eight days, the iron in the bone marrow has passed out completely into the circulating red cells, and the resultant RBCU curve forms a plateau, which is not usually effected by the slow process of red cell destruction unless random destruction is present. From Figure I, the amount of radio iron circulating at this time represents the RBCU $\left[I_u \text{ Cpm} \right]$.

$$\therefore \quad N_{BM_t \to \infty} = \frac{k_1}{k_1+k_2} = \frac{k_1}{\lambda_1} = I_u \qquad (3)$$

Thus k_1 and k_2 can be calculated from a knowledge of λ_1 and I_u using equation (3). From this, the rate of input of radioactive iron into the BM can be calculated

i.e. rate of input $= k_1 N_p = \frac{d N_{BM}}{dt}$

4-2 Bone marrow compartment and RBCU curve

4-2-1 A number of assumptions have been made in preparing a model to describe the bone marrow.

a) New cells are born at a constant rate in the BM. This assumption is reasonable under steady state conditions, but need not be under non steady state conditions, i.e. where production exceeds destruction or the reverse.

b) The maturing erythroblasts in the BM have an average maturation period of 6.5 days, and that each BM unit (as defined in 3-b) behaves in the same way in regard to iron uptake as a function of maturation.

c) The probability of iron uptake by an erythroblast unit during maturation is assumed to be a parabolic function which varies according to the stage of maturation of the erythroblast. Autoradiography studies (see section 3-b-5 of review) have shown iron uptake to be maximal in the primitive erythroblast and to decline to zero as the cell matures. As a result of mitosis however, the number of cells

derived from the same erythroblast precursor increases so that the amount of iron taken up by these cells, as a function of time, is probably maximal during the mid stage of their development. This function, i.e. amount of iron uptake as a function of time, is assumed to be parabolic.

d) The initial slope of the plasma iron clearance curve represents the rate at which labelled iron is incorporated into maturing erythroblasts.

e) All compartments, described in Figure I, are assumed to be "lumped" so that space dependent effects are assumed to be negligible. This assumption is demanded by the complexity of the physiological system.

From assumption (d) for the early part of iron uptake after radio iron injection, the first exponential of the plasma iron clearance curve represents the rate of incorporation of iron into maturing erythroblasts (see section 3-a). The total amount of radioactivity (x in Cpm) taken up at time (t) by the erythroblasts can be defined as:-

$$x_t = C_o (1 - e^{-k_1 t}) \qquad (4)$$

where k_1 is the rate constant of BM iron uptake (as described in section 4-1)

C_o is the amount of iron label initially injected in Cpm.

t is time in days.

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where k_1 is the rate constant of BM iron uptake (as described in section 4-1)

C_o is the amount of iron label initially injected in Cpm.

t is time in days.

The rate of radio iron uptake $\left[R_u(t) \right]$ by the erythroblasts at time (t) is given by differentiating equation (4).

$$\therefore \quad R_u(t)/t = \frac{dx/t}{dt} = C_o k_1 e^{-k_1 t} \qquad (5)$$

The amount of radio iron taken up by the (i-th) group of maturing units born (t) days earlier (x_i Cpm) is, using assumptions (a) to (d), given by the product of the amount of labile radio iron in the plasma, (x) and the probability (Pr) that this group of units will take up the available radio iron at their stage of maturation (Pr_i).

$$\therefore \quad x_i = Pr.x$$

$$\therefore \quad dx_i = \frac{\delta x_i}{\delta Pr} \cdot dPr + \frac{\delta x_i}{\delta x} \cdot dx$$

Hence, the rate of uptake in Cpm/day is

$$\frac{dx_i}{dt} = \frac{x.dPr}{dt} + \frac{Pr.dx}{dt} \qquad (6)$$

In order to compute the rate of output $\left[R_o(t) \right]$ of label from the BM in Cpm/day at time (t), all the groups of units o, 1, 2, 3, ...i, ...n born t^o, t^1, t^2, t^3, ...t^i, ...t^n units of time earlier which are taking up radio iron must be considered. Thus, at time $t = t_o$, (the time of injection of radio iron) the output is given by equation (6) as:-

At time

$$t = t_o,\ Ro(t) = \frac{dzo}{dt} = x_o \frac{dPr_n}{dt} + Pr_n . \frac{dx_o}{dt}$$

