THE MYELOPROLIFERATIVE DISORDERS: AN INVESTIGATION INTO THEIR NATURAL HISTORY AND AETIOLOGY.

by

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Introduction

In January, 1957, in a medical ward in the Western Infirmary of Glasgow, I saw a patient with myeloproliferative disease. This patient had neither typical chronic myeloid leukaemia nor typical myelofibrosis but had features of each disease. The interest of this 'intermediate' form of disease was pointed out to me by Dr. Abraham Goldberg who also encouraged me to investigate some of the clinical problems of myeloproliferative disease. This I did for over three years during which time more problems presented and consequently the scope of the study expanded until twenty seven patients had been investigated from various aspects.

The main theme of this thesis is concerned with the question of relationship of the myeloproliferative disorders. This problem was investigated by a comparison of firstly, the clinical features, secondly, the haematological findings and thirdly the mechanisms of blood

/production and destruction in the various myeloproliferative disorders.

The results of studies of other points of interest, such as the occurrence of infection and hypogammaglobulinaemia, the problem of gout and uric acid metabolism and the causation of megaloblastic erythropoiesis, are reported. Lastly, the clinical and haematological response to therapy, in particular splenectomy and splenic irradiation, and the effect of treatment on the course of the disease are discussed. Conclusions are then drawn as to the possible aetiology of the myeloproliferative group of disorders.

Acknowledgments

I wish to record my gratitude to Professor E. J. Wayne for permission to study the patients under his care and for the facilities of his department; also to Dr. J. A. W. McCluskie, in whose unit most of this work was done, for allowing me to study the remaining patients who were under his care and for his helpful advice and encouragement during this work.

I should also like to thank the Department of Medical Physics for their willing help with the radioisotope investigations and the Departments of Radiology and Radiotherapy of the Western Infirmary for their cooperation in the investigation and treatment of the patients.

The Department of Biochemistry of the Western Infirmary carried out the total protein, uric acid and serum iron investigations and I am grateful to Dr. E. B. Hendry and his staff for their willing cooperation in these studies. I should also like to thank

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I am grateful to Dr. I. A. Cook for his kindness in making available to me the results of the coagulation studies and other information on the patient with megakaryocytic thrombocythaemia. Dr. J. F. Adams performed the serum B_{12} assays and Dr. D. L. Mollin the folic acid assays and to both I wish to extend my thanks.

I should also like to thank Dr. D. L. MacQueen for access to the earlier records of some patients and Mr. J. T. Brown for carrying out the bone marrow trephine biopsies.

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The Department of Medical Illustration of the University of Glasgow are responsible for all other figures and I am much indebted to Mr. G. Donald and his staff for their very

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I am very grateful to Miss M. Bain for the trouble she has taken in the preparation and typing of this thesis and for the valuable assistance she has given in many other ways.

Lastly, I wish to record my gratitude to Dr. Abraham Goldberg. Dr. Goldberg first introduced me to the interesting problems of myeloproliferative disease and since then has proved to be a source of enthusiasm and encouragement during this study. I am very grateful to him for his helpful advice and practical assistance and also for the knowledge he has so readily passed to me.

C O N T E N T S

'V O L U M E I'

SECTION I

THE BACKGROUND

Chapter 1.

HISTORICAL BACKGROUND OF .

MYELOPROLIFERATIVE DISEASE.

Historical

The obliteration of the bone marrow by an overgrowth of fibrous tissue and bone is known as myelosclerosis. This condition may be a primary disease or may be secondary to other diseases. There are many causes for secondary myelosclerosis e.g. metastatic carcinomatous deposits in the marrow from primary growths in lung, thyroid, breast, stomach, prostate and adrenal glands; the results of the action of toxic agents such as aniline, arsenic, benzine, fluorine, phosphorus and X-irradiation; and other diseases such as Hodgkin's disease, myelomatosis, Gaucher's disease, osteomalacia and syphilis. Crail, Alt and Nadler (1948) considered that tuberculosis was aetiologically significant and reported several cases with this association. A complete review of conditions giving rise to diffuse or focal myelosclerosis has been made by Erf and Herbut (1944).

Myelosclerosis, however, also occurs in two forms as a primary disease. Both forms give rise to splenomegaly and a leucoerythroblastic

/anaemia but they are clinically and aetiologically distinct diseases. The first is the rare and hereditary condition which occurs in children and young adults and is known as Osteopetrosis or the Marble-bone Disease of Albers-Schönberg (1904). The second form of primary myelosclerosis is seen in adults; it is not hereditary and appears to bear no relation to Marble-bone disease. The aetiology of this condition is unknown and the disease is characterised by a slowly progressive splenomegaly, a leucoerythroblastic blood picture and overgrowth of the bone marrow by fibrous and osteoid tissue. Myelofibrosis is that same condition without proliferation of bone in the marrow cavity.

Wood (1871) discussed "A Splenic Variety of Pseudo-Leukaemia" and described gelatinous degeneration of the bone marrow in association with splenomegaly. It is possible that his patients had primary myelosclerosis but the first report of the definite association of generalised osteosclerosis, hepatosplenomegaly

Zwei Fälle von Leukämie mit eigenthümlichem Blut- resp. Knochenmarksbefund.

Von Dr. G. Heuck, klin. Assistenzarzt.

(Aus der medic. Klinik zu Heidelberg.)

Bekanntlich sind in den letzten Jahren beim Studium des in vieler Beziehung immer noch dunklen Krankheitszustandes der Leukämie die Untersuchungen des Blutes auf's Neue, die des Knochenmarks zuerst mit besonderem Eifer betrieben worden, und sehr wichtige, für das Verständniss dieser Affection höchst bedeutungsvolle Befunde sind dabei zu Tage getreten.

Was zunächst die Blutuntersuchungen betrifft, so hat man sich neuerdings vorzugsweise mit dem Verhalten der rothen Blutkörperchen beschäftigt, da es sich herausgestellt hat, dass dieselben nicht nur bei Leukämie, sondern auch bei einer ganzen Reihe anderer, zu Anämie führender Krankheitszustände unter Umständen sehr auffallende Veränderungen erleiden, Veränderungen, die bei der erstgenannten Erkrankung früher wohl deshalb übersehen worden sind, weil man wesentlich nur den weissen Blutkörperchen Aufmerksamkeit zugewendet hatte.

So mannichfaltig nun aber auch die Beobachtungen von abnormem Verhalten der rothen Blutkörperchen sind und soviel man sich bisher mit der Erklärung derselben abgemüht hat, so wenig wissen wir doch im Ganzen über die Bedingungen, unter denen jene Veränderungen zu Stande kommen, und erst ein weiteres klinisches Studium, insbesondere eine genauere Beachtung dessen, ob diese eigenthümlichen Blutbefunde mit bestimmten anderen Krankheits-

Fig. 1 The first report of the association of osteosclerosis, hepatosplenomegaly and a leukaemic blood picture.

/and a leukaemic blood picture is generally credited to Heuck (1879) (fig.1). He considered that this was a chance association of chronic myeloid leukaemia with osteosclerosis. In 1908, however, Donhauser suggested that a primary interference in the bone marrow caused secondary and compensatory erythropoiesis to appear in the liver and spleen and so led to enlargement of these organs. This explanation was accepted for many years while more cases were gradually reported under a variety of names such as megakaryocytic myelosis, (Hewer, 1937) aleukaemic megakaryocytic myelosis, (Favre et al, 1934; Lindeboom, 1938) chronic non leukaemic myelosis, (Carpenter & Flory, 1941) agnogenic myeloid metaplasia (Jackson et al, 1940) and myeloid megakaryocytic hepatosplenomegaly (Downey and Nordland, 1939). In 1939 however, Vaughan and Harrison challenged this view and pointed out that considerable enlargement of the spleen could occur at a time when the bone marrow was still cellular. They felt that splenic enlargement was due not simply to compensatory erythropoiesis but to the fundamental

/megakaryocytic thrombocythaemia and erythraemic myelosis, were related disorders of development of the primitive mesenchyme cell. Dameshek (1951) considered them to be variable manifestations of a single myeloproliferative stimulus and in his opinion there was no fine dividing line between them and many transition forms existed. In the same year Wintrobe (1951) felt that there was no sound evidence for this suggested relationship between myelosclerosis and chronic myeloid leukaemia. More recently Leonard, Israels and Wilkinson (1957) in a study of 28 cases of myelosclerosis considered that these diseases were clinically, pathologically and perhaps even biochemically distinguishable and not simply different facets of a single disease. They went even further in relation to myelosclerosis and stated that their findings supported the previous conception that marrow failure caused blood formation to appear in the spleen and liver.

It is clear, then, that there has been considerable controversy about the relationship

/or lack of relationship of what are now termed the myeloproliferative disorders. This controversy has persisted up to the present and the recent study of Leonard et al in 1957 provides data supporting Donhauser's theory of almost fifty years earlier. In view of the conflicting opinions it would seem that any advancement in knowledge of this subject and any answer to the question of relationship must depend on the use of more dynamic methods of study which, in conjunction with detailed clinical, haematological and biochemical assessment would provide fundamental information about this interesting group of diseases.

Chapter 2

MATERIALS AND METHODS

THE PATIENTS

The twenty seven cases of myeloproliferative disease used in this study were patients admitted to either of two medical units in the Western Infirmary, Glasgow, during the period 1st. January 1957 to 1st. March 1960. All patients admitted to these two units and found to have myeloproliferative disease were included in the investigation with the exception of a few who were too ill to justify delay in treatment.

PERIPHERAL BLOOD AND MARROW

The routine haematological investigations were carried out by the standard methods as described by Dacie (1956) and Whitby and Britton (1957). Bone marrow smears were prepared from marrow obtained by needle puncture of the sternum. No patient was considered to have a 'dry tap' until several punctures had been carried out at each of two or more sites. Iliac crest trephine was carried out by open operation under local anaesthesia.

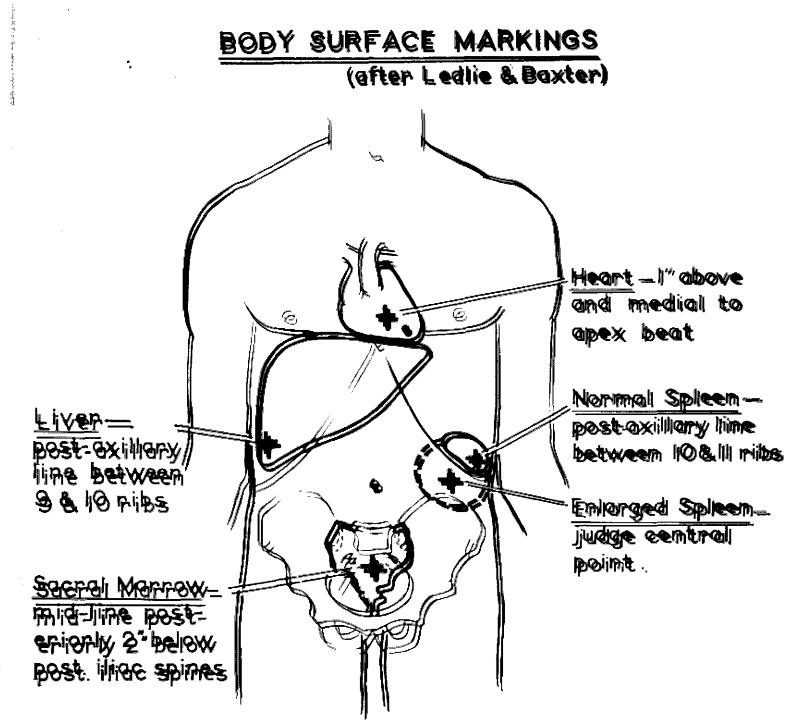

Fig. 2 The counting sites for body uptakes of radioactive iron.

/SPLENIC PUNCTURE

Splenic puncture was carried out under local anaesthesia by the abdominal route as described by Chatterjea et al (1952).

RADIOISOTOPE STUDIES

The radioiron (^{59}Fe) and radiochromium (^{51}Cr) used for these studies were obtained from the Radiochemical Centre, Amersham.

Radioiron

10 uc of radioactive iron (^{59}Fe) in the form of ferric chloride was injected intravenously in a 10 ml solution. A directional scintillation counter was used to record the radioactivity over the heart, the spleen, the liver and the sacrum as described by Ledlie and Baxter (1954) and Wetherley-Mein et al (1956). The heart was used as a measure of whole blood activity, the sacrum as a measure of marrow activity and the liver and spleen as possible sites of erythropoiesis; the counting sites are illustrated in fig.2. Changes in activity over these areas were recorded at intervals over a period of 100 hours and

/were corrected for background radiation. The results are plotted with radioactivity on a linear ordinate scale against time on a log abscissa scale.

Samples of blood were withdrawn at intervals up to 14 days after the administration of radioiron to determine the utilisation of the injected radioiron for haemoglobin formation. In calculating the utilisation from the maximum blood radioactivity (usually about the tenth day) correction was made for total body haematocrit, background radiation and the natural decay of the radioiron. The result is expressed as a percentage of the dose of radioiron injected.

Radiochromium

The radioactive chromium (^{51}Cr) technique was based on the method of Mollison and Veall (1955) using heparin as the anticoagulant. A dose of 100 uc ^{51}Cr was used in the form of sodium chromate. A sample of 20 ml of the patients' blood was incubated with the radiochromium for 45 minutes and then having been washed 3 times in saline the cells were

/reinjected intravenously in normal saline.

Red cell volume was calculated from the radioactivity in a sample of venous blood obtained 20 minutes after the injection of the labelled cells, by the dilution principle. Plasma volume was calculated from the red cell volume by use of the venous haematocrit corrected for total body haematocrit and for trapped plasma (Chaplin and Mollison, 1952).

A measure of red cell survival was obtained by the examination for radioactivity of samples of blood taken off at intervals usually of 1 - 2 days until the blood radioactivity was reduced to 50 per cent of that found in the first sample. The time taken for this to occur was expressed in days ($T\frac{1}{2}$). For convenience the results are plotted with radioactivity as a percentage of the first sample on a log ordinate scale and time in days on a linear abscissa scale. Corrections were made for haematocrit readings and for the natural decay of the isotope.

Sequestration studies were carried out by counting the radioactivity over praecordium,

/liver and spleen as described by Jandl et al (1956) at intervals of a few days after injection of the labelled cells. A spleen/liver ratio of 2.5/1 was considered the lower limit of significant sequestration (Goldberg and Seaton (1960).

Apparatus

Body counts were carried out with a directional scintillation counter. The radioactivity of the blood samples was measured in a well counter using an Ecko automatic scaler type N530A. All radioactive counts were corrected for background radiation which was taken as the mean of two readings, one before and one after the sample counts. All samples were counted for 300 seconds, and body counts were taken for 100 seconds.

Radioactive ^{58}Co vitamin B_{12} absorption tests

Radioactive vitamin B_{12} was obtained from the Radiochemical Centre, Amersham, and diluted to a working concentration of 0.5ug, specific activity 0.5 uc, in 5 ml. saline. The oral dose of 5 ml of this solution was given in

/200 ml. water, the patient having fasted overnight. When intrinsic factor was used it was given in a dose of 30 mgm. suspended in the vitamin B_{12} solution a few minutes before ingestion. 1000 ug vitamin B_{12} was injected intramuscularly 2 hours later and the urine was then collected for 24 hours. The total radioactivity excreted in 24 hours in the urine was then estimated and expressed as a percentage of the dose given. In normal subjects over 7 per cent of the dose given was excreted within 24 hours. This urinary excretion test is based on the method of Schilling (1953) and further details are as described by Adams and Seaton (1961).

VITAMIN B_{12} ASSAY

Serum vitamin B_{12} levels were estimated by microbiological assay using Euglena Gracilis, based on the method of Hutner et al (1956).

SERUM PROTEINS

Biochemical estimation of the total serum proteins was carried out by the biuret method as described by Gornall et al (1949). Serum

/protein filter paper electrophoresis was performed using the horizontal paper strip method, and after staining, quantitative estimation of the various fractions was carried out using the EEL scanner.

URIC ACID ESTIMATIONS

Serum uric acid

Estimation of serum uric acid was carried out by the method of Caraway (1955).

Urinary uric acid

The urine was first diluted 1 in 200 and then uric acid content was estimated by the method of Caraway (1955).

FAECAL UROBILINOGEN

The urobilinogen content of faeces was estimated by the method of Maclagan (1946)

SECTION II

A COMPARISON OF CHRONIC MYELOID LEUKAEMIA AND MYELOFIBROSIS

Chapter 3

THE CLINICAL PICTURES OF

CHRONIC MYELOID LEUKAEMIA

AND

MYELOFIBROSIS

Although chronic myeloid leukaemia has been reported in a baby of only three months of age (Keith, 1945) this is quite exceptional and the disease is essentially one of middle or later life and occurs usually between the ages of thirty and sixty years (Scott, 1957). In myelofibrosis the incidence is, if anything, rather later and the disease is found mostly in patients over forty five years of age (Mulcahy, 1957). In this series the age of the patients with chronic myeloid leukaemia ranged from thirty three to fifty nine years (mean 51.8 years) and of those with myelofibrosis from forty two to sixty four years (mean 56.9 years) at the time of diagnosis. The sex incidence is about equal in both diseases although in the patients described here there was a preponderance of females in each disease; four out of five patients with chronic myeloid leukaemia were females and seven out of nine patients with myelofibrosis were females. The age and sex incidence is seen in table 1. The discrepancy is attributable to the relatively

Case	Sex	Age (yrs.)	Diagnosis
J.C.	female	42	Mf.
A.S.	female	49	Mf.
M.G.B.	female	63	Mf.
T.Q.	female	56	Mf.
M.C.	female	62	Mf.
S.H.	female	64	Mf.
R.C.	female	58	Mf.
W.P.	male	56	Mf.
J.G.	male	62	Mf.
C.L.	female	47	C.M.L.
E.W.	female	69	C.M.L.
J.L.	male	33	C.M.L.
A.R.	female	59	C.M.L.
M.M.	female	51	C.M.L.

Table I Age and sex incidence of the patients with chronic myeloid leukaemia and myelofibrosis. (C.M.L. = chronic myeloid leukaemia; Mf.= myelofibrosis).

/small number of cases of each disease included in the investigation.

The onset of symptoms in either disease is usually insidious and estimates of time of onset, duration of the disease and length of survival of the patients are rendered of doubtful value. Furthermore, changes may take place in the blood, even before the onset of symptoms. The chance finding of an unexplained leucocytosis up to nearly four years before any further evidence of chronic myeloid leukaemia has come to light has allowed the development of the disease to be observed. (Wintrobe and Hashenbush, 1939). Thus, chronic myeloid leukaemia may exist for some time, and possibly a long time, before the onset of symptoms. This slow progression of the disease sometimes results in the patient paying little attention to symptoms until, after a considerable time, they have become troublesome. This insidious nature of these disorders is confirmed by the incidental finding of splenomegaly in patient S.H. with myelofibrosis during the investigation of true

	Number of patients affected	
	C.M.L.	Mf.
abdominal swelling or discomfort	3	6
symptoms of anaemia	4	6
asthenia	3	5
ankle swelling	2	3
pallor	2	6
loss of weight	4	6
bleeding tendency	2	1
thrombotic tendency	1	1
bone pain	0	1
gout	0	1

Table 2 Main symptoms in the five patients with chronic myeloid leukaemia and the nine patients with myelofibrosis.