At time

$$t = t_1,\ Ro_1(t) = \frac{dz_1}{dt} = \left[x_o . d . \frac{Pr_{n-1}}{dt} + Pr_{n-1} . \frac{dx_o}{dt} \right] + \left[x_1 . \frac{dPr_n}{dt} + Pr_n . \frac{dx_1}{dt} \right]$$

At time

$$t = t_2,\ Ro_2(t) = \frac{dz_2}{dt} = \left[x_o . \frac{dPr_{n-2}}{dt} + Pr_{n-2} . \frac{dx_o}{dt} \right] + \left[x_1 . d \frac{Pr_{n-1}}{dt} + Pr_{n-1} . \frac{dx_o}{dt} \right] + \left[x_2 . d . \frac{Pr_n}{dt} + Pr_n . \frac{dx_2}{dt} \right]$$

At time $t = t_i$,

$$Ro_i(t) = \frac{dz_i}{dt} = \sum_{j=o}^{i} \left[xj . \frac{dPr_{n-i+j}}{dt} + Pr(n-i+j) \frac{dxj}{dt} \right] \quad (1$$

Here, Pr_n is the probability of iron uptake at the stage of full maturation in the BM when the mature red cell is about to be released. Cells at this stage are, therefore,

the only ones contributing to radio iron output at $t = t_o$. These cells 'see' the initial labile radio iron concentration x_o.

At time $t = t_1$, a fraction after t_o, cells which had this fraction of time $t_1 - t_o$ left to mature will be released and will have been exposed to concentrations of labile radio iron x_o when the probability is Pr_{n-1} (at time $t = t_o$) and x_1 when the probability is Pr_n.

This process is generalised into the summation formula (formula (7)).

Assuming that the functions $Pr_{(t)}$ and $x_{(t)}$ are continuous and differentiable, the summation of equation (7) may be expressed as:-

$$Ro(t) = \frac{dz}{dt}/t = \int_o^t \left[x(\tau) \frac{dPr[L-(t-\tau)]}{dt} + Pr\left[L-(t-\tau)\right] \frac{dx(\tau)}{dt} \right] d\tau \quad \ldots(8)$$

Where τ is the dummy variable of integration and L is the erythroblast maturation period which appears to be 6.5 days as previously indicated.

One can substitute from equations (4) and (5) to get:-

$$\frac{dz}{dt}/t = C_o \int_o^t \left[\left[1 - e^{-k_1 \tau} \right] . \frac{dPr\left[L-(t-\tau)\right]}{d\tau} + k_1 e^{-k_1 \tau} . Pr\left[L-(t-\tau)\right] \right] d\tau \quad \ldots(9)$$

This equation expresses the rate of red cell output of radio iron (Cpm) from the BM in terms of the plasma iron clearance constants and the probability of iron uptake distribution at time (t) after the initial injection of radio iron. A further assumption, implicit in equation (9), is that mature cells are released from the BM only once they have reached full maturity. This may not always occur in stress situations where earlier release of "stress" reticulocytes has been shown to occur. These cells will, however, still require 6.5 days from the time of birth of their original erythroblast precursor until they reach ultimate maturity.

4-2-(2) Probability Distribution.

From assumption (c) in 4-2-(1), the probability distribution curve is parabolic.

$$Pr(t) = a_o + a_1 t + a_2 t^2 ,$$

$$0 \leq t \leq L \qquad \ldots\ldots(10)$$

$$Pr(t) = 0, \quad t < 0$$

$$Pr(t) = 0, \quad t > L$$

Since (L) is known - (6.5 days) i.e. BM maturation period

$$Pr/_{t=0} = Pr/_{t=L} = 0$$

$$\int_0^{L} Pr(t)\,dt = 1$$

The quadratic constants a_o, a_1 and a_2 are readily evaluated in terms of (L). From the first of the above conditions substituted in (10), $a_o = 0$, and the remaining two conditions give:-

$$a_1 + a_2 L = 0 \quad (L \neq 0 \text{ as discussed})$$

$$\frac{a_1 L^2}{2} + \frac{a_2 L^3}{3} = 1$$

Solving these two equations simultaneously gives

$$a_1 = t/L^2$$

$$a_2 = -t/L^3$$

Equation (10) can now be used in equation (9) to calculate R_o from the BM in terms of the plasma iron clearance constants C_o and k_1 (experimentally determined) and this probability distribution.

This equation is only valid if there is no feedback of labile radio iron from ineffective erythropoiesis or early random red cell destruction, as discussed in 1. However, this feedback may be incorporated as will be discussed.

4-3 Red Blood Cell Utilisation Curve.

In order to compute R_o as a function of time, equation (9) can be integrated i.e.

$$\frac{dz}{dt}\Big/_t = C_o \int_o^t \left[\left(1 - e^{-k_1\tau}\right) \cdot \frac{dPr\left[L-(t-\tau)\right]}{d\tau} + k_1 e^{-k_1\tau} Pr\left[L-(t-\tau)\right] \right] d\tau \quad \ldots.(9)$$

$$\therefore \quad 2(t) = C_o \int_o^t \int_0^T \left[(1 - e^{-k_1\tau}) \frac{dPr\left[L-(t-\tau)\right]}{d\tau} + k_1 e^{-k_1\tau} Pr\left[L-(t-\tau)\right] \right] d\tau \quad \ldots.(11)$$

Where 2(t) has units of Cpm.

It is the aim, in developing this model, to generate the best fit between the observed RBCU curve, and the predicted RBCU curve using feedback of radio iron from ineffective erythropoiesis and early random red cell destruction as a variable parameter. This was achieved using digital techniques on a Univac 1106 computer.

5 Computational Method.

Input data for the model are:-

a) the plasma iron clearance constants

i.e. amount injected = 100%

concentration at t_o = 100%

slope of initial exponential of PIC curve.

b) the RBCU curve for each subject.

This is derived as derived under methods. Each patient has RBCU values calculated from each specimen taken. These were usually taken every day in the first week, and on alternate days in the second week. For the generation of a best least squares polynomial fit between the model curves and the experimental curves, it was necessary to smooth the experimental curve and put in more data. Each individual's RBCU curve was plotted and the best eyeball fit of the data was made. Points at 0.5 day intervals were read off the resultant curve and recorded for each subject on data cards.

The basic procedure is as follows:-

(1) For zero feedback, the output RBCU curve is calculated using equation (10) and (11).

(2) The SD between the predicted and experimentally derived curves are computed.

(3) The feedback fraction is increased (this fraction representing the amount of radio iron label released,

either by ineffective erythropoiesis or early random red cell destruction, back to the plasma and then back to the BM) by increments of 0.01%.

(4) Steps (1), (2) and (3) are repeated until the standard deviation (SD) has been minimised. The feedback value giving the minimum SD between the computed and experimentally derived RBCU curves is a parameter describing the state of erythropoiesis in a particular subject. The greater the feedback fraction (expressed as a fraction of the total bone marrow output), the greater the degree of ineffective erythropoiesis or early random red cell destruction in that particular individual.

A simplified flow chart of the computer programme for the model is shown in Figure III and IV. Integration of equation (10) is performed numerically using Simpson's rule. In order to compare predicted and experimental RBCU curves, the experimental values are fitted using a second order polynomial least squares interpolation method devised by P.T. Haddingham, the author of the mathematics of this model. The use of this programme is shown in the flow chart. The fitting procedure involves taking groups of five successive points of data

i.e.

$$(P_1 - P_5)\ (P_2 - P_6)\ (P_3 - P_7)\ (P_2 - P_2 + 4)\ (P_{n-4} - P_n)$$

and fitting a second order polynomial to each group. Each polynomial is identified by its central pivot point, respectively P_3, P_4, P_{2+2} , P_{n-2}, about which the data is fitted.

A further computational improvement would involve streamlining the method of seeking an optimum fit between experimental and predicted RBCU values using optimal dynamic programming.[188b]

Figure III Flow chart of computational method. Part 2.