(C.M.L. = chronic myeloid leukaemia; Mf. = myelofibrosis).

/peripheral vascular disease. This patient had had anorexia, weight loss, tiredness and dyspnoea on exertion for approximately three years and had not sought medical advice until her activity was severely limited by intermittent claudication. Patient R.C. with myelofibrosis had symptoms of anaemia, anorexia, weight loss, dyspepsia and angina of effort for three years and patient M.M. with chronic myeloid leukaemia had symptoms for two years before the first attendance at hospital.

The main symptoms of the patients with chronic myeloid leukaemia and myelofibrosis are demonstrated in Table 2.

The initial symptoms in chronic myeloid leukaemia are commonly those of anaemia, asthenia, loss of weight and abdominal swelling or discomfort; bone pain, a bleeding tendency and ankle oedema occur less commonly. Of the five patients with chronic myeloid leukaemia, four had weight loss, four had symptoms of anaemia and three complained of abdominal swelling or discomfort and weakness or tiredness.

/Of the more unusual symptoms, spontaneous bruising and ankle oedema were each noted in two patients and another two patients were aware of increasing pallor. In the male an early symptom may be priapism (Whitby and Britton, 1957) and this occurred in the one male patient.

In myelofibrosis the presenting symptoms are very similar, namely, asthenia, symptoms of anaemia, weight loss and abdominal swelling or discomfort and occasionally bleeding tendency, bone pain and ankle swelling. Abdominal discomfort or swelling, symptoms of anaemia, loss of weight and pallor were each present in six of the nine patients with myelofibrosis, asthenia occurred in five and ankle swelling in three cases. A bleeding tendency and bone pain each were noted once. These features are in agreement with these found by Korst et al (1956) in a series of 23 cases of myelofibrosis.

Although these symptoms appear to give a classical picture the diagnosis cannot always be made with certainty on the history alone

/and several interesting points emerge from this. One patient, J.L., a male of thirty three years of age, complained of loss of over one stone in weight during the preceding three months. He maintained that his appetite was good and his only other complaint was of breath-:lessness on exertion. As a result, he was referred to an Endocrine Clinic as a case of suspected thyrotoxicosis. This, on consideration of the brief history alone, is understandable but on physical examination the error was apparent. The mistake in initial diagnosis was due to the occurrence of marked loss of weight in the presence of a good appetite. Weight loss occurred in six of the nine patients with myelofibrosis. One patient, S.H., lost as much as four stones over a 3 year period and another J.G., lost weight much more rapidly, 1 stone being lost in two months. The same variation was seen in chronic myeloid leukaemia where C.L. lost four stones in weight in four months and three other patients had loss of weight. It would seem obvious that

/in debilitating disease this could be due to lack of food but this is not the case here as only half of these patients admitted any deterioration of appetite. The basal metabolic rate is commonly raised in chronic myeloid leukaemia (Riddle and Sturgis, 1927) and it is generally accepted that increased metabolism is the cause of loss of weight as in thyrotoxicosis. That this is not due to increased activity of the thyroid gland has been shown by studies of gland uptakes with radioactive iodine (Albright and Middleton, 1950).

Scott (1957) noted the occurrence of spontaneous haemorrhage in as much as 26.1 per cent of 177 patients with chronic myeloid leukaemia and yet the explanation of this common feature remains obscure (Thomas et al, 1960). Despite this bleeding tendency venous thrombosis sometimes occurs. This paradoxical situation was confirmed by the occurrence of spontaneous bruising in two patients and priapism, which is almost certainly the result

/of thrombosis, in another. The association of a haemorrhagic tendency and thrombotic tendency in the one disease was also seen in the patients with myelofibrosis where one patient had marked spontaneous bruising and another phlebothrombosis.

The abdominal discomfort often experienced by patients with chronic myeloid leukaemia or myelofibrosis is usually due to the gross enlargement of the spleen associated with these diseases. Less often, however, pain is of a sharper nature and is then aggravated by respiration. This type of pain, accompanied by splenic friction, was found in two patients with chronic myeloid leukaemia and one with myelofibrosis. While splenic infarcts are common in chronic myeloid leukaemia (Whitby and Britton, 1957) and are no doubt the cause of this pain, they are not common in myelosclerosis and perisplenitis may then be the cause (Leading Article, Brit. med. J., 1956).

Chronic myeloid leukaemia and myelofibrosis are the two conditions which, in this country,

	Grade 0	Grade I	Grade II	Grade III
C.M.L.	0	I	2	2
Mf.	0	I	4	4

Table 3 Splenomegaly in chronic myeloid leukaemia and myelofibrosis. (C.M.L.= chronic myeloid leukaemia; Mf.= myelofibrosis).

/are by far the most common causes of a massive splenomegaly. It does not follow that the spleen must be grossly enlarged before the diagnosis can be considered and as splenic enlargement takes some time to develop it seems possible that in the fairly early stages of these diseases the size of the spleen may bear some relation to the duration or severity of the disease. The size of the spleen has been estimated as follows:

Grade 0 The spleen is not palpable.

Grade I The spleen is enlarged to a point between the costal margin and the umbilicus.

Grade II The spleen is enlarged to a point below the umbilicus but does not cross the midline.

Grade III The spleen is enlarged to a point below the umbilicus and crosses the midline.

The spleen was palpable in all cases but a considerable variation in size was found in both diseases (Table 3). Gross splenomegaly

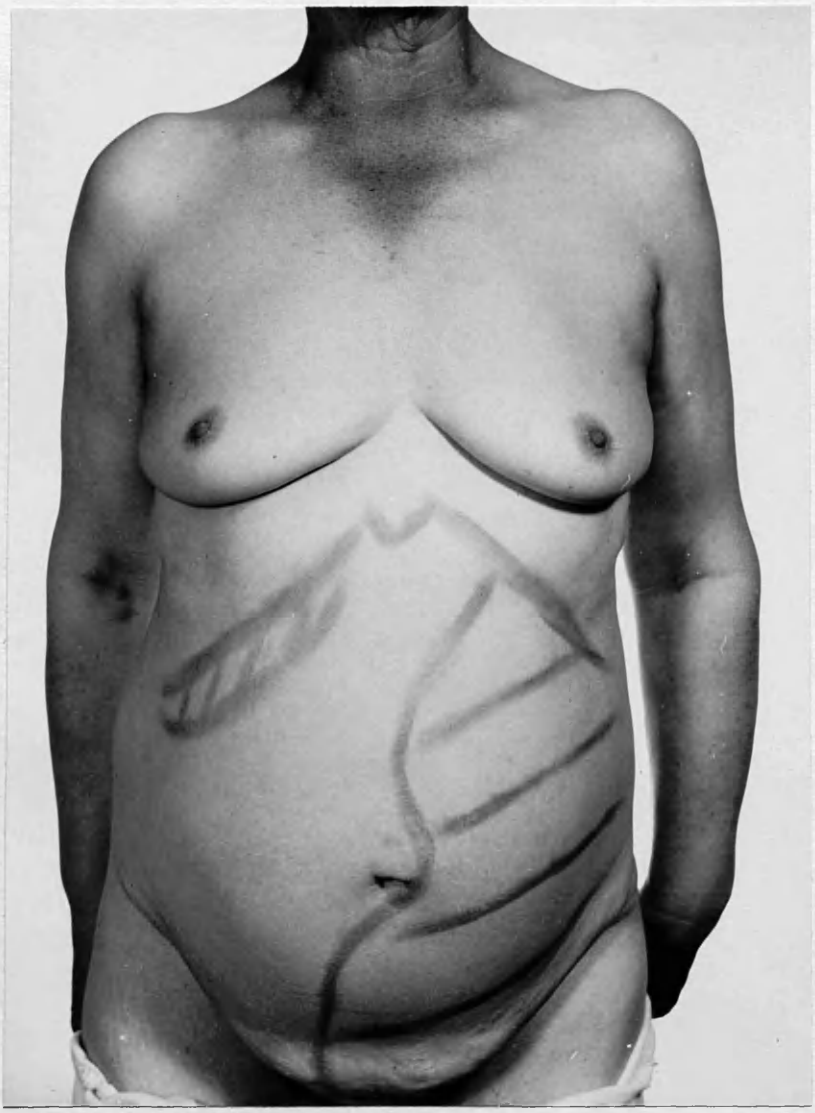

Fig. 3 Grade III splenomegaly.(Patient A.R.)

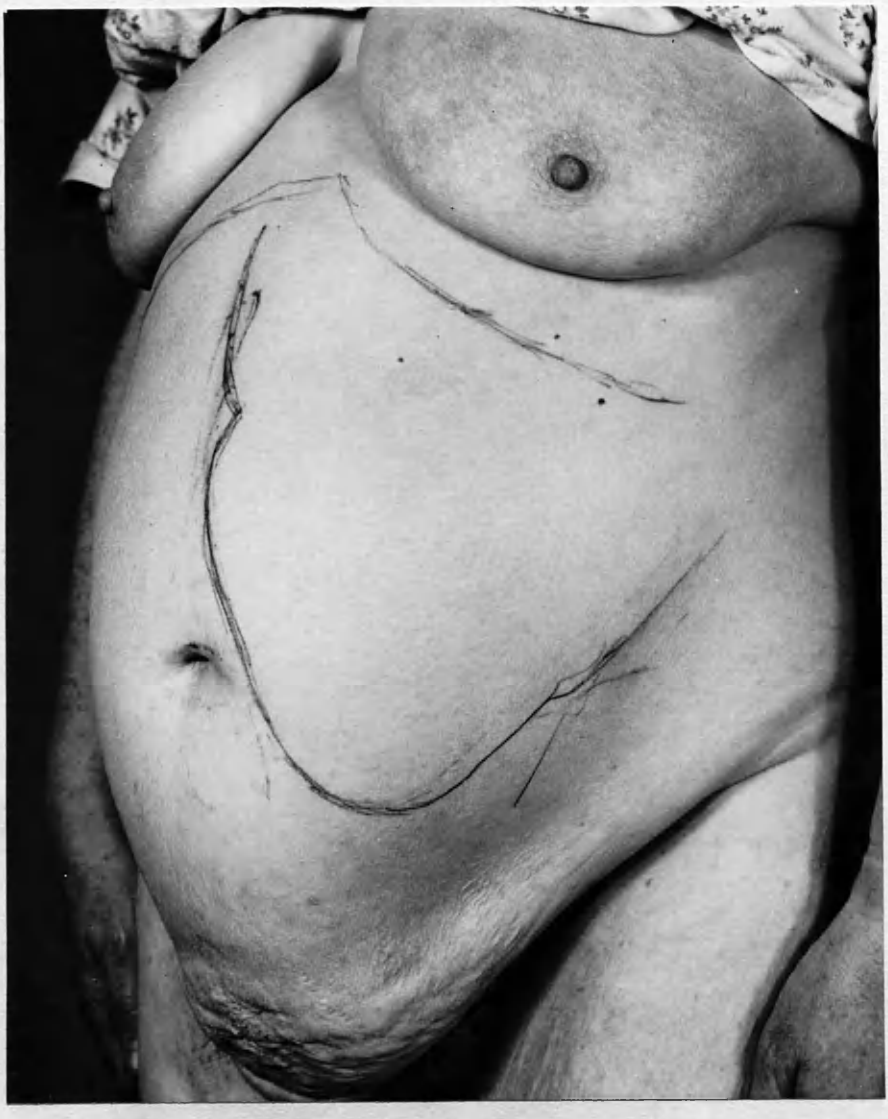

Fig.4 Grade II splenomegaly. (Patient R.C.)

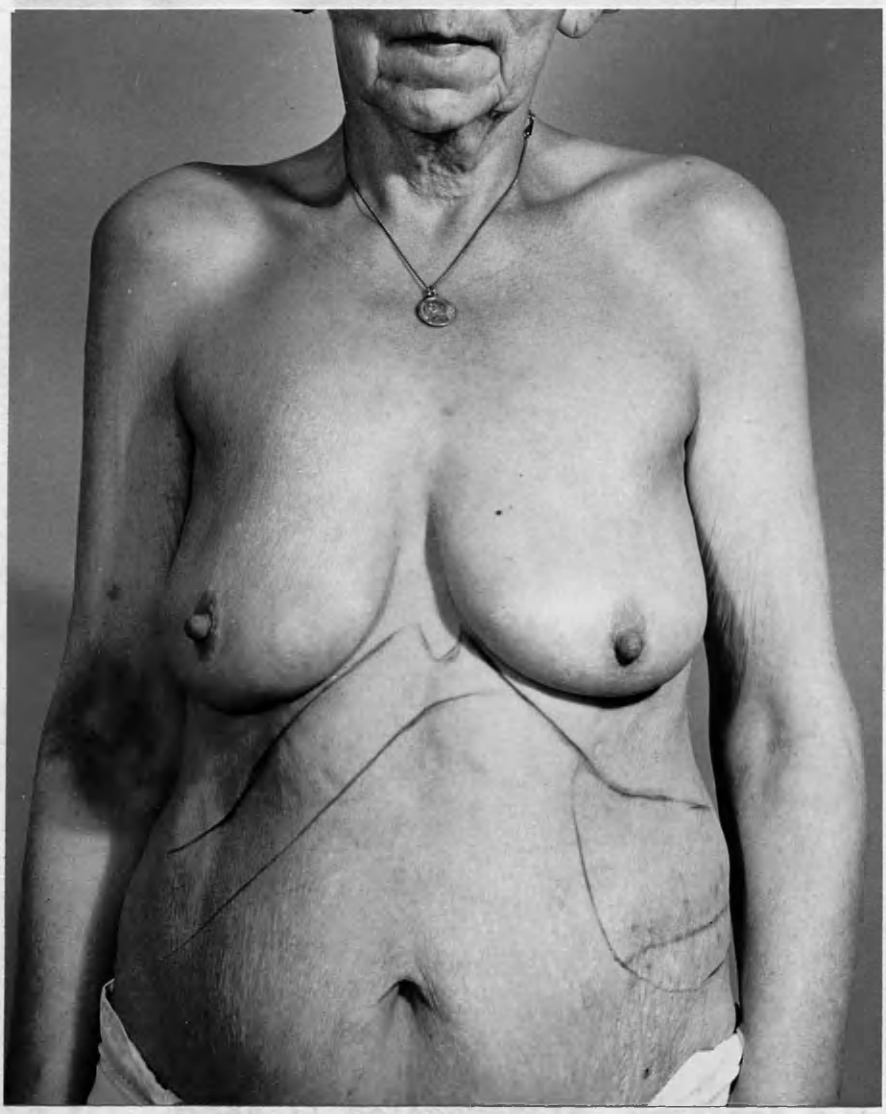

Fig.5 Grade I splenomegaly. (Patient S.H.)

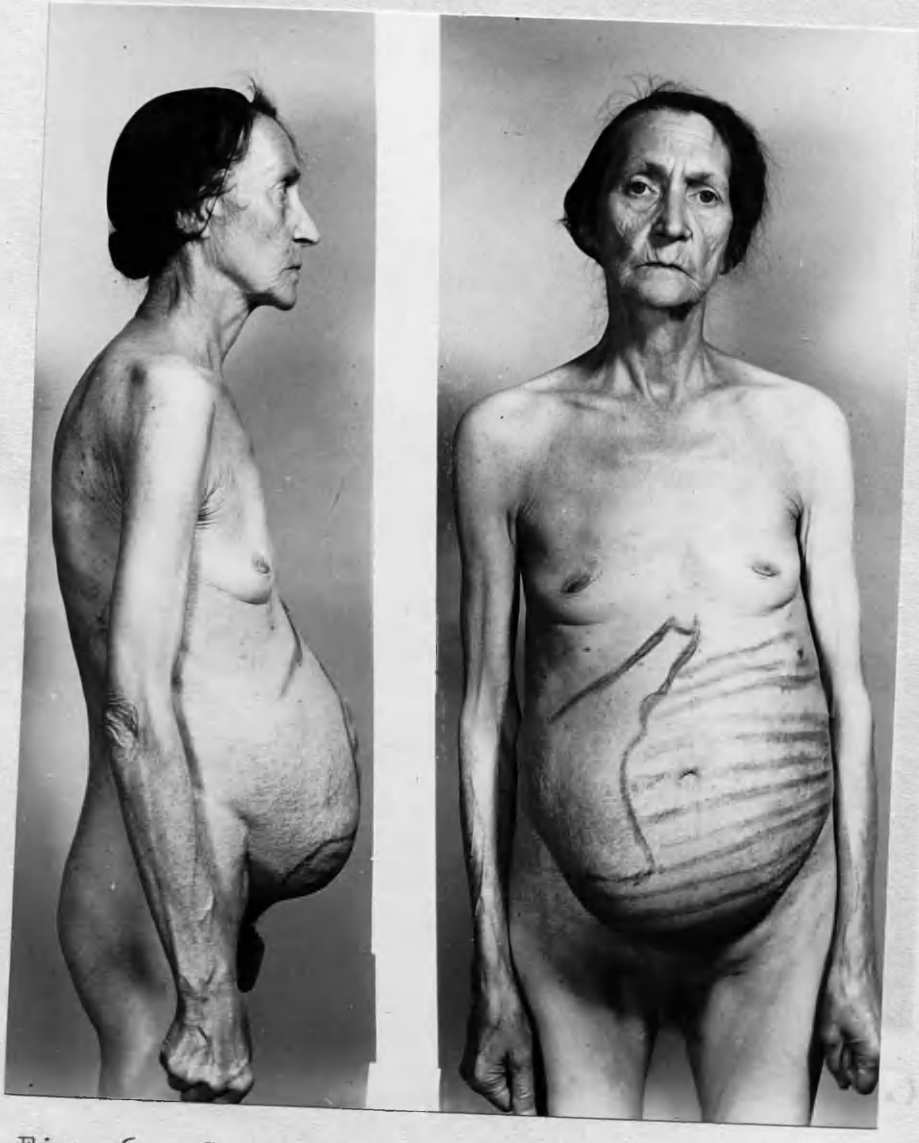

Fig. 6 Gross splenomegaly (Patient M.C.)