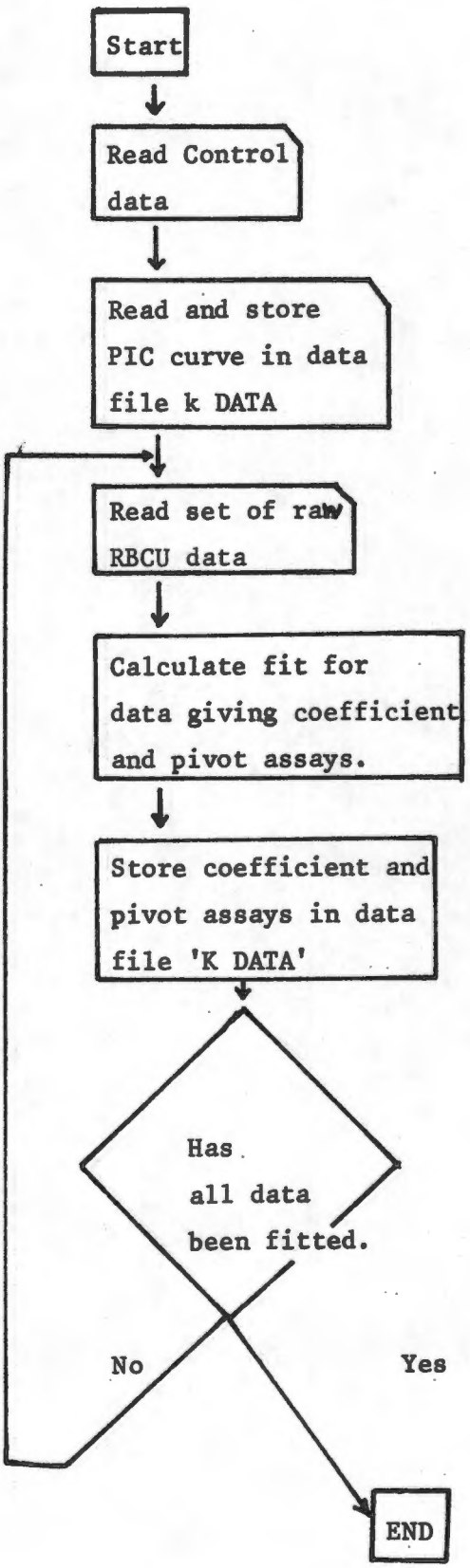
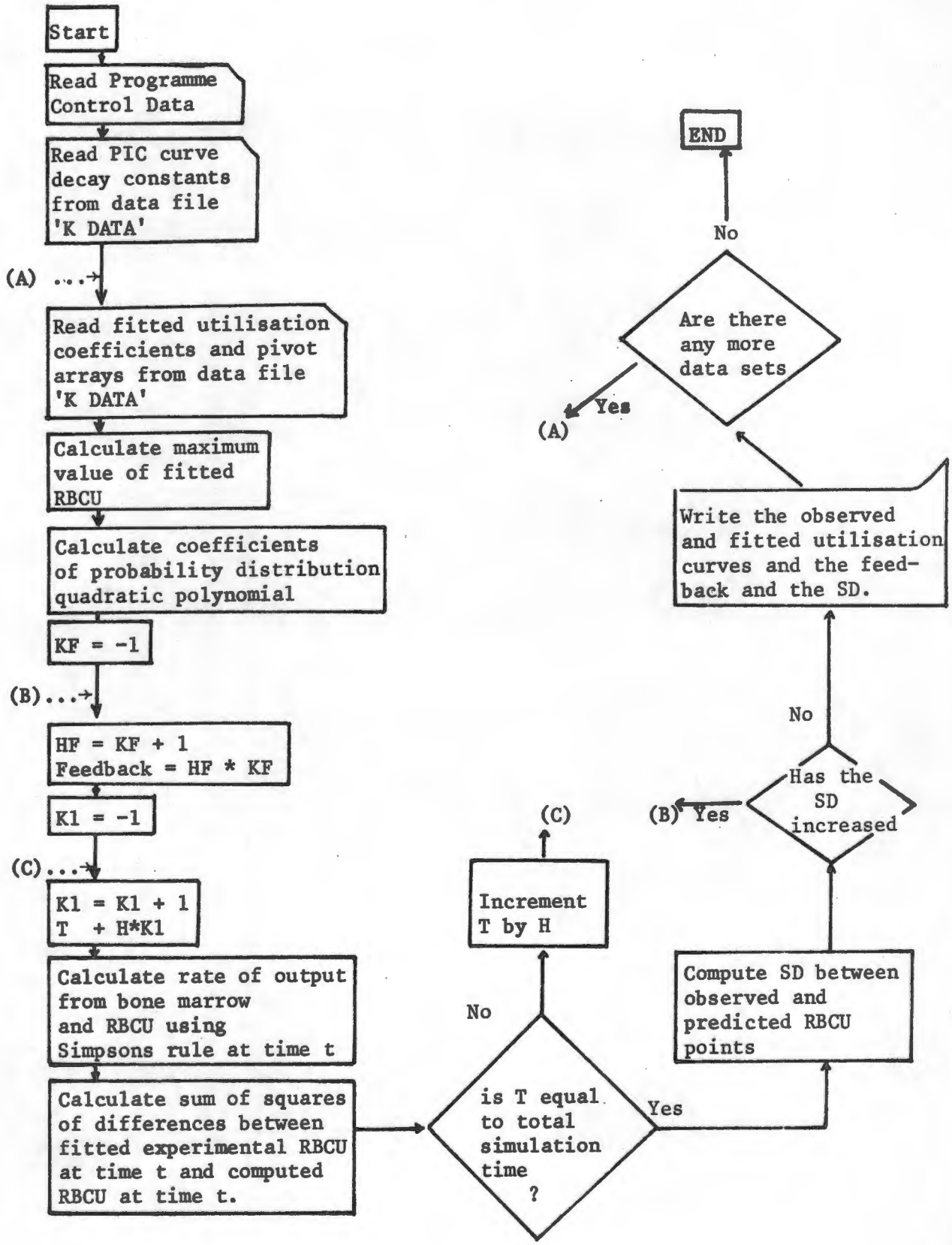


Figure IV Flow Chart of Computational Method . Part I.



6. CRITICISM OF THE MODEL

The limitations imposed upon this analytical system are of two kinds.

(1) Experimental

a) The plasma iron clearance curve can be accurately measured, and we are happy that, for the purposes of this model, very little error is present in the measurements of this parameter.

b) The RBCU curve, as fitted by us (section 5-b), may contain a fair amount of experimental error. Even at plateau parts of the RBCU curve we often find a 4 - 5% range in RBCU values. The only way that I have been able to smooth this is by 'eyeball' techniques using RBCU values calculated experimentally as often as possible. This method, while not ideal, appears to me to give a reasonably good fit to the experimental curve without excessive manipulation of the data.

2) Theoretical

We have assumed a maturation time of 6.5 days, and have likewise assumed that this maturation occurs completely in the bone marrow. That this is not so, is well known, as relatively immature reticulocytes may be released into the circulation under 'stress' situations. This model could be expanded to incorporate a parameter describing this premature release which would then shift the computed RBCU curve to the left. At this stage I believe that,

the fewer variable parameters introduced into this model, the more easily will I be able to interpret the data. In some subjects, however, when fractional feedback was increased from zero, an increase occurred in the SD between predicted and experimental RBCU curves. This probably represents premature reticulocyte release from the BM, but may in addition be caused by the shift to the left in the RBCU curve caused by early random haemolysis (see thesis section IV-5).

Ideally, one would like to be able to compute what the RBCU curve of each patient should look like in the absence of abnormal erythropoiesis and haemolysis. By varying the feedback fraction, one could then compute a curve matching the experimental curve, and this value of feedback would then be used to measure in a quantitative way, the proportion of erythroblast iron uptake going effectively into the circulation, if we assume that ineffective erythropoiesis and random early red cell destruction represent a continuum of the same process. This would, however, necessitate some further study on the effect of effective erythropoietic activity upon the RBCU curve. It is possible that my regression equation, describing in normal patients the RBCU values as a function of plasma iron concentration, approaches this ideal. However, we cannot assume that the low RBCU values in aplastic anaemia represent an increase of ineffective erythropoietic activity.

Presumably, they represent an increase of non.erythroid iron uptake relative to normal patients at the same plasma iron concentration. I have been unable to study this in greater depth, and have assumed for now that NEIT depends solely upon the plasma iron concentration, and is not influenced by the disease state. This assumption may be wrong. In order to make this model work to reveal truely useful information, therefore, some method of measuring predicted RBCU values depending solely upon changes in effective erythroid activity as opposed to changes in plasma iron concentration must be generated. This I have not been able to do. Until this is done, the value derived for feedback describes only the shift of experimental RBCU curves to the right caused by ineffective erythropoiesis, and quantitates this in terms of a feedback fraction. The greater this feedback fraction, the more severe is thought to be the extent of ineffective erythropoiesis. At best, this data is only partially the truth, but it does have the merit of putting numbers or orders of severity upon a parameter which is extremely difficult to describe by other methods.

In addition, the model has used all the input and output data, and has generated an intermediary function describing what happens in the bone marrow. To my knowledge this is the first attempt to do this, and seems to have some merit itself, by matching the plasma and red cell ferro-kinetic behaviour of an individual.

Analysis of the data obtained utilising this model can be seen in Chapter V of this thesis.

APPENDIX C.

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