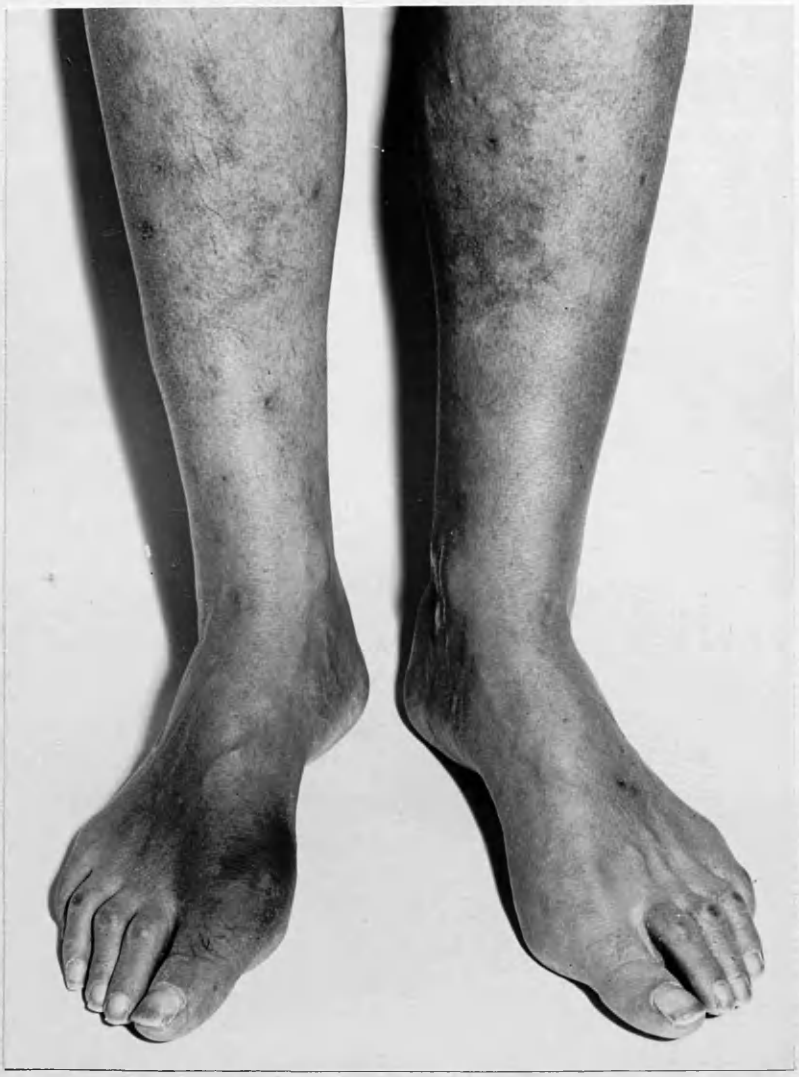

Fig. 7 Gout in myelofibrosis. (Patient J.C.)

/considered here and six of the nine with myelofibrosis had a palpable liver.

Lymphadenopathy was found in three patients with chronic myeloid leukaemia and in four with myelofibrosis but the nodes, with the exception of those in M.M. who had chronic myeloid leukaemia, were never large.

Other features are occasionally found. Cough was present in one patient with each disease; true peripheral vascular disease in one patient with myelofibrosis and hypertension (B.P. 210/110 mm. Hg.) in another. Pigmentation of the skin was reported in a case of myelofibrosis by Beattie and Withey (1953) and this unusual feature was found in one patient. Gout is a recognized complication of myeloproliferative disease due to the increased uric acid metabolism and this was seen in one patient with myelofibrosis (fig. 7).

Summary

Chronic myeloid leukaemia and myelofibrosis are diseases of middle and later life.

In both diseases the presenting symptoms are usually those of asthenia, anaemia and abdominal swelling or discomfort. Loss of weight may be a striking feature of either disease and occasionally there may be the paradoxical situation of either a bleeding or a thrombotic tendency.

The spleen in both disorders is usually greatly enlarged and may reach enormous proportions. The liver is less constantly palpable and lymphadenopathy is sometimes found.

There is, therefore, a similarity in the age and sex incidence, the presenting symptoms and the physical signs in chronic myeloid leukaemia and in myelofibrosis.

Chapter 4

THE HAEMATOLOGICAL FINDINGS IN CHRONIC MYELOID LEUKAEMIA AND MYELOFIBROSIS

The diagnosis of many diseases of the blood rests mainly on the clinical picture, the examination of the blood and the histological appearances of the bone marrow. Occasionally other special investigations are required for precise diagnosis or for the elucidation of problems which have arisen in a particular disease or patient. When two diseases have similar clinical pictures the haematological and other investigations then assume considerable importance. As marked similarity has been shown to exist in the clinical features of chronic myeloid leukaemia and myelofibrosis, the results of haematological investigation are of great interest and importance.

The striking feature on examination of the blood of patients with chronic myeloid leukaemia is the great number of circulating leucocytes. The white cells usually number from 100,000 per c.mm. to 400,000 per c.mm. though counts up to and over one million cells have been recorded (Scott, 1957). This high count was found in all five cases, the levels ranging from

Patient	White cells per c.mm.	Neutrophils %	Eosinophils %	Basophils %	Lymphocytes %	Monocytes %	Myeloblasts %	Myelocytes %	Metamyelocytes %	Smudge cells %	Nucleated red cells/100 W.B.C.
E.W.	135,000	64	1	6	1	4	0	7	17	0	4
J.L.	340,000	35.5	3.5	2.5	1	1	3	32.5	21	0	0.5
C.L.	150,000	46	4	20	4	1	0	1.5	10	0	1
A.R.	300,000	45	2	2	1	0	3	38	9	0	1
M.M.	105,000	51	1	0.5	15	1	1.5	16	14	0	5

Table 4 The differential counts of white cells in 5 patients with chronic myeloid leukaemia.

Patient	White cells per c.mm.	Neutrophils %	Eosinophils %	Basophils %	Lymphocytes %	Monocytes %	Myeloblasts %	Myelocytes %	Metamyelocytes %	Smudge cells %	Nucleated red cells/100 W.B.C.
E.W.	135,000	64	1	6	1	4	0	7	17	0	4
J.L.	340,000	35.5	3.5	2.5	1	1	3	32.5	21	0	0.5
C.L.	150,000	46	4	20	4	1	0	1.5	10	0	1
A.R.	300,000	45	2	2	1	0	3	38	9	0	1
M.M.	105,000	51	1	0.5	15	1	1.5	16	14	0	5

Table 4 The differential counts of white cells in 5 patients with chronic myeloid leukaemia.

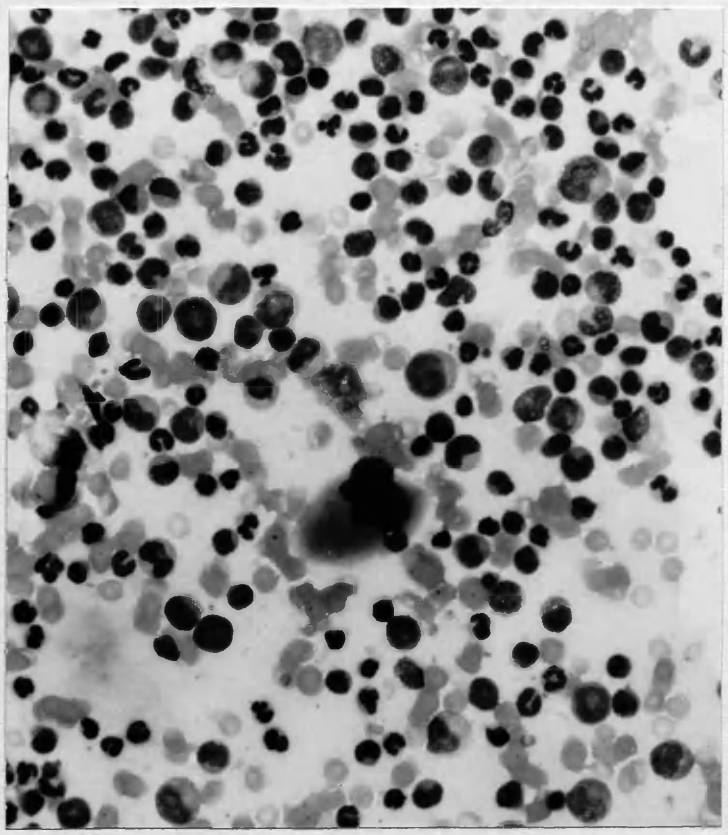

Fig. 8 Marrow in chronic myeloid leukaemia. (Patient J.L.) (May-Grunwald-Giemsa x 375)

/Occasional nucleated red cells may be found in the peripheral blood (Scott, 1957) and platelets are usually abundant except in the terminal stages. The striking feature of a blood film of typical chronic myeloid leukaemia is the greatly increased number of leucocytes. In such cases the diagnosis can usually be made confidently from the blood and bone marrow examination is seldom necessary. If marrow examination is made then gross myeloid hyperplasia is seen (fig. 8) and the diagnosis is established.

In myelofibrosis the white cell count is often within the normal range in contrast to the greatly elevated counts of chronic myeloid leukaemia. Although Hickling (1953) reported a count of 70,000 cells per c.mm.the highest figure reported in a series of 28 cases investigated by Leonard, Israels and Wilkinson (1957) was 42,000 per c.mm. Most authors are agreed that the white cell count is usually normal or slightly elevated but that counts of over 50,000 cells per c.mm. are occasionally

Patient	White cells per c.mm.	Neutrophils %	Eosinophils %	Basophils %	Lymphocytes %	Monocytes %	Myeloblasts %	Myelocytes %	Metamyelocytes %	Smudge cells %	Nucleated red cells/100 W.B.C.
J.C.	73,000	38	0.5	3	3.5	1	5	19	24	6	3.5
T.Q.	2,700	61	0	4	32	1	0	0	2	0	5
M.C.	30,000	52	0.5	2.5	2.5	2.5	1	10	20	9	3
S.H.	9,200	51	1	2.5	30	15.5	0	0	0	0	0
A.S.	12,500	39	1	2	6	0	2	23	27	0	2
J.G.	21,000	45	1	17	12	0	0	7	18	0	2
R.C.	11,600	89	0	1	9	1	0	0	0	0	4
M.G.B.	2,000	73	1	2	23	1	0	0	0	0	17
W.P.	2,100	6	0	1	25	9	12	10	0	37	17

Table 5 The differential counts of white cells in 9 patients with myelofibrosis.

/seen. A count of 100,000 cells per c.mm. appears to be a convenient dividing line between chronic myeloid leukaemia and myelofibrosis. Korst, Clatanoff and Schilling (1956) reported two patients with white cell counts of 112,500 and 232,000 per c.mm. but these high levels are quite exceptional. Of the nine cases reported here only one had a white cell count of over 50,000 cells per c.mm. and none had counts of 100,000 per c.mm. Primitive white cells were often present and the results of differential white cell counts are seen in table 5. Anaemia was always present and the haemoglobin levels ranged from 4.1 g per cent to 12.4 g per cent (mean 9.2 $\pm$ 2.5). Iron deficiency was usually found but on examination of the blood film the mature red cells often showed marked poikilocytosis and 'tear drop' cells (Cook et al, 1953) were commonly seen (fig. 9). A characteristic feature is the finding of nucleated red cells in the peripheral blood. Although they may be scanty or occasionally absent they are present usually

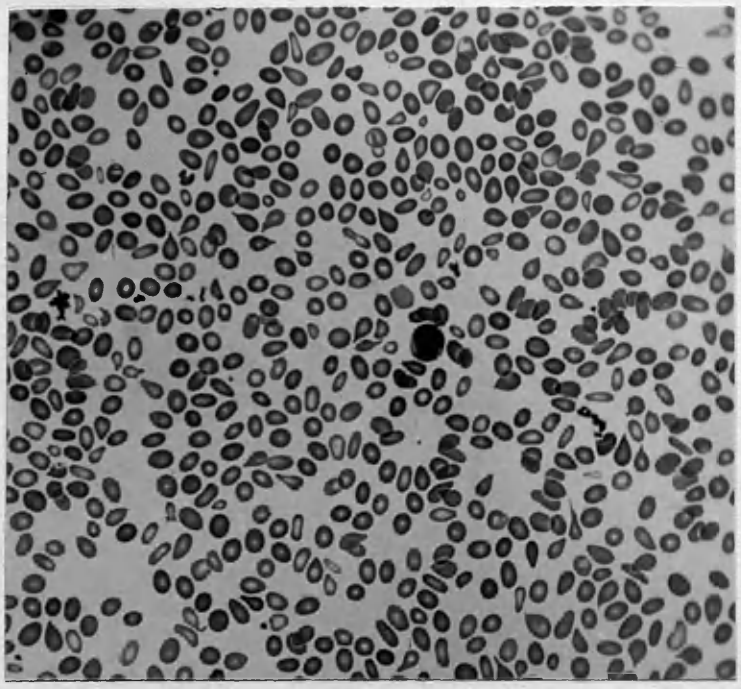

Fig. 9 Marked anisopoikilocytosis with 'tear drop' cells in myelofibrosis. (Patient R.C.) (Leishman x 400).

/in considerably higher proportions than are found in chronic myeloid leukaemia. Two patients had 17 nucleated red cells per 100 white cells in the blood but Leonard et al (1957) report a patient with as many as 61 per 200 white cells. The origin of these immature cells and the mechanism of their release into the blood is obscure but it has been suggested by Rosenthal (1950) that they may originate in sites of extramedullary erythropoiesis.

Platelet numbers are variable and range from levels of thrombocytopenia to those of thrombocythaemia. Often, however, the platelets are seen to be abnormal, as was noted in two of these patients, and occasionally megakaryocytes or fragments of megakaryocytes are found in the blood.

Thus, the blood picture in myelofibrosis is characteristically that of leuco-erythroblastosis which was defined a quarter of a century ago by Vaughan (1936) as "an anaemia characterised by the presence in

/the peripheral blood of unusually immature red cells and a few immature white cells of the myeloid series. The anaemia is not necessarily severe, nor is there usually a marked leucocytosis."

The diagnosis of myelofibrosis, however, cannot be made with certainty purely from the examination of the blood. The nature of the condition demands examination of the bone marrow. The conventional marrow puncture technique usually yields a 'dry tap' although a few drops of marrow or blood may occasionally be obtained, whereas in chronic myeloid leukaemia ample marrow is normally obtained without difficulty. A 'dry tap' will arouse considerable suspicion but a marrow trephine biopsy is required to substantiate the diagnosis of myelofibrosis.

Histological examination of the marrow trephine specimen usually reveals a hypoplastic marrow although there may be areas of hyperplasia. This appears to indicate that at least in the early stages the disease is of patchy

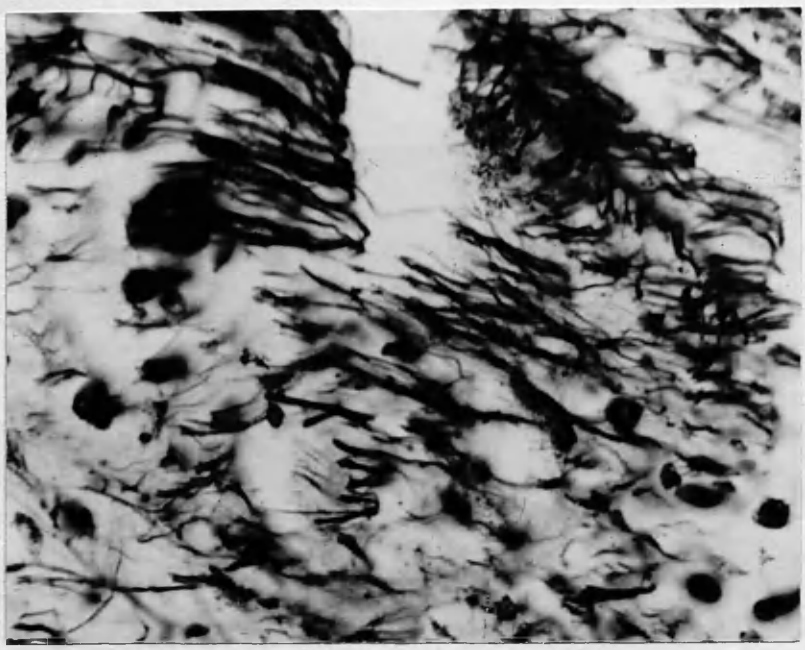

Fig. 10 Iliac crest marrow showing an increase in reticulum in myelofibrosis. (Patient S.H.) (x 675).

/distribution. The hypoplasia is most marked for erythropoietic tissue which may be virtually absent but there is commonly some increase in leucopoietic tissue and megakaryocytes until the later stages of the disease. An increase in reticulum, which may be quite striking (fig. 10), usually is seen and, of course, by definition fibrous tissue is present. Bony trabeculae may be broadened and interlaced and give rise to marked increase in osteoid tissue.

Leonard, Israels and Wilkinson (1957) attempted to classify the histological appearances seen in the marrow trephine specimens in myelosclerosis according to the stage of the disease and they describe bony changes only in the intermediate and late stages. Thus, they consider 'myelofibrosis' as an early stage of 'myelosclerosis'. This suggests that the disease is not fully developed until bony changes are present. Many patients, however, never develop bony changes even after several years and may die with pure 'myelofibrosis'.

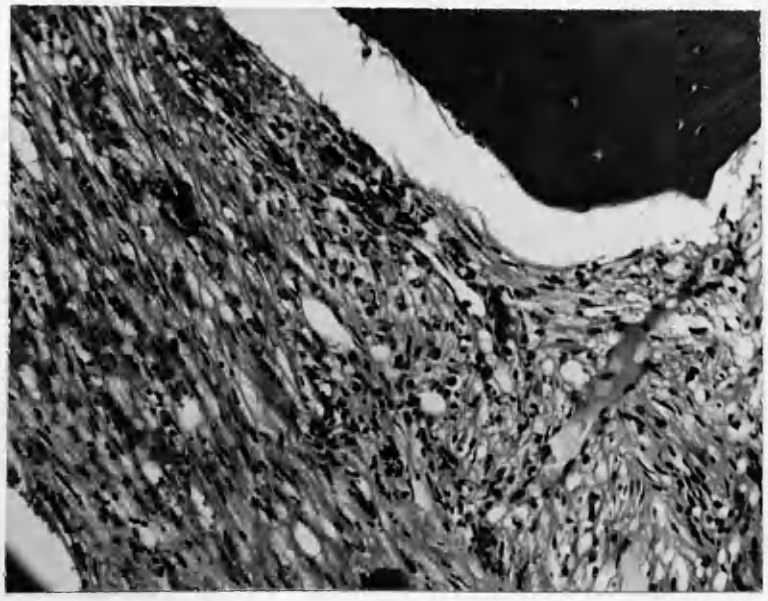

Fig. 11 Marrow trephine showing the early stage of myelofibrosis (Patient J.C.) (H. & E. x 150).

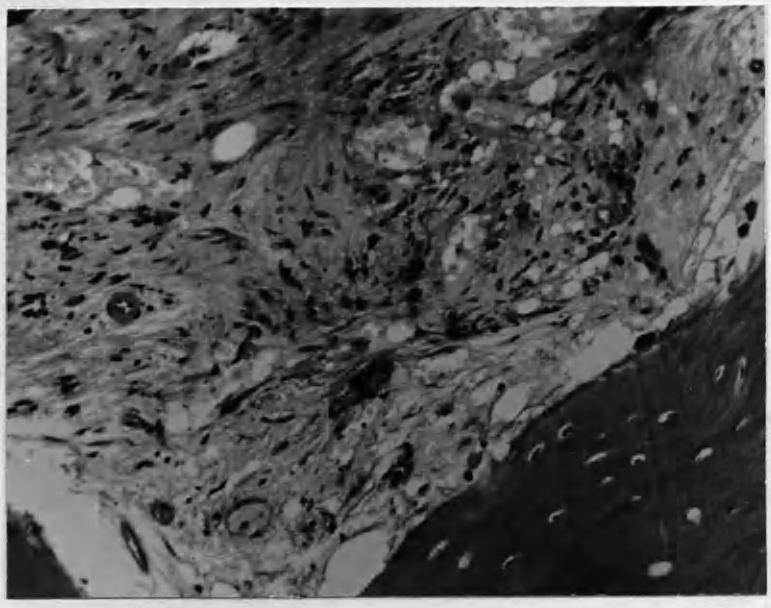

Fig. 12 Marrow trephine showing the intermediate stage of myelofibrosis. (Patient M.G.B.) (H. & E. x 210).

/It would seem, therefore, more satisfactory to regard the disease as 'myelofibrosis' and to accept 'myelosclerosis' as an occasional complication due to the involvement of the bony trabeculae in an otherwise identical disease. For this reason the whole group hereafter is regarded as myelofibrosis and the term 'myelosclerosis' is used only when it is desired to stress the occurrence of bony changes.

The histological classification of Leonard, Israels and Wilkinson does give however, a very satisfactory method of comparing the disease process from one patient to another. They described three stages. In the early stage they noted hypoplasia with patchy fibrosis and an increase in the number of megakaryocytes present but normal haemopoietic tissue was seen in some parts of the specimen (fig. 11). The intermediate stage was characterised by widespread fibrosis, increase in megakaryocytes and some increase in bony trabeculae (fig. 12). The late stage showed broad bony trabeculae,

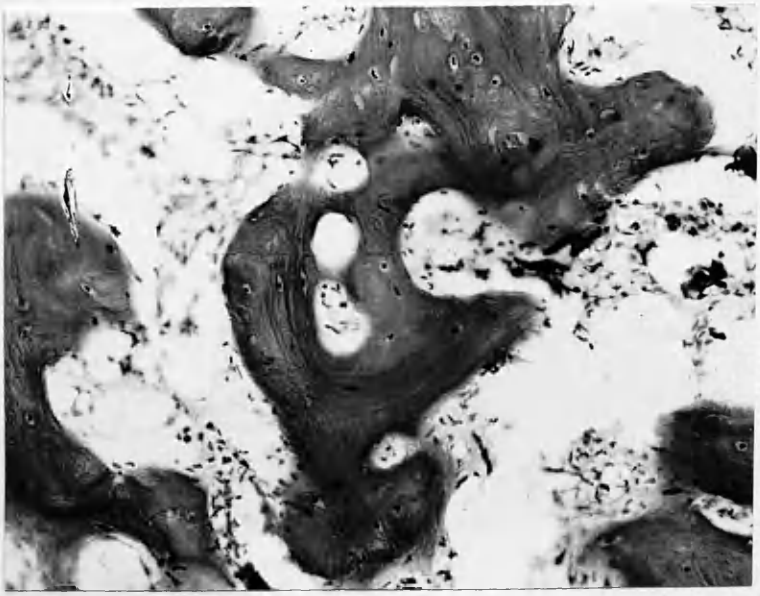

Fig. 13 Marrow trephine showing the late stage of myelofibrosis (Patient A.S.) (H. & E. x 130).

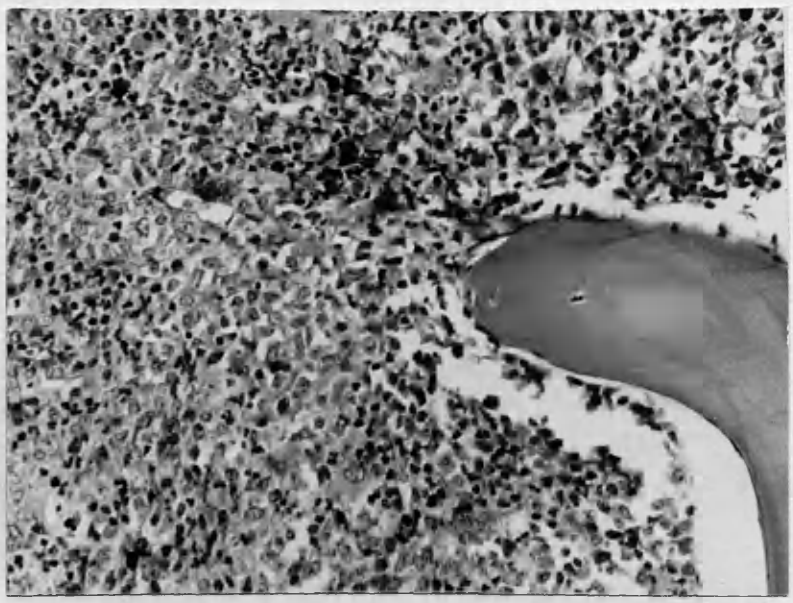

Fig. 14 Marrow trephine in chronic myeloid leukaemia. (Patient A.R.) (H. & E. x 210).

/acellular fibrosis, virtual absence of haemopoietic tissue and scanty or absent megakaryocytes (fig. 13). Using this classification, the histological appearances of the marrow trephine on the eight patients on whom this investigation was carried out showed four patients to be in the early stage, two in the intermediate stage and two in the late stage of the disease. This distribution is not surprising because, firstly, the biopsies were done in most instances when the patient presented for the first time and, secondly, as mentioned previously, some patients do not develop bony changes and so will never qualify for classification in the late stage. It is likely, then, that these patients form a representative group.

Marrow trephine biopsy was carried out on three patients with chronic myeloid leukaemia. In each case there was gross myeloid hyperplasia, but no obvious increase in megakaryocytes, no fibrosis and no increase in bony trabeculae. An illustration of the histological appearance

/of the trephine biopsy in one of these patients is seen in fig. 14. In one case there was an occasional focus of increased reticulum but this was not comparable to that seen in myelofibrosis.

There is, then, a striking difference in the histological appearances of the marrow in chronic myeloid leukaemia and of that in myelofibrosis and this is a simple and satisfactory way in which the two diseases can be differentiated.

The gross enlargement of the spleen in both diseases suggests that it is the site of a major pathological disturbance. It is generally accepted that in myelosclerosis this is due to myeloid metaplasia (Wintrobe, 1956) though Engell (1947) stated that myelosclerosis could exist without myeloid metaplasia. Moeschlin (1951) considered that the diagnosis could be determined by examination of splenic aspirate but Block and Jacobson (1950) went even further and stated that microscopic examination of the splenic tissues was the most important step in diagnosis. Splenic

Patient	Diagnosis	Neutrophils %	Eosinophils %	Basophils %	Lymphocytes %	Monocytes %	Myeloblasts %	Myelocytes %	Metamyelocytes %	Nucleated red cells /100 W.B.C.
C.L.	C.M.L.	26	5	1	5	5	6	14	15	22
E.W.	C.M.L.	45	0	4	5	6	3	11	25	1
J.G.	Mf.	32	0	4	29	3	0	9	22	1
S.H.	Mf.	14	2	1	34	4	1	10	8	26
M. C.	Mf.	28	3	2	52	1	3	5	3	3

Table 6 Splenograms on 2 patients with chronic myeloid leukaemia and 3 with myelofibrosis. (**C.M.L.= chronic myeloid leukaemia; Mf.= myelofibrosis**).

/puncture was carried out on two patients with chronic myeloid leukaemia and four with myelofibrosis and the results of differential counts of five of these are seen in table 6. The striking difference is the proportion of lymphocytes found. In chronic myeloid leukaemia they are very much less than normal and Moeschlin (1951) interpreted this as indicative of disruption of the splenic architecture. If this is the case then the splenic architecture is better preserved in myelofibrosis where the proportion of lymphocytes is higher and this is in agreement also with the experience of Leonard, Israels and Wilkinson (1957). In chronic myeloid leukaemia the great majority of the cells are of the granulocyte series but in myelofibrosis the numbers of granulocytes and lymphocytes, though variable, often are not greatly different. The results of these splenic punctures agree well with those of the other workers mentioned but the counts of nucleated red cells are rather lower. If splenic erythropoiesis is indicated by the

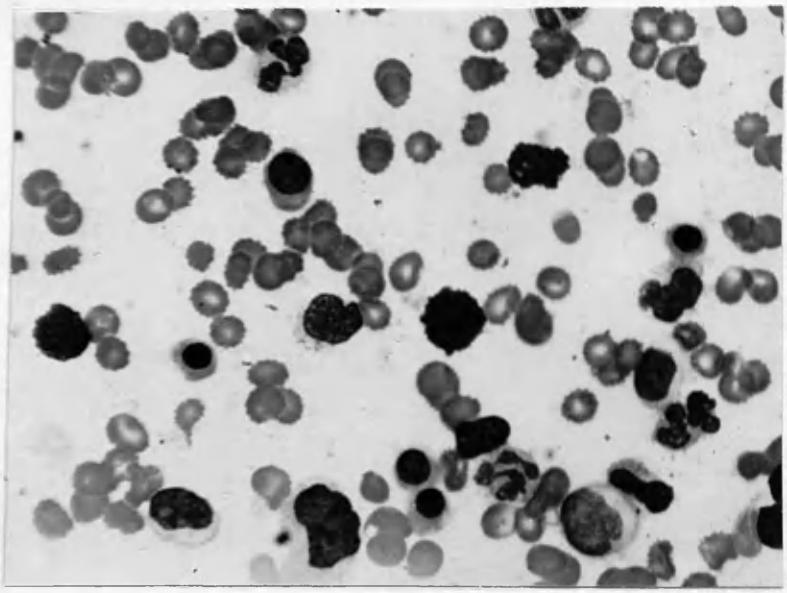

Fig. 15 Splenic aspirate showing nucleated red cells in myelofibrosis. (Patient S.H.) (Leishman x 525).

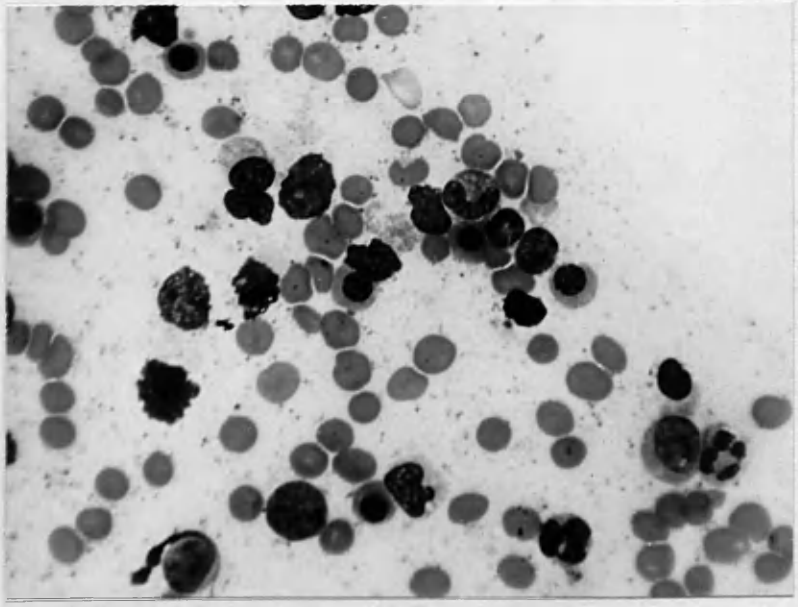

Fig. 16 Splenic aspirate showing nucleated red cells in chronic myeloid leukaemia. (Patient C.L.) (Leishman x 525).

/presence of moderate numbers of nucleated red cells in the splenic aspirate then this is not a necessary accompaniment of myelofibrosis as only one of the four cases so investigated showed this (fig. 15). Significant numbers of nucleated red cells were found also on splenic puncture of one patient with chronic myeloid leukaemia (fig.16).

This suggests that the occurrence of splenic erythropoiesis is not a prerogative of myelofibrosis but can occur in some cases of chronic myeloid leukaemia. The number of patients investigated by splenic puncture is small because the procedure is contraindicated in the presence of recent splenic infarcts or a bleeding diathesis. Furthermore, it was considered that splenic histology might be significantly altered, and therefore unacceptable, if the spleen had been previously subjected to irradiation. Splenic erythropoiesis, can, however, be estimated by other methods and this subject is considered in more detail later.

/ Anaemia is usually caused by lack of production of red cells, blood loss or an increased rate of haemolysis. In chronic myeloid leukaemia and myelofibrosis haemorrhage is rarely a factor in the production of anaemia and although a few patients admitted to easy bruising none admitted to external bleeding and there was no reason to suspect blood loss. Lack of red cell production is a possible factor and this could be related to a dyshaemopoietic defect; but it is not difficult to visualise the mechanical problem of erythropoiesis in a marrow cavity obliterated by fibrous or bony tissue to the degree seen in some patients. This is simulated in chronic myeloid leukaemia by the gross myeloid hyperplasia which has been thought to cause "crowding out" of the red cell precursors. The enlargement of the spleen has been considered to be related to extramedullary erythropoiesis compensatory to the marrow destruction; this extra source of red cells raises a further complication to the problem of the mechanism of

/the anaemia in these diseases; furthermore, the question of increased haemolysis has yet to be raised.

The whole problem of the causation of anaemia is better considered after red cell production and destruction have been investigated by more definitive studies involving the use of radioactive substances. The routine haematological investigations however, provide the means to precise diagnosis and give information of value in assessing a possible relationship between chronic myeloid leukaemia and myelofibrosis.

Summary

The results of examination of the blood, the bone marrow and splenic aspirate are compared in chronic myeloid leukaemia and myelofibrosis.

In both disease anaemia is usually present but the striking difference is seen in the white cell count which is greatly increased in chronic myeloid leukaemia. A count of 100,000 cells per c.mm. appears to be a convenient dividing line. The anaemia of myelofibrosis is leuco-erythroblastic and nucleated red cells are seen in higher proportion than occurs in chronic myeloid leukaemia. Platelet levels are variable in both diseases.

Sternal puncture characteristically yields a 'dry tap' in myelofibrosis whereas ample marrow is usually obtained in chronic myeloid leukaemia. Marrow trephine biopsy is necessary for the diagnosis of myelofibrosis and the histological findings are discussed.

The results of splenic puncture suggest that splenic architecture is better preserved in myelofibrosis and indicate that splenic

/erythropoiesis may occur in either disease.

Thus the two diseases can be differentiated on haematological grounds. Although there are similarities in the blood pictures the correct diagnosis can often be made from this alone but marrow trephine biopsy is required to substantiate the diagnosis of myelofibrosis. Splenic erythropoiesis does not appear to be essential for the diagnosis of myelofibrosis and the findings suggest that it occurs in chronic myeloid leukaemia.

Chapter 5

STUDIES WITH RADIOACTIVE IRON AND CHROMIUM IN CHRONIC MYELOID LEUKAEMIA AND MYELOFIBROSIS

The advent of radioactive substances has allowed a new and more dynamic approach to the problems of blood production and destruction. Prior to this reliance was placed mainly on the routine examination of the blood and the histological appearances of the bone marrow for estimation of blood production, and on pigment excretion studies and the application of the method of differential agglutination of red cells for estimation of blood destruction. Although these procedures provided valuable information, and may still constitute an important part of investigation, there remained a distinct lack of knowledge of the quantitative aspects of blood production and of the survival of the patient's own cells in his own circulation. The use of radioactive iron (^{59}Fe) and radioactive chromium (^{51}Cr) has allowed a study of these problems, because, while behaving in all respects like the naturally occurring non-radioactive elements, the emission of radioactivity permits tracking of their metabolic pathways in the living subject.

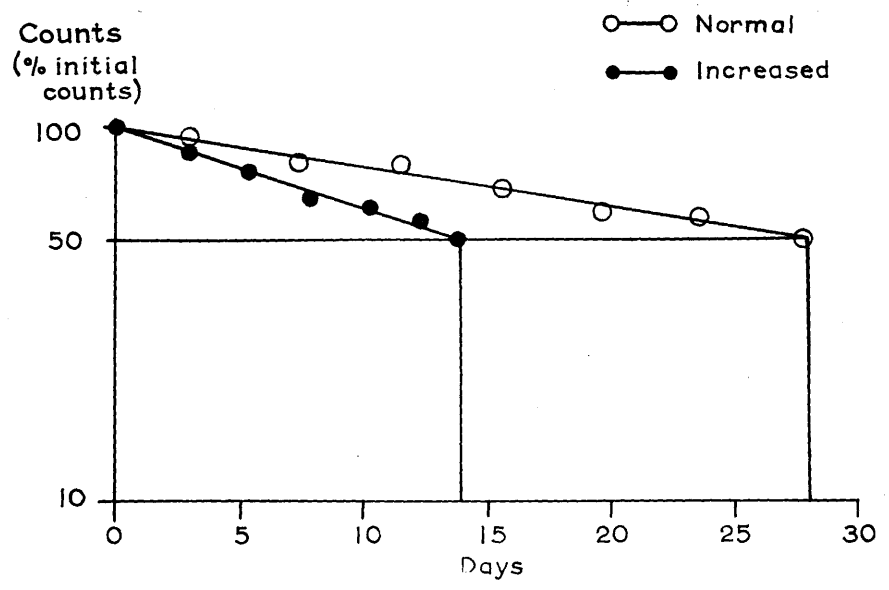

Fig. 17 Illustration of normal and increased rates of haemolysis as determined by the radiochromium technique.

/ In 1950 Gray and Sterling demonstrated the ability of radioactive chromium (^{51}Cr) in the form of sodium chromate to 'label' the red cells by attachment to the globin moiety. When a patient's red cells are labelled in this way and reinjected into the circulation elution of chromium into the plasma occurs at the rate of about one per cent per day (Jones and Mollison, 1956). This is insignificant over the short period required for mixing and so accurate blood volume measurements can be obtained by application of the dilution technique (Sterling and Gray, 1950). Decrease of radioactivity in the blood is otherwise due to the natural decay of the radioactive chromium and to the removal from the circulation of cells containing it. In the normal subject the radioactivity is reduced to half ($T\frac{1}{2}$) in 25 - 30 days but in patients with increased haemolysis the half life may be much less (fig.17). Furthermore, if during this period the red cells are being sequestered and destroyed by the spleen, there

/will be a marked accumulation of radioactivity in the spleen as compared to the liver or heart (Jandl et al, 1956). The spleen/liver ratio in normal subjects at $T\frac{1}{2}$ is less than 1.5/1 and definite sequestration and destruction of red cells by the spleen is considered to be present when the ratio is 2.5/1 or more.

Thus, the use of radioactive chromium allows measurement of the blood volume, indicates the rate of red cell destruction and provides information about the spleen as an organ of sequestration and destruction of red cells.

When radioactive iron (^{59}Fe) is injected intravenously in the form of ferric chloride it immediately labels the plasma iron pool and then passes within one hour to the sites of haemoglobin formation. If the radioactivity is counted at intervals for a period of 100 hours over the heart, (as a measure of blood radioactivity) the sacrum (as normal site of blood formation), the liver and spleen, (as possible sites of erythropoiesis) the results

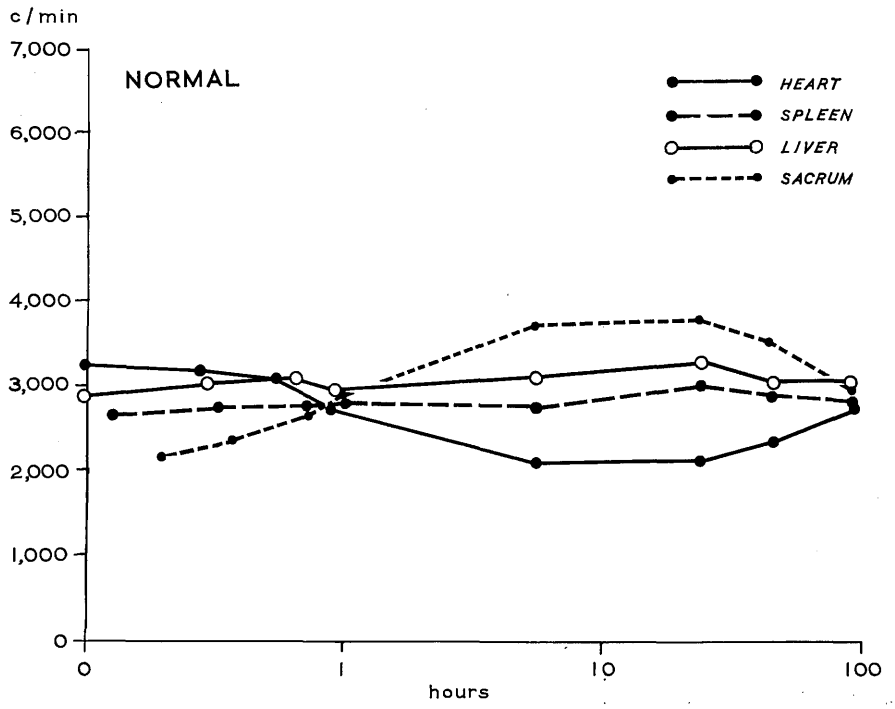

Fig. 18 Normal surface pattern of radioiron uptake.

(reproduced by permission from 'Clinical Radiology).

/can be plotted on a graph to give a radioiron 'profile' for each patient (Ledlie and Baxter, 1954). The 'profile' for a normal subject (fig.18) shows a fall in heart counts and a rise in sacral radioactivity as the iron passes from the plasma to the marrow. There is a later decrease in marrow counts and a rise of heart counts as the iron, incorporated within newly formed red cells, returns to the blood. There is no significant change in the radioactivities of the spleen or liver. Thus, a radioiron profile may indicate the site or sites of erythropoiesis provided it is remembered that the surface counts are affected also by the radioactivity of the blood flowing through the organs. Wetherley-Mein, Hutt, Langmead and Hill (1956) describe four such profiles: the normal, the aplastic, the hypoplastic and the extramedullary.

If samples of blood are examined at intervals the radioactivity of the blood will be found to increase for about ten days (fig.19) (Wetherley-Mein et al 1956) as the red cells

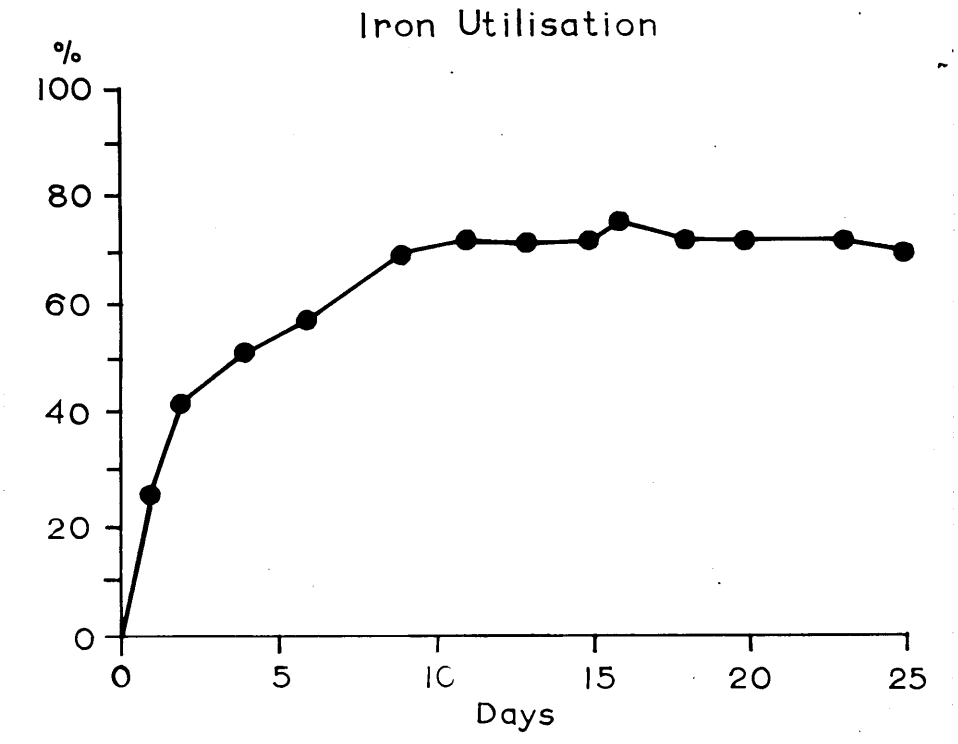

Fig. 19 Normal utilisation of injected radioiron.

/containing the radioactive iron are released into the blood stream. This gives a measure of the utilisation of the injected iron for haemoglobin formation which is 68-83% of the injected dose in normal subjects (Finch et al, 1949). The utilisation of injected iron depends mainly on the rate and amount of red cell production and on the state of the iron stores. When the iron stores are low the iron is incorporated rapidly into haemoglobin and so gives a high utilisation which, however, does not indicate that the amount of erythropoiesis is greater than normal. Provided the state of the iron stores is considered the iron utilisation can give an indication of the amount of red cell production.

Thus, information on the amount and sites of red cell production and of the rate and sites of red cell destruction can be gained from these investigations with radioiron and radiochromium.

Results

Radioiron studies were carried out on the

/patients with chronic myeloid leukaemia and those with myelofibrosis. One patient with chronic myeloid leukaemia died of a cerebro-vascular accident before the iron utilisation could be completed and before radiochromium studies were commenced. Red cell survival was estimated on all patients and sequestration studies were carried out on eight patients with myelofibrosis. As each patient presents a different problem in relation to anaemia, and as these investigations provide so much information about the mechanisms of blood production and destruction, the results obtained for each patient are considered separately. Iron deficiency was present only where indicated..

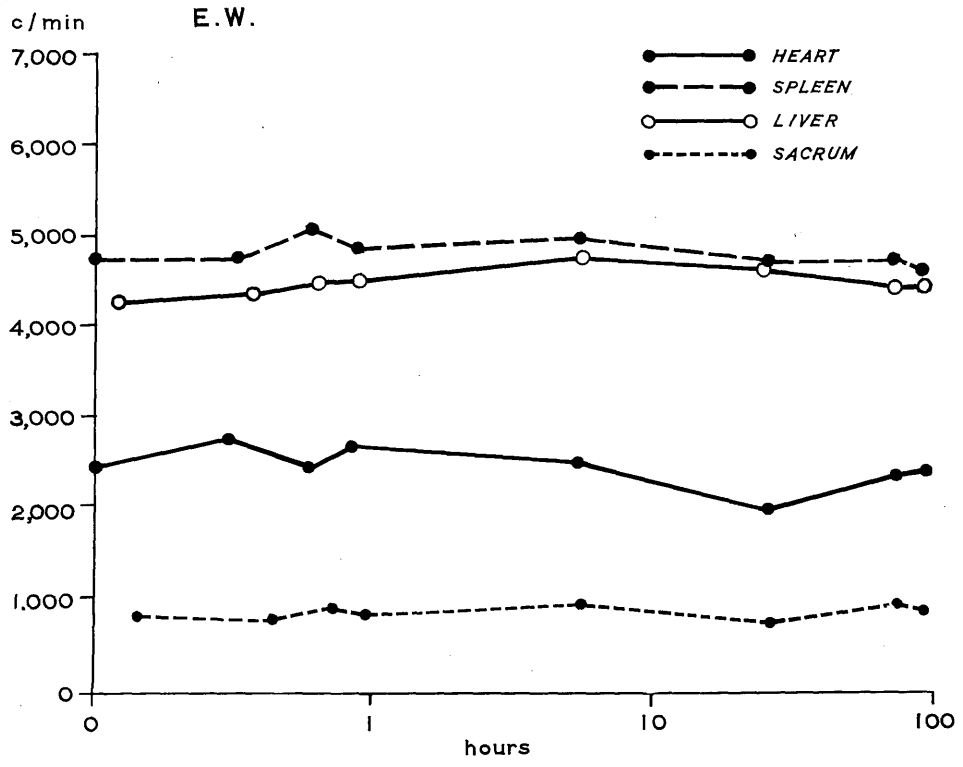

Fig. 20 Radioiron profile in patient E.W. (chronic myeloid leukaemia).

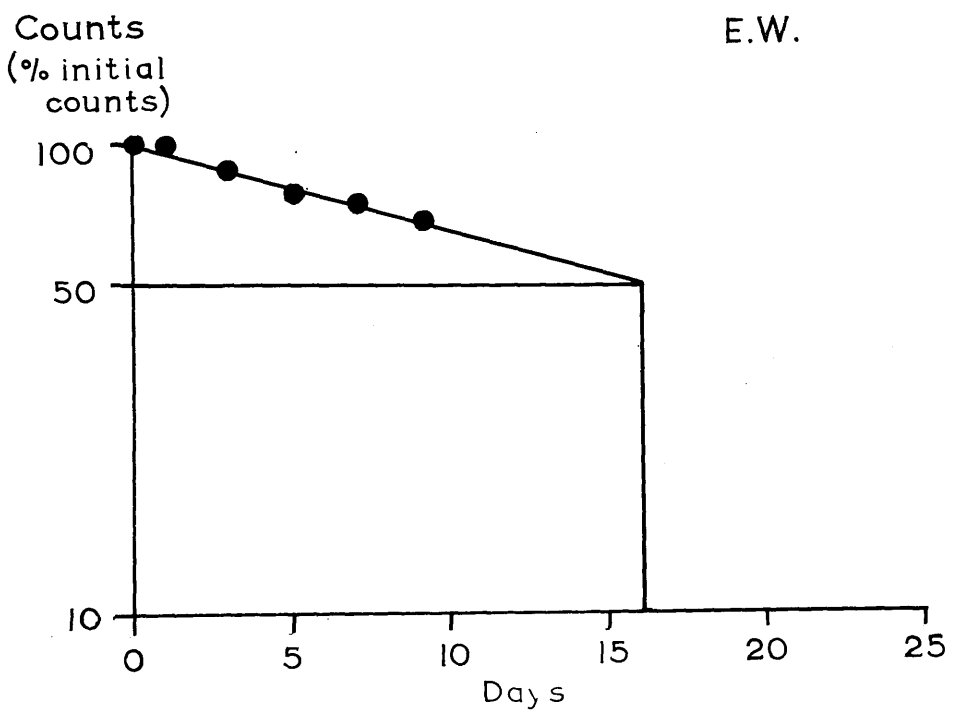

Fig. 21 Red cell survival by radiochromium technique in patient E.W.

/Chronic Myeloid Leukaemia

E.W. - Chronic Myeloid Leukaemia

The radioiron profile is seen in fig. 20 and shows no significant increase in radioactivity over marrow, spleen or liver. This indicates markedly reduced or absent marrow erythropoiesis and there is no evidence of extramedullary erythropoiesis. Lack of splenic erythropoiesis was confirmed by splenic puncture. Utilisation of the injected radioactive iron was 31% (normal 68 - 83%) and this low figure indicates a diminished amount of red cell production. The red cell survival as estimated by radiochromium was 16 days (fig.21) and this is evidence of a markedly increased rate of red cell destruction.

Thus, the anaemia in this patient is caused by a combination of marrow hypoplasia and an increased rate of haemolysis.

There is no splenic erythropoiesis.

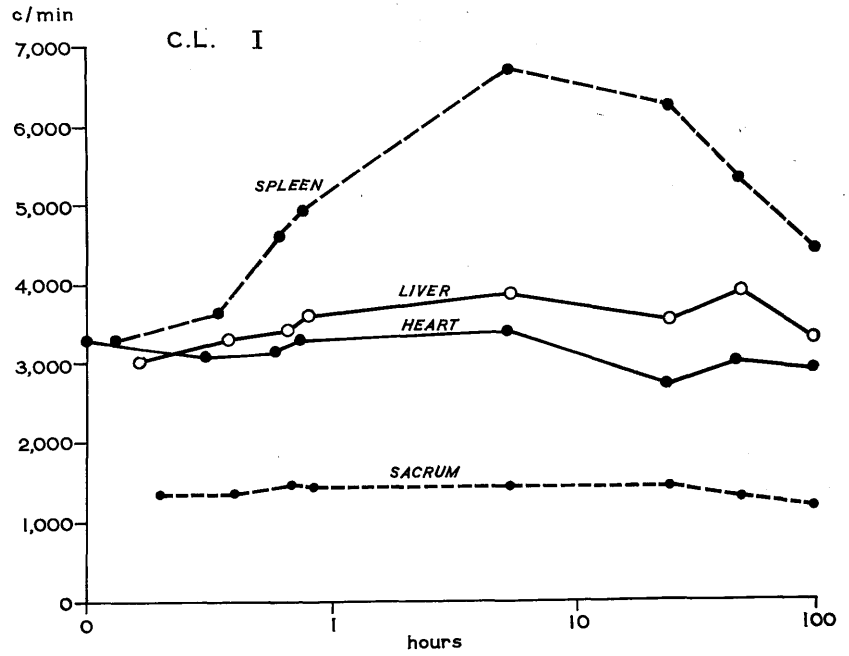

Fig. 22 Radioiron profile in patient C.L. (chronic myeloid leukaemia)

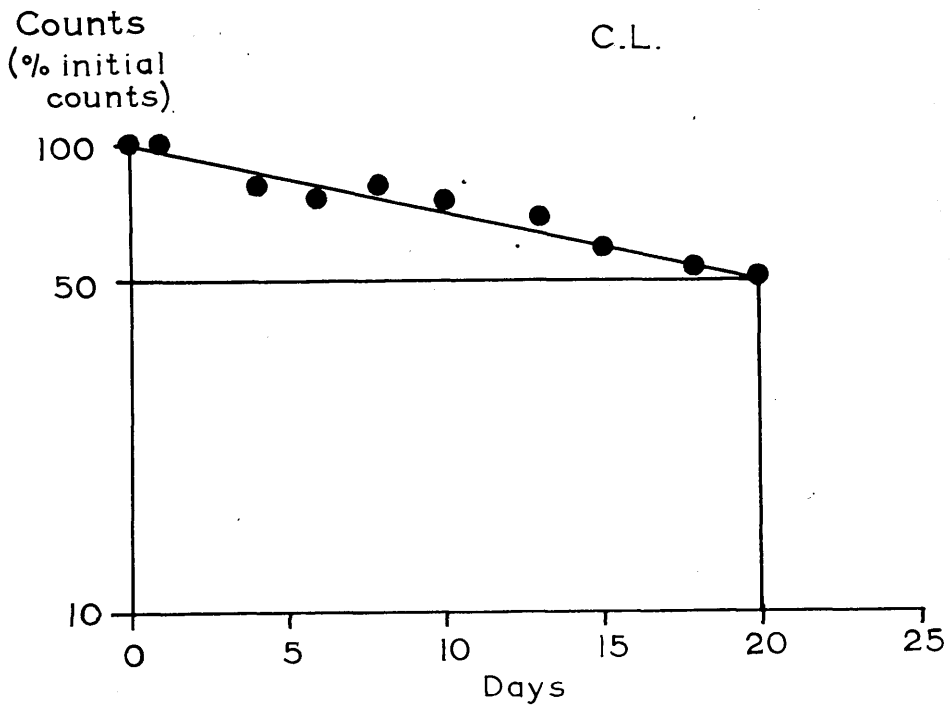

Fig.23 Red cell survival by radiochromium technique in patient C.L.

C.L. - Chronic Myeloid Leukaemia

The pattern of iron uptakes is seen in fig. 22. In this patient there is no significant alteration in marrow level but a marked increase of radioactivity is seen over the spleen. This evidence of splenic erythropoiesis was confirmed by splenic puncture. The iron utilisation was 73% and red cell half life was 20 days. (fig.23).

Thus the anaemia in this patient is related to marrow hypoplasia associated with increased haemolysis. The normal utilisation of iron occurs in the absence of iron deficiency and indicates that the total red cell production must be around normal. The spleen, therefore, must play an important part in erythropoiesis.

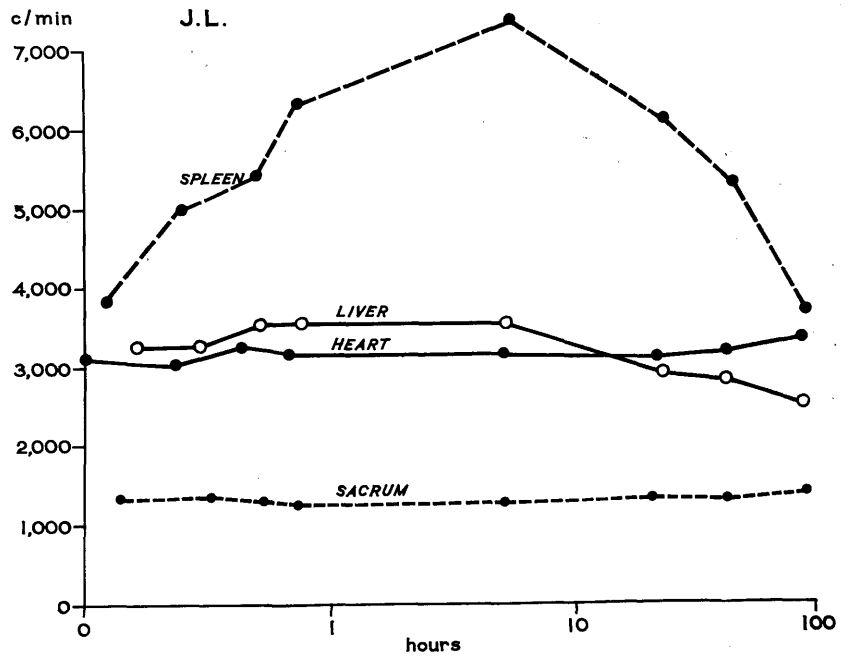

Fig. 24 Radioiron profile in patient J.L. (chronic myeloid leukaemia)

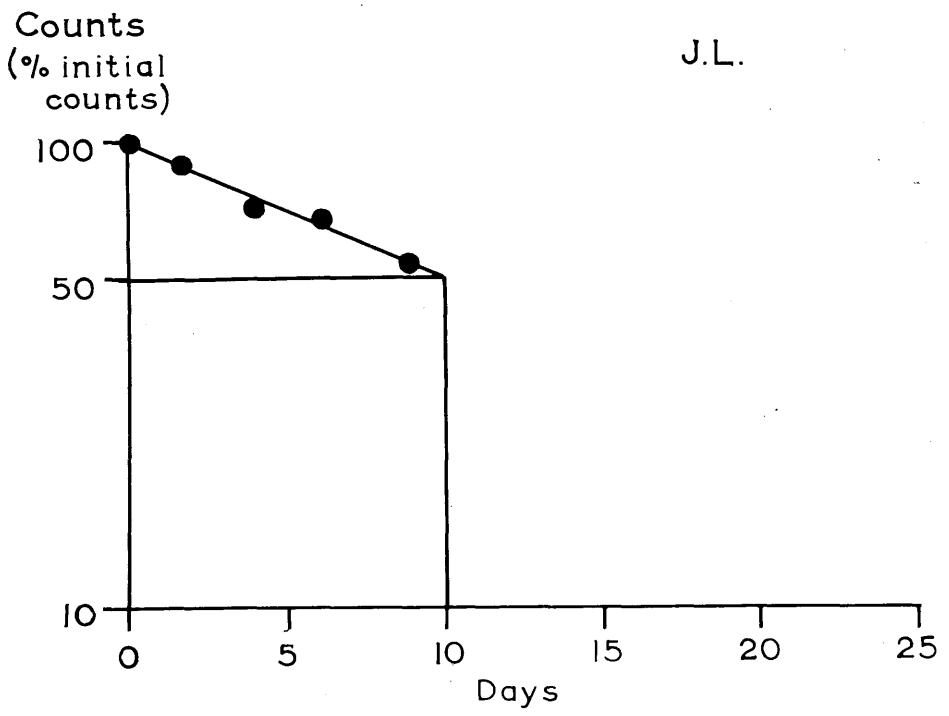

Fig. 25 Red cell survival by radiochromium technique in patient J.L.

J.L. - Chronic Myeloid Leukaemia

In this patient the radioiron profile indicates diminished marrow production of red cells but the spleen appears to be very active in erythropoiesis. (fig.24). The iron utilisation was very high (96%) but this is artificially high because of deficiency of iron, (serum iron 20ug, total iron binding capacity 450ug, haemoglobin 6.1 g%, M.C.H.C. 30.5%). The red cell half life was only ten days (fig.25).

Here the anaemia is due to marrow hypoplasia and a greatly increased rate of haemolysis. Extramedullary erythropoiesis occurs in the spleen, the iron utilisation is very high but in view of the iron deficiency, the total red cell production is not increased and is almost certainly reduced.

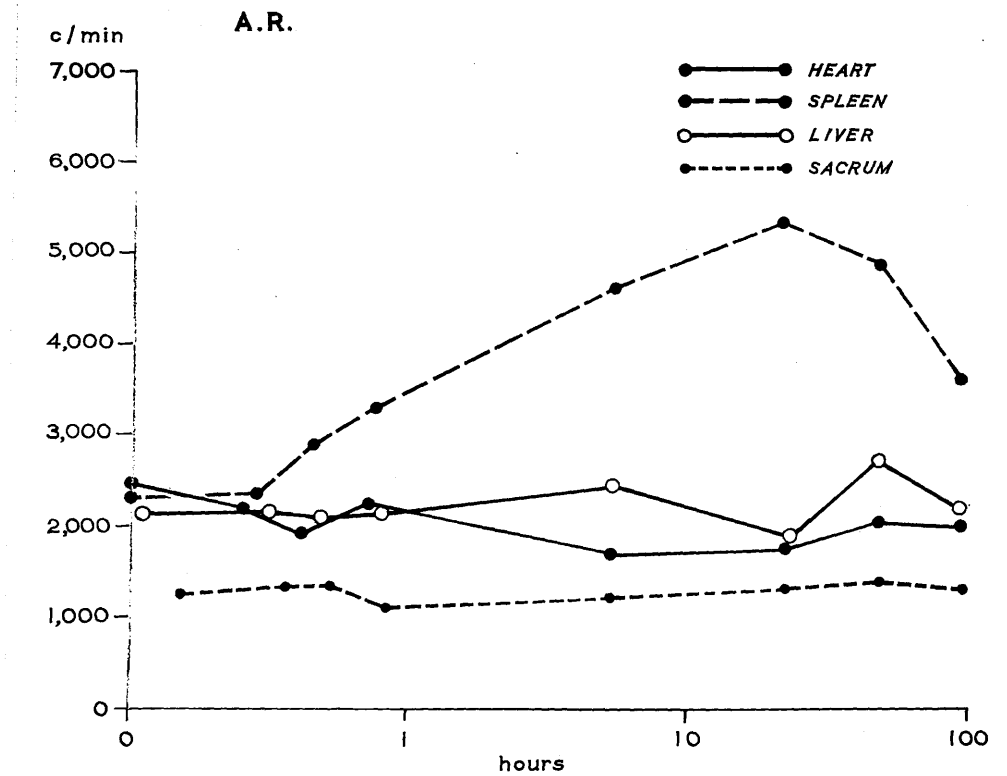

Fig. 26 Radioiron profile in patient A.R.
(chronic myeloid leukaemia)

A.R. - Chronic Myeloid Leukaemia

This patient unfortunately died of a cerebral vascular accident during investigation and consequently the only result is that of the pattern of radioiron uptakes (fig.26). The marrow uptake is not significant but the high splenic uptake indicates that the spleen had a major erythropoietic function.

Thus, the anaemia probably is due to marrow hypoplasia.

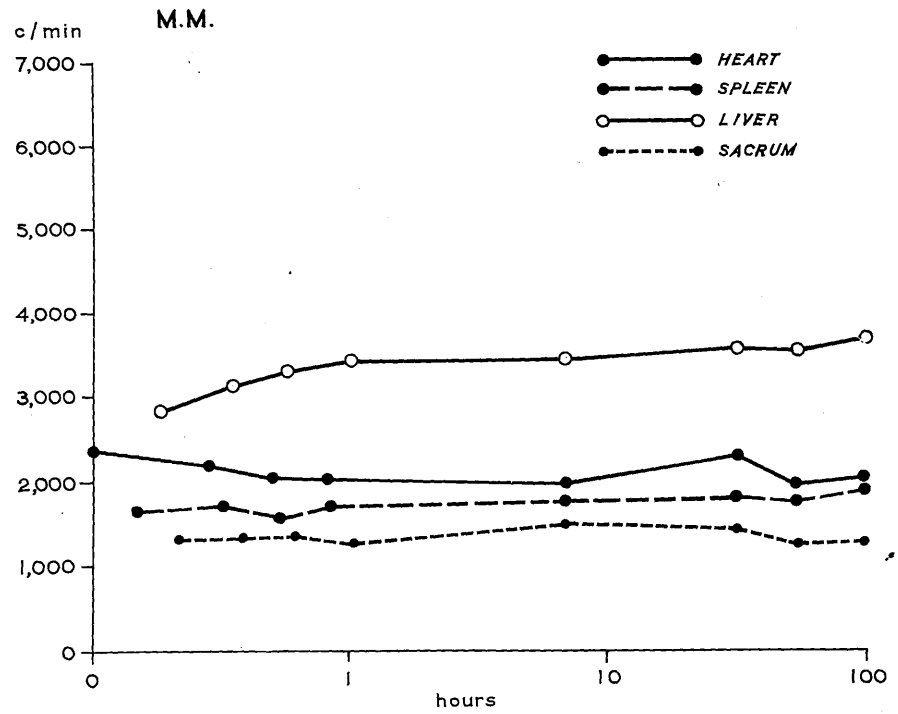

Fig. 27 Radioiron profile in patient M.M. (chronic myeloid leukaemia)

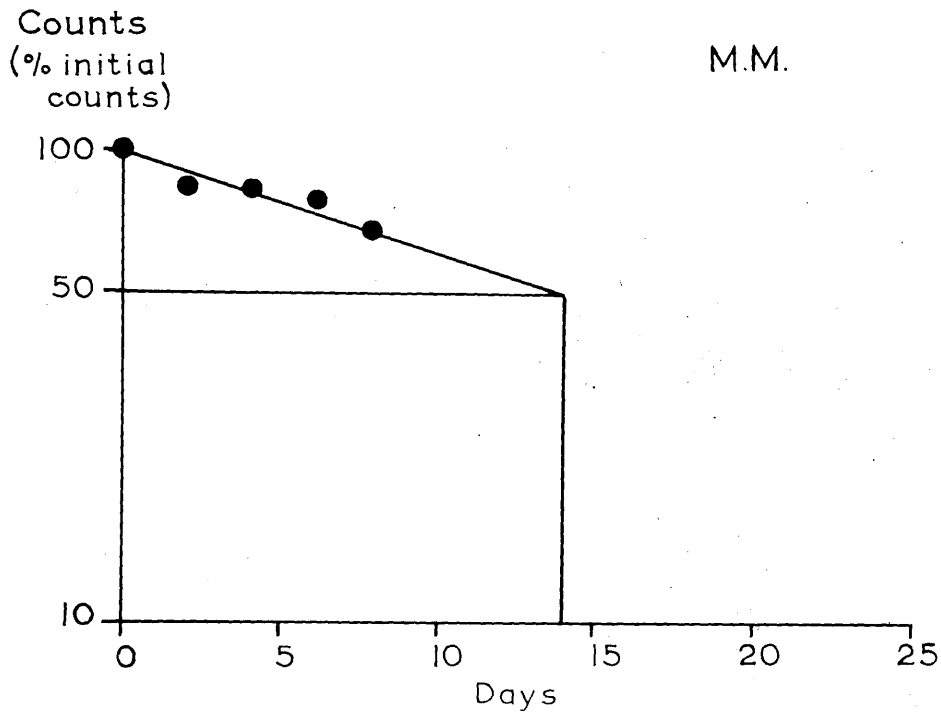

Fig. 28 Red cell survival by the radiochromium technique in patient M.M.

M.M. - Chronic Myeloid Leukaemia

The radioiron profile (fig.27) shows absence of any significant uptake in radioiron in any of the possible sites of erythropoiesis. The utilisation of the injected radioactive iron is very low (24%) and there is an increased rate of haemolysis ($T\frac{1}{2}$ = 14 days) (fig.28). In this patient there is marrow hypoplasia and no evidence of extramedullary erythropoiesis and the total amount of red cell production is greatly reduced. Increased haemolysis also appears to be a major factor in the causation of anaemia.

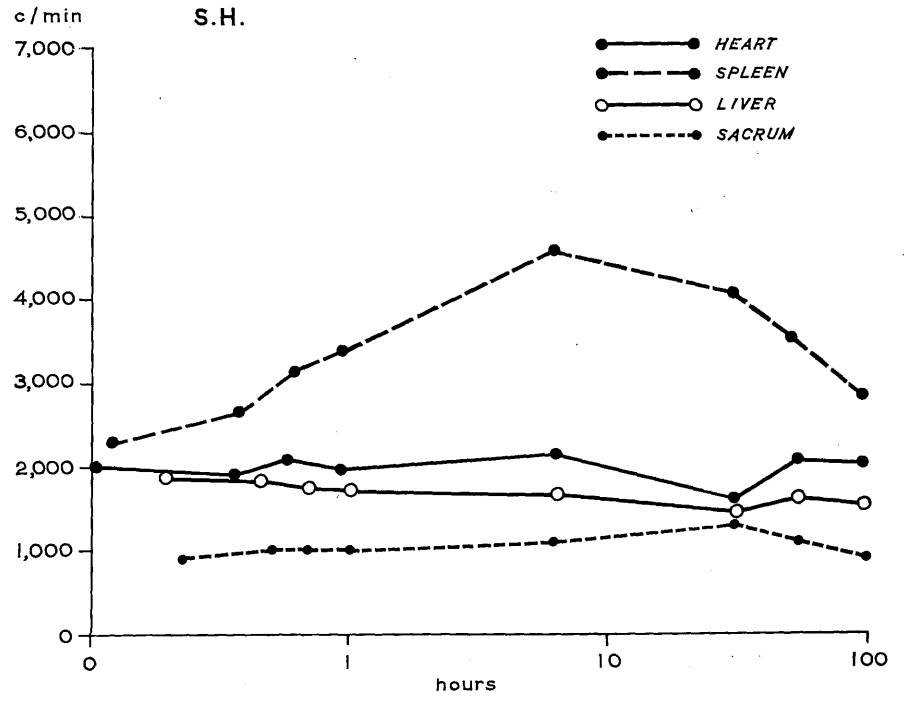

Fig. 29 Radioiron profile in patient S.H. (myelofibrosis). (reproduced by permission from 'Clinical Radiology')

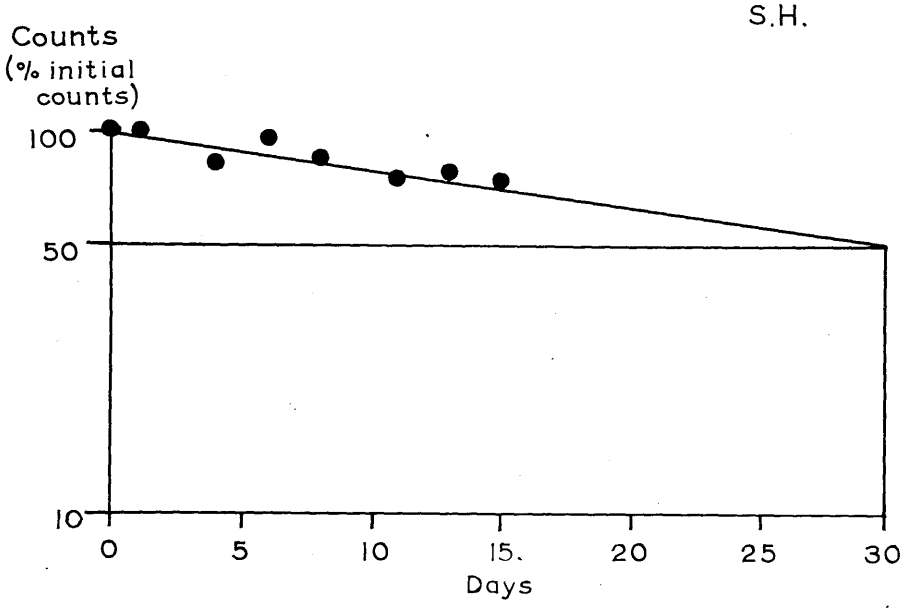

Fig. 30 Red cell survival by the radiochromium technique in patient S.H.

Myelofibrosis

S.H. - Myelofibrosis

The radioiron profile is seen in fig.29 and demonstrates marked increase in splenic radioactivity but no significant alteration in marrow counts. The iron utilisation was normal (80%) and the red cell survival time, $T\frac{1}{2}$, was also normal (30 days) (fig.30).

The anaemia is due to marrow hypoplasia and there is no haemolytic factor. There is marked splenic erythropoiesis, confirmed by splenic puncture and this is probably the main source of red cells.

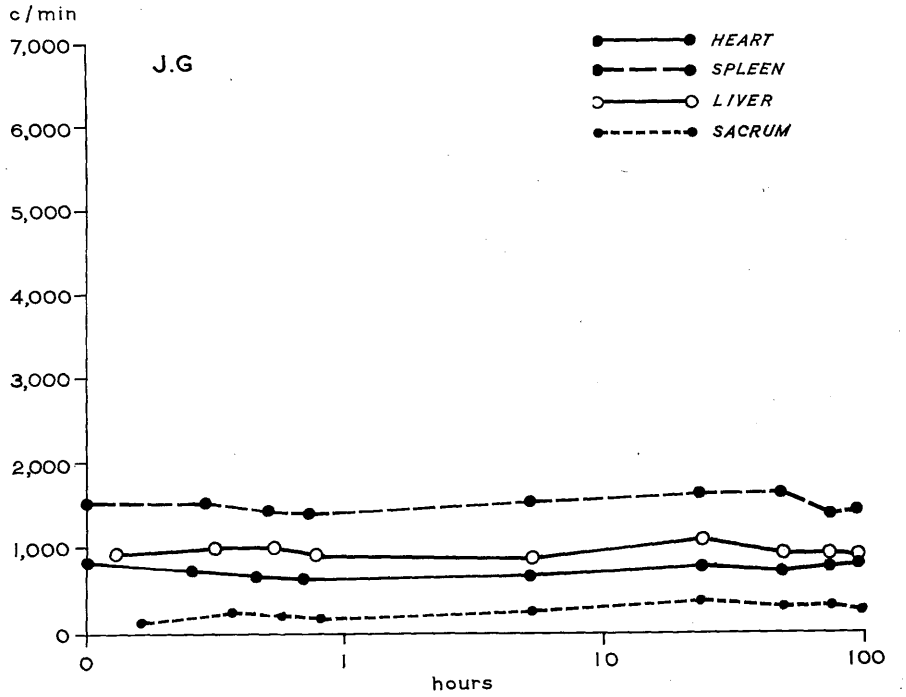

Fig. 31 Radioiron profile in patient J.G. (myelofibrosis)

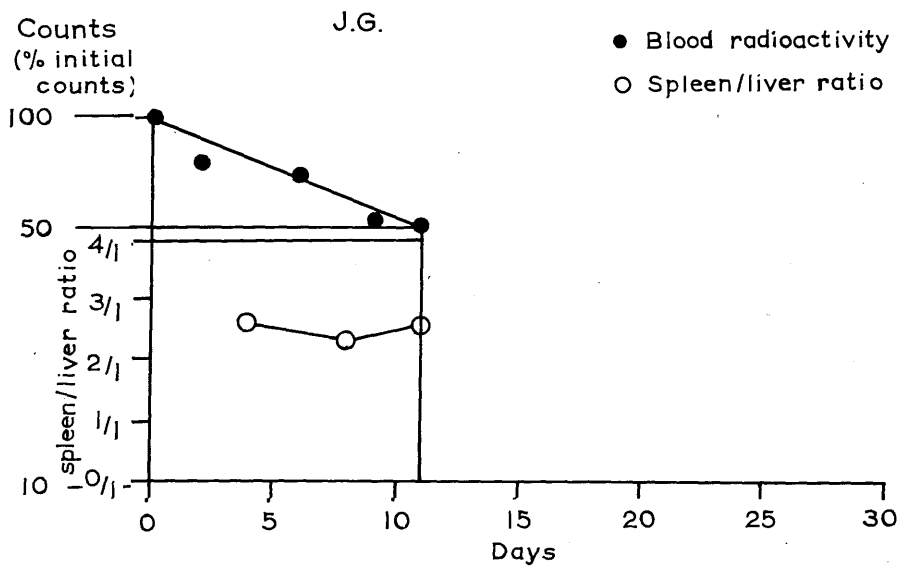

Fig. 32 Sequestration studies and red cell survival by the radiochromium technique in patient J.G.

J.G. - Myelofibrosis

The pattern of radioiron uptakes (fig.31) shows no significant alteration in the levels of radioactivity in any organ. Iron utilisation was low (33%). Red cell half life estimation was 17 days but at a later date was further reduced to 11 days at which time the spleen/liver ratio was 2.5/1. (fig.32).

Thus the anaemia is due to a combination of marrow hypoplasia and an increased rate of haemolysis. The spleen plays no significant part in blood production, confirmed by splenic puncture, but appears to be the site of red cell sequestration and destruction.

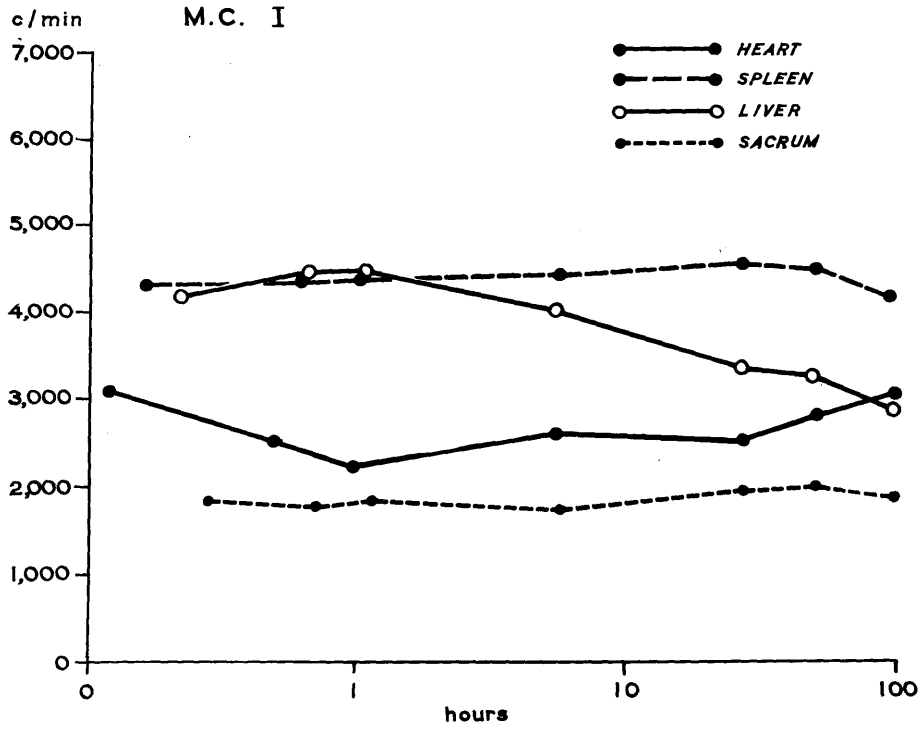

Fig. 33 Radioiron profile in patient M.C. (myelofibrosis)

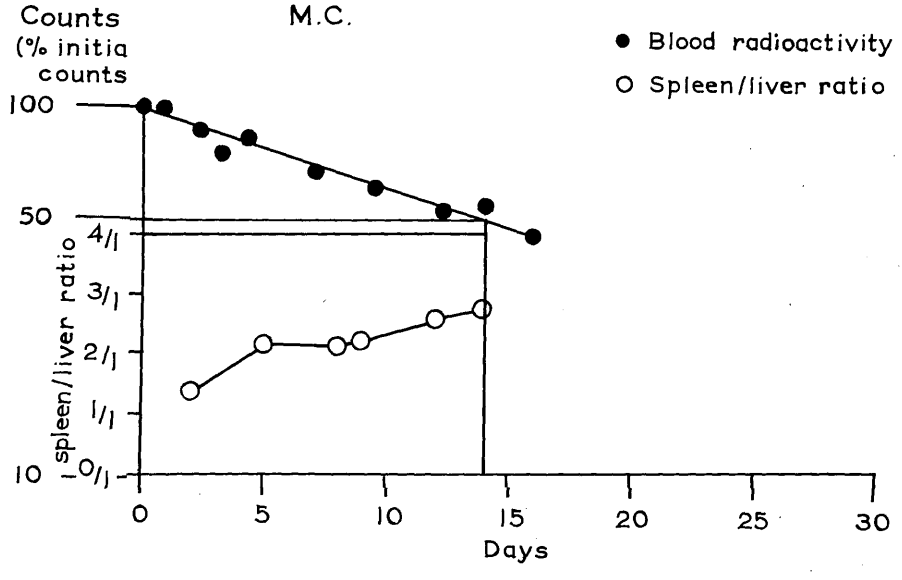

Fig. 34 Sequestration studies and red cell survival by the radiochromium technique in patient M.C.

M.C. - Myelofibrosis

The radioiron profile is seen in fig.33 and shows no significant uptake in any organ. The iron utilisation is 100% due to severe iron deficiency (serum iron 35ug, total iron binding capacity 235ug, haemoglobin 9.2 g%, M.C.H.C. 25.5%) Radiochromium studies showed $T\frac{1}{2}$ to be 13 days but at a later date it was 25 days and the spleen/liver ratio at this time was 2.65/1 (fig.34).

Thus the presenting anaemia is due to marrow hypoplasia and increased haemolysis. The spleen has no erythropoietic function but is later shown to be an organ of sequestration and destruction of blood.

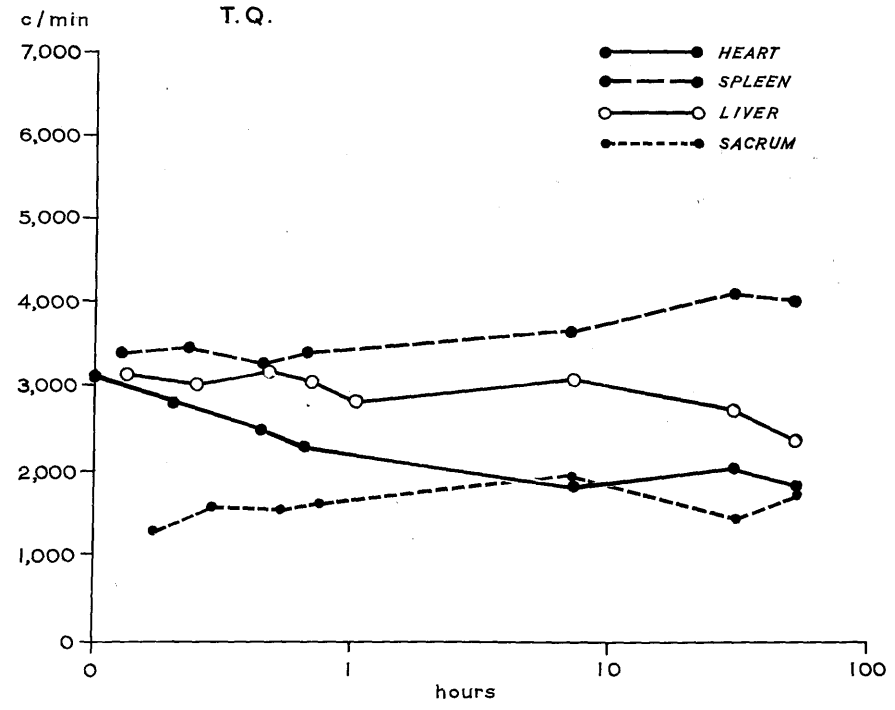

Fig. 35 Radioiron profile in patient T.Q. (myelofibrosis)

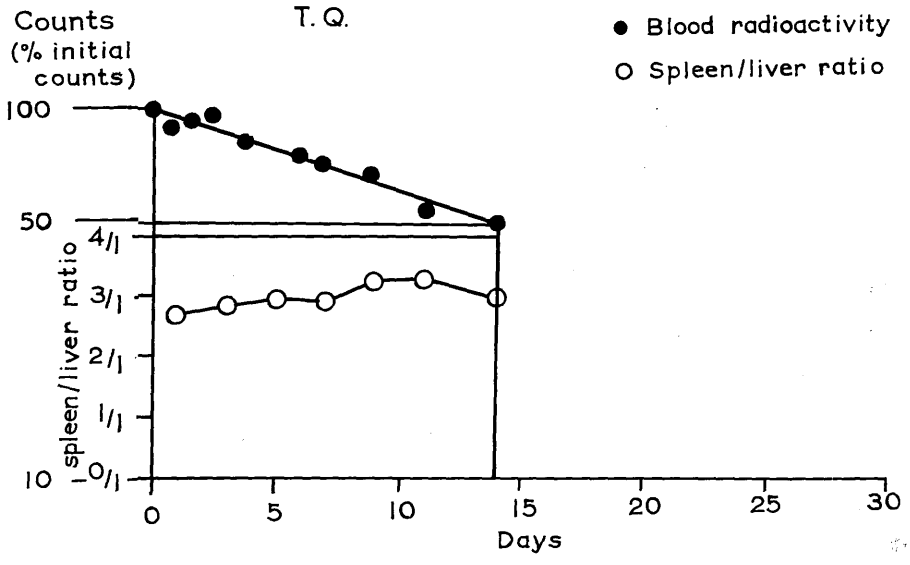

Fig. 36 Sequestration studies and red cell survival by the radiochromium technique in patient T.Q.

T.Q. - Myelofibrosis

In this patient the radioiron profile (fig.35) shows some increase in marrow and splenic uptakes of radioactive iron. The iron utilisation is only 42%. The radiochromium studies are seen in fig.36. Increased haemolysis is present ($T\frac{1}{2}$ = 14 days) and spleen/liver ratio at $T\frac{1}{2}$ is 3/1.

The anaemia is therefore due to a combination of hypoplasia and an increased rate of haemolysis. The marrow shows some sign of continuing activity but the total red cell production is obviously low as the iron utilisation is only 42%. The spleen shows evidence of erythropoiesis, though splenic puncture failed to reveal this, but it is mainly an organ of erythrocyte sequestration and destruction.

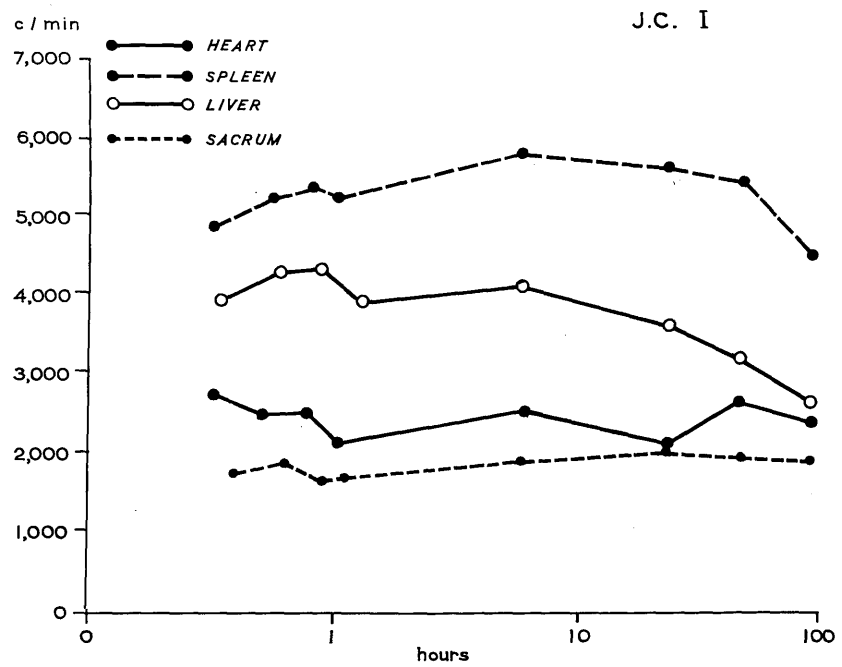

Fig. 37 Radioiron profile in patient J.C. (myelofibrosis)

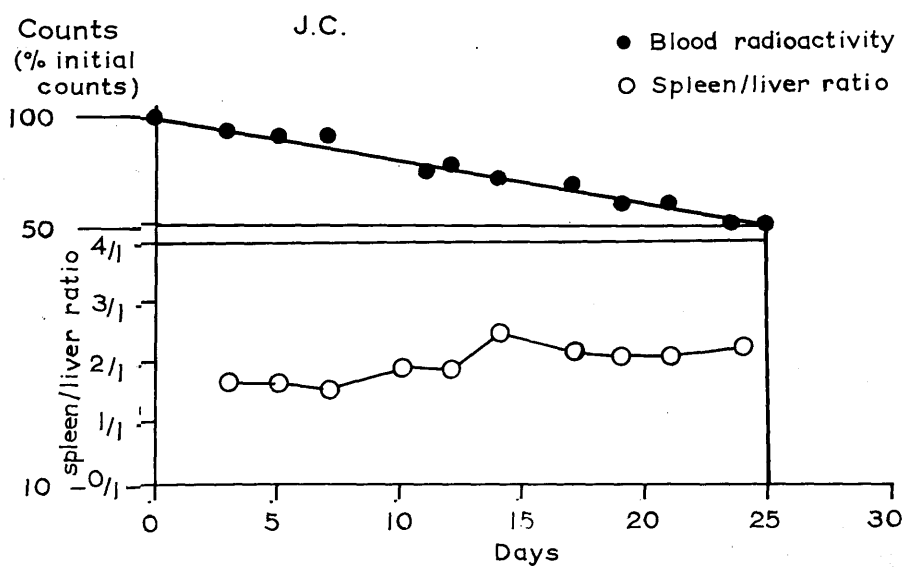

Fig. 38 Sequestration studies and red cell survival by the radiochromium technique in patient J.C.

J.C. - Myelofibrosis

The iron uptake patterns are demonstrated in fig.37. There is no increase in marrow radioactivity but an increase in splenic activity is seen. The iron utilisation is rather low at 55% and the red cell half life is 22 days. At a later date $T\frac{1}{2}$ was 25 days and the spleen/liver ratio was then 2.3/1 (fig.38).

These results indicate that the presenting anaemia is due to marrow hypoplasia but slightly increased haemolysis may be a minor contributory factor. The spleen has moderate erythropoietic activity but no significant sequestration is demonstrated.

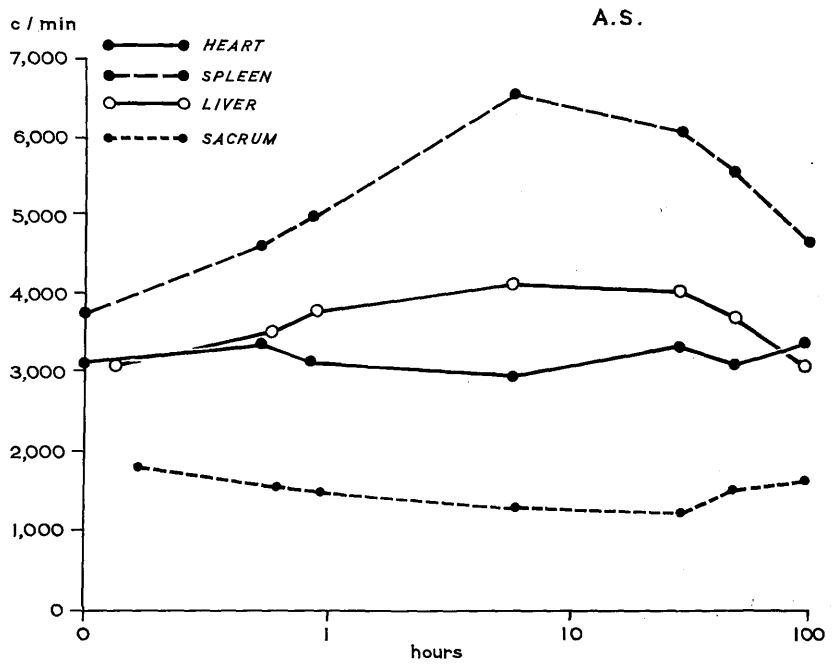

Fig. 39 Radioiron profile in patient A.S. (myelofibrosis)

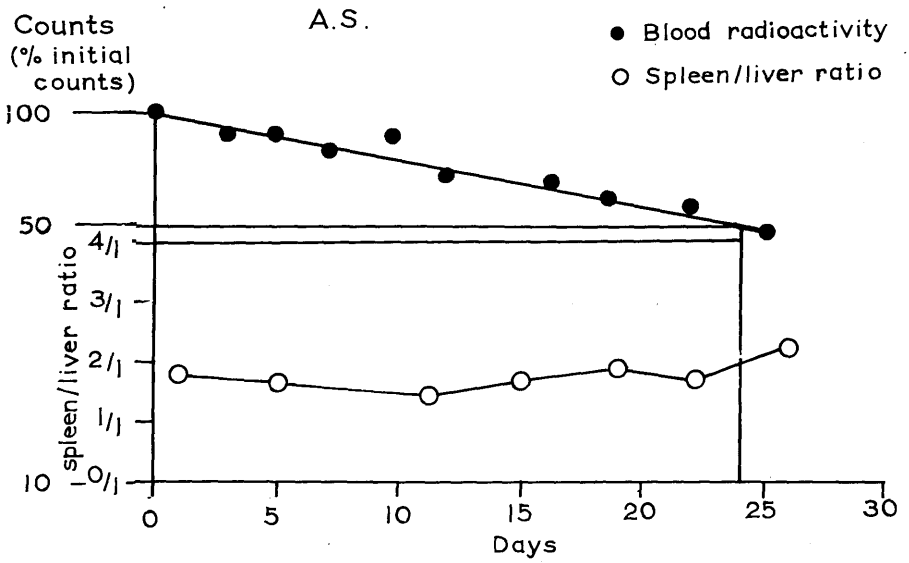

Fig. 40 Sequestration studies and red cell survival by the radiochromium technique in patient A.S.

A.S. - Myelofibrosis

The radioiron profile is shown in fig.39. There is marked increase in splenic radioactivity but no significant alteration in marrow counts. A normal utilisation of radioiron of 64% is present and $T\frac{1}{2}$ is virtually normal at 24 days. The spleen/liver ratio at $T\frac{1}{2}$ is 2/1 (fig.40).

The anaemia of this patient is due principally to marrow hypoplasia and there is no significant increase in the rate of haemolysis.

The spleen has a marked erythropoietic function and does not appear to sequester red cells.

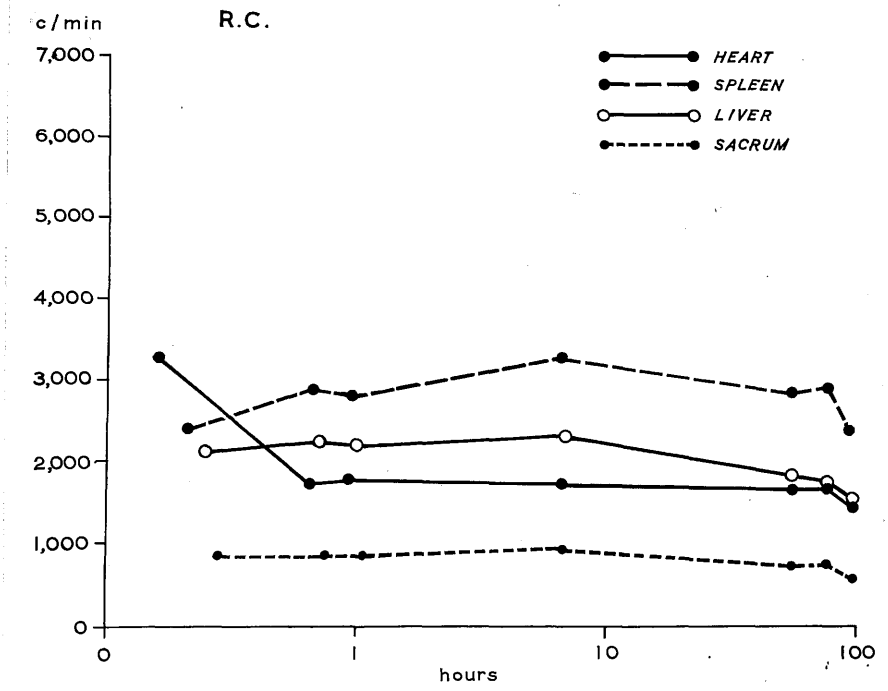

Fig. 41 Radioiron profile in patient R.C. (myelofibrosis)

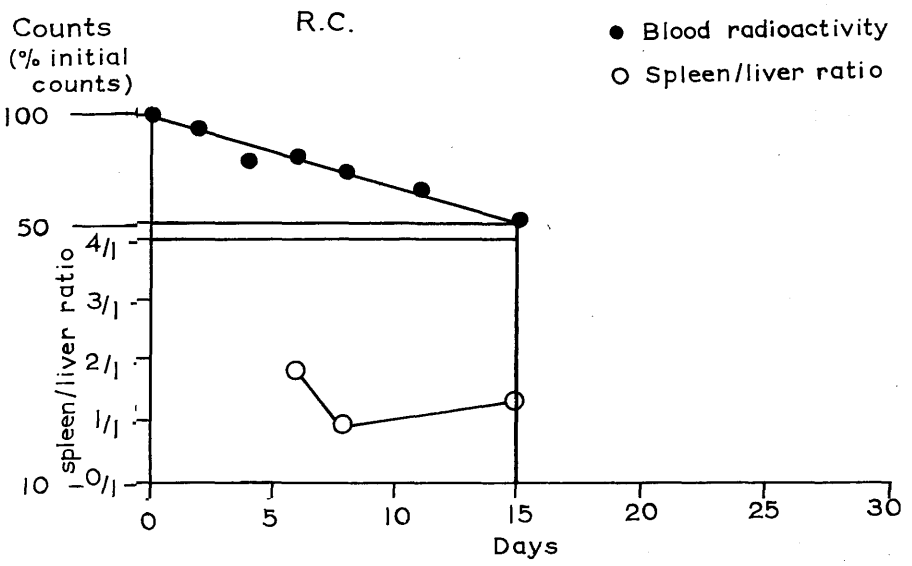

Fig. 42 Sequestration studies and red cell survival by the radiochromium technique in patient R.C.

R.C. - Myelofibrosis

The radioiron profile is seen in fig.41. There is no increase of marrow radioactivity but there is some uptake of radioactive iron in the spleen. The iron utilisation is rather low (53%) despite iron deficiency (serum iron 45ug, total iron binding capacity 480ug, haemoglobin 10.4 g%, M.C.H.C. 26%) and the red cell half life is 15 days which is considerably reduced. At $T\frac{1}{2}$ the spleen/liver ratio is 1.32/1 (fig.42).

Thus, the anaemia of this patient is due to a combination of hypoplasia and increased red cell destruction. The spleen has some erythropoietic function but is not an organ of sequestration of red cells.

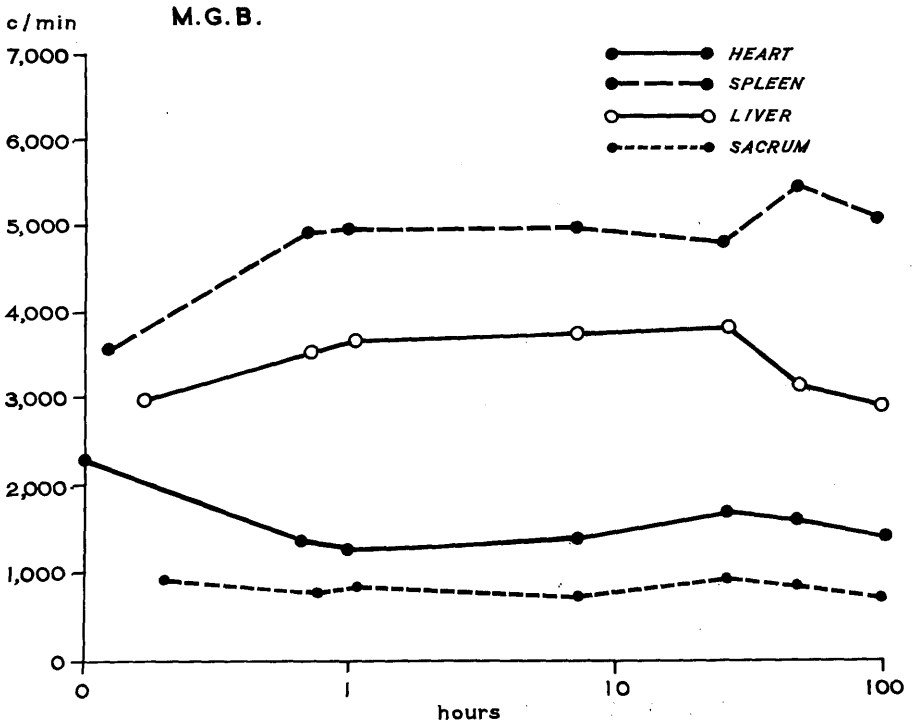

Fig. 43 Radioiron profile in patient M.G.B. (myelofibrosis)

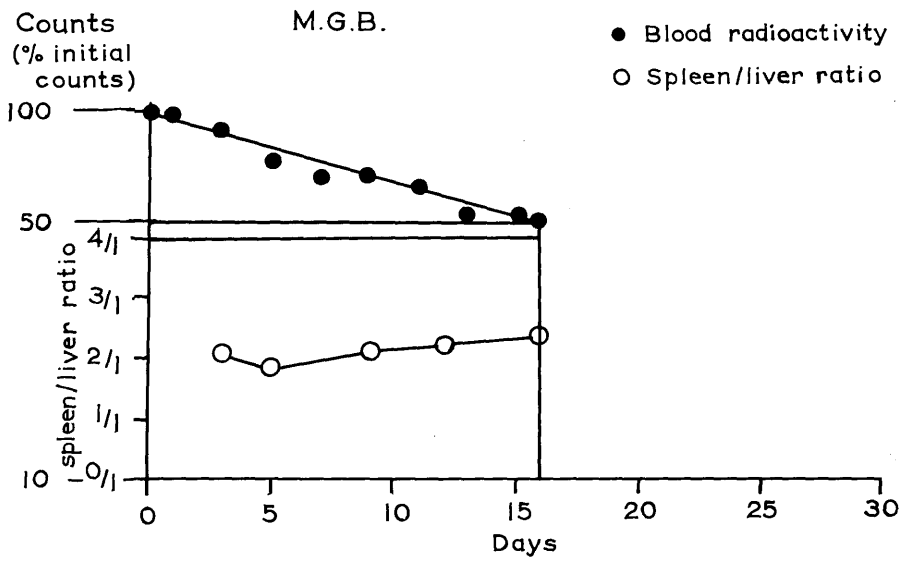

Fig. 44 Sequestration studies and red cell survival by the radiochromium technique in patient M.G.B.

M.G.B. - Myelofibrosis

The radioiron profile (fig.43) shows no significant increase in marrow radioactivity but there is a moderate rise of splenic radioactivity. The iron utilisation of only 21% is very low. Red cell survival is diminished ($T\frac{1}{2}$ = 16 days) and the spleen/liver ratio at $T\frac{1}{2}$ is 2.4/1 (fig.44).

The anaemia is due to hypoplasia and excessive haemolysis. In view of the very low iron utilisation the total red cell production must be greatly reduced. The spleen has an erythropoietic function and sequestration of red cells is not definitely present.

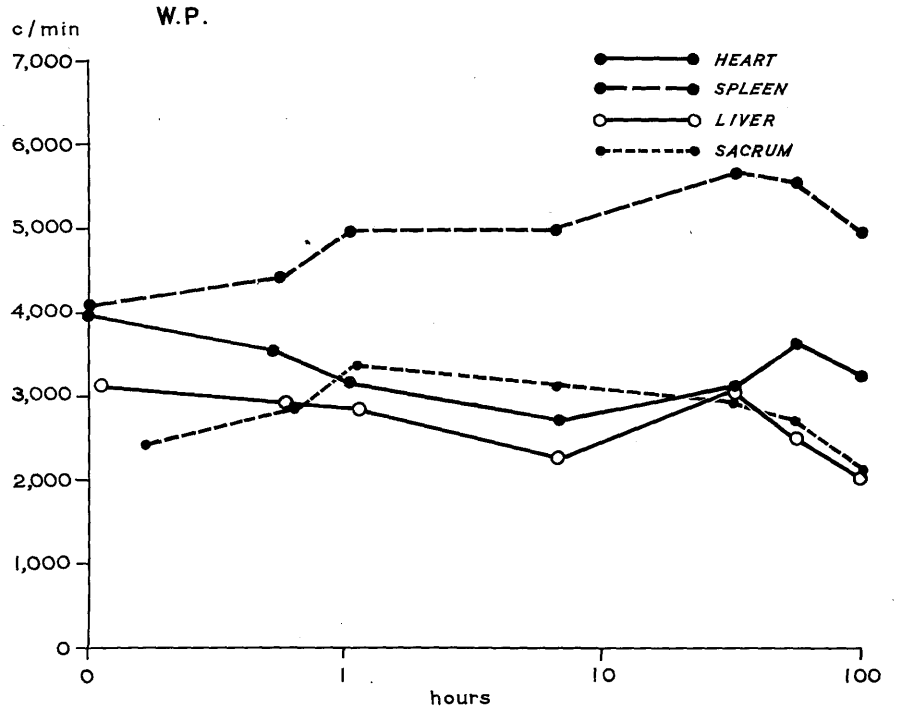

Fig. 45 Radioiron profile in patient W.P. (myelofibrosis)

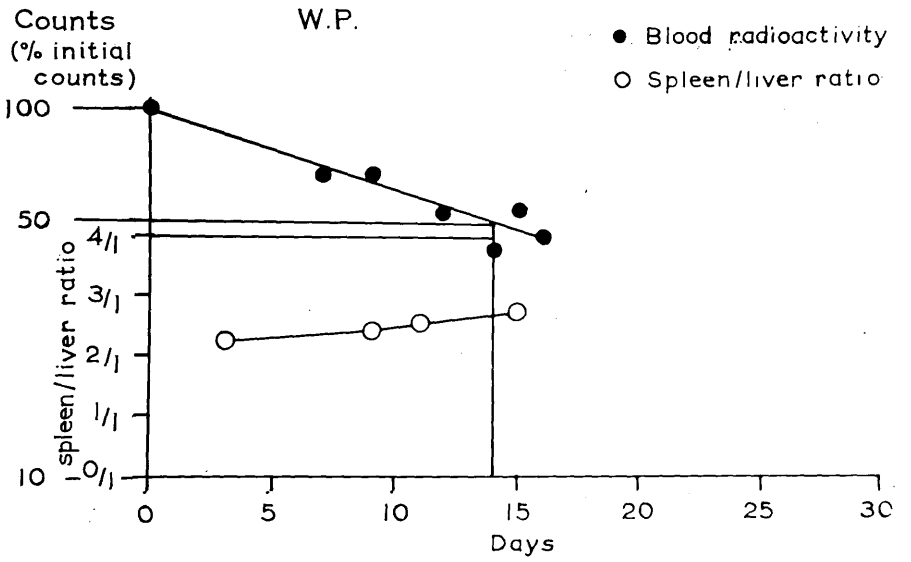

Fig. 46 Sequestration studies and red cell survival by the radiochromium technique in patient W.P.

W.P. - Myelofibrosis

The radioiron profile is seen in fig.45. There is a rise in radioactivity in the marrow and to a greater degree in the spleen. The iron utilisation is 63% and the red cell half life is 14 days. At $T\frac{1}{2}$ the spleen/liver ratio is 2.7/1 (fig.46).

Thus, a major factor in the production of anaemia in this patient is increased haemolysis. There is evidence of some continuing erythropoietic activity in the marrow and the iron utilisation is almost normal which indicates, in the absence of iron deficiency, that total red cell production cannot be greatly reduced and may be a minor factor only in the causation of anaemia.

The spleen has the dual function of production and destruction of red cells.

Patient	^{59}Fe uptakes		^{59}Fe Utilisation (%)	^{51}Cr $T\frac{1}{2}$ (days)
	Marrow	Spleen		
E.W.	0	0	31	16
J.L.	0	+++	96	10
C.L.	0	+++	73	20
A.R.	0	+++	-	-
M.M.	0	0	24	14

Table 7 Summary of Radioiron and Radiochromium studies in chronic myeloid leukaemia.

Discussion

Radioisotopic investigations have allowed a determination of erythrocyte survival and a study of the dynamics of erythropoiesis. The results obtained are a great advance on what could be inferred from routine haematological procedures and provide more detailed knowledge of the mechanisms of anaemia both in individual patients and in particular diseases.

A summary of the results in the chronic myeloid leukaemia group is seen in table 7. The lack of uptake of radioactive iron by the bone marrow is a feature of all five cases and this indicates that marrow erythropoietic activity is greatly reduced. It must be remembered that the utilisation of injected iron is dependent on the state of the iron stores as well as on the amount of red cell production. When iron deficiency is present or when erythropoiesis is greater than normal the utilisation of iron will be high. Therefore, if the iron utilisation is high and iron deficiency is present to account for this,

/the utilisation is no guide as to the amount of erythropoiesis. On the other hand if utilisation is high in the absence of iron deficiency then the amount of erythropoiesis must be greater than normal. When iron utilisation is low the state of iron stores is irrelevant as red cell production must be diminished. Thus the iron utilisation may be used as an indication of the total amount of red cell production provided the state of the iron stores is kept in mind. It cannot, however, be used in a quantitative manner, with any accuracy unless elaborate corrections are made.

It is of interest that the very low iron utilisation figures of 24 per cent and 31 per cent are seen in two patients with iron deficiency and no demonstrable activity in marrow or spleen. There is, therefore, in these patients greatly reduced red cell production. In the three other patients, marrow hypoplasia is again present but there is evidence of considerable splenic erythropoiesis. In one of these patients there is no iron deficiency

Patient	^{59}Fe Uptakes		^{59}Fe Utilisation (%)	Initial ^{51}Cr $T\frac{1}{2}$ (days)	^{51}Cr sequestration Spleen/liver ratio
	Marrow	Spleen			
J.C.	0	++	55	22	2.3 : 1
T.Q.	+	+	42	14	3 : 1
M.C.	0	0	100	13	2.65 : 1
S.H.	0	+++	80	30	-
A.S.	0	+++	64	24	2 : 1
J.G.	0	0	33	17	2.5 : 1
R.C.	0	+	53	15	1.32 : 1
M.G.B.	0	++	21	16	2.4 : 1
W.P.	+	++	63	14	2.7 : 1

Table 8 Summary of Radioiron and Radiochromium studies in Myelofibrosis

/yet the utilisation of iron is in the normal range and this is evidence that the spleen plays a major part in red cell production. Thus, splenic erythropoiesis may compensate (at least partly) for diminished marrow production.

The red cell survival times in the four patients so investigated demonstrate an increased rate of haemolysis, sometimes to a severe degree, in all. Thus, the anaemia of chronic myeloid leukaemia is due usually to a combination of marrow hypoplasia and an increased rate of haemolysis. Splenic erythropoiesis occurs in some patients with chronic myeloid leukaemia and may partly compensate for the marrow deficiency.

A summary of the results obtained in the nine patients with myelofibrosis is seen in table 8.

Marrow hypoplasia is present in seven of the nine patients but in the other two there is evidence of some continuing activity. The iron utilisation is low in approximately half

/of the patients in all of whom the total red cell production must be less than normal, and in the others it is probably less than normal. The spleen, though sometimes a major source of red cells, does not always take part in erythropoiesis.

An increased rate of haemolysis occurs in most of the patients with myelofibrosis and in some the spleen is the site of erythrocyte sequestration and destruction.

Thus the anaemia of myelofibrosis is due usually to a combination of marrow hypoplasia and an increased rate of haemolysis. Splenic erythropoiesis is not necessarily present in myelofibrosis but occurs in most cases and may help to compensate for the diminished marrow production. Sequestration and destruction of red cells may also occur in the spleen.

It is clear that the radioisotopic studies have provided real information about the basic mechanisms concerned with the production of anaemia. On comparison of the two groups of results there is seen to be no specific pattern

/of radioiron uptakes for either disease. Reduced marrow function occurs in all cases, though in two patients with myelofibrosis there is evidence of some continuing erythropoiesis, and the anaemia of both diseases appears to be due usually in part to hypoplasia and in part to increased haemolysis, either of which may be the dominant factor. The spleen may be the seat of erythropoiesis in either disease and this may compensate to some extent the reduced marrow production.

While the evidence of splenic erythropoiesis as derived from histological examination of splenic aspirate agrees well with that of splenic uptake of radioactive iron, the more accurate estimate of the amount of red cell production is undoubtedly that of radioiron. Histological study of splenic aspirate demonstrates the approximate proportion of red cell precursors in the spleen but there is no indication of the proliferative activity of these cells or of their release from the site of origin. The radioactive iron, however,

/travels to the sites of red cell formation and is incorporated in haemoglobin in the newly formed red cells. The accumulation of radioactive iron in an organ indicates that it is the site of erythropoiesis and thereafter the rise of blood radioactivity due to cells containing the radioisotope proves that there is no failure of release of the cells from their site of origin. This is, therefore, a more accurate estimate of the functional and effective erythropoiesis.

On the basis of the radioiron results splenic erythropoiesis is present to a moderate or marked degree in three of the patients with chronic myeloid leukaemia and five of those with myelofibrosis. Rosenthal (1950) suggested that the immature cells in the blood are derived from the sites of extramedullary erythropoiesis because these sites lack the regulatory release mechanism of the bone marrow. If this is true then there should be some correlation between the degree of splenic erythropoiesis and the numbers of immature cells in the blood. This

Patient	^{59}Fe Splenic Uptake	Nucleated red cells (per c.mm.)	Reticulocytes (per c.mm.)
M.M.	0	5.250	<76,000
J.L.	+++	1,700	147,000
C.L.	+++	1,500	140,000
E.W.	0	5,400	75,000
A.R.	+++	3,000	130,000
M.C.	0	900	196,000
J.C.	++	2,555	144,000
J.G.	0	420	330,000
R.C.	+	464	82,000
T.Q.	+	135	286,000
M.G.B.	++	340	52,000
S.H.	+++	0	<80,000
W.P.	++	357	704,000
A.S.	+++	250	<84,000

Table 9 A comparison of ^{59}Fe splenic uptake and nucleated red cell and reticulocyte counts in chronic myeloid leukaemia and myelofibrosis.

/correlation is absent both in chronic myeloid leukaemia and in myelofibrosis when the radioiron result is compared with the numbers of circulating nucleated red cells with or without reticulocytes (Table 9). Thus it seems unlikely that the immature cells are derived solely from the extramedullary sites of erythropoiesis. It is more probable that their occurrence in the blood is related entirely to the efforts of all sites of haemopoiesis, including those in the bone marrow, to compensate for the reduced marrow cavity and the consequent anaemia, by releasing cells before they are fully mature.

In myelofibrosis the spleen is, in some cases, a site of erythropoiesis but in seven patients there is also increased haemolysis. The role of the spleen as an organ of erythrocyte destruction has yet to be considered. The sequestration studies show accumulation of radiochromium in the spleen to a level indicative of splenic sequestration, and destruction of labelled red cells in about half of those cases

/with an increased rate of haemolysis. Thus, the spleen in myelofibrosis may act either as an organ of red cell production or as an organ of red cell destruction. In some of the patients with splenic sequestration there was also splenic erythropoiesis indicating that in some patients the spleen may act in both capacities at the same time.

Donhauser (1908) first suggested that blood formation occurred in the spleen as a compensatory mechanism and Marson and Meynell in 1952 stated that it was then generally accepted that splenomegaly was compensatory to marrow involvement. These radioisotopic studies show that extramedullary erythropoiesis may compensate partly for diminished blood formation in the marrow: but as splenic erythropoiesis is absent in some patients who have marked splenomegaly and severe marrow cavity obliteration, it cannot be simply compensatory to marrow involvement.

Summary

The radioactive iron and chromium studies carried out in the patients with chronic myeloid leukaemia and those with myelofibrosis provide information on the mechanisms of anaemia in these diseases.

The radioiron studies demonstrate that the same pattern of iron uptakes occur in both diseases. Splenic erythropoiesis may be present in either condition and the studies do not substantiate the suggestion that the spleen in myelofibrosis is merely an organ of compensatory erythropoiesis.

The basic mechanisms of anaemia are the same in each disease, namely, a combination of hypoplasia and increased haemolysis.

Although the spleen in myelofibrosis may act as a source of red cells it is sometimes seen to act as an organ of destruction and these two functions may co-exist.

Chapter 6

'INTERMEDIATE' CASES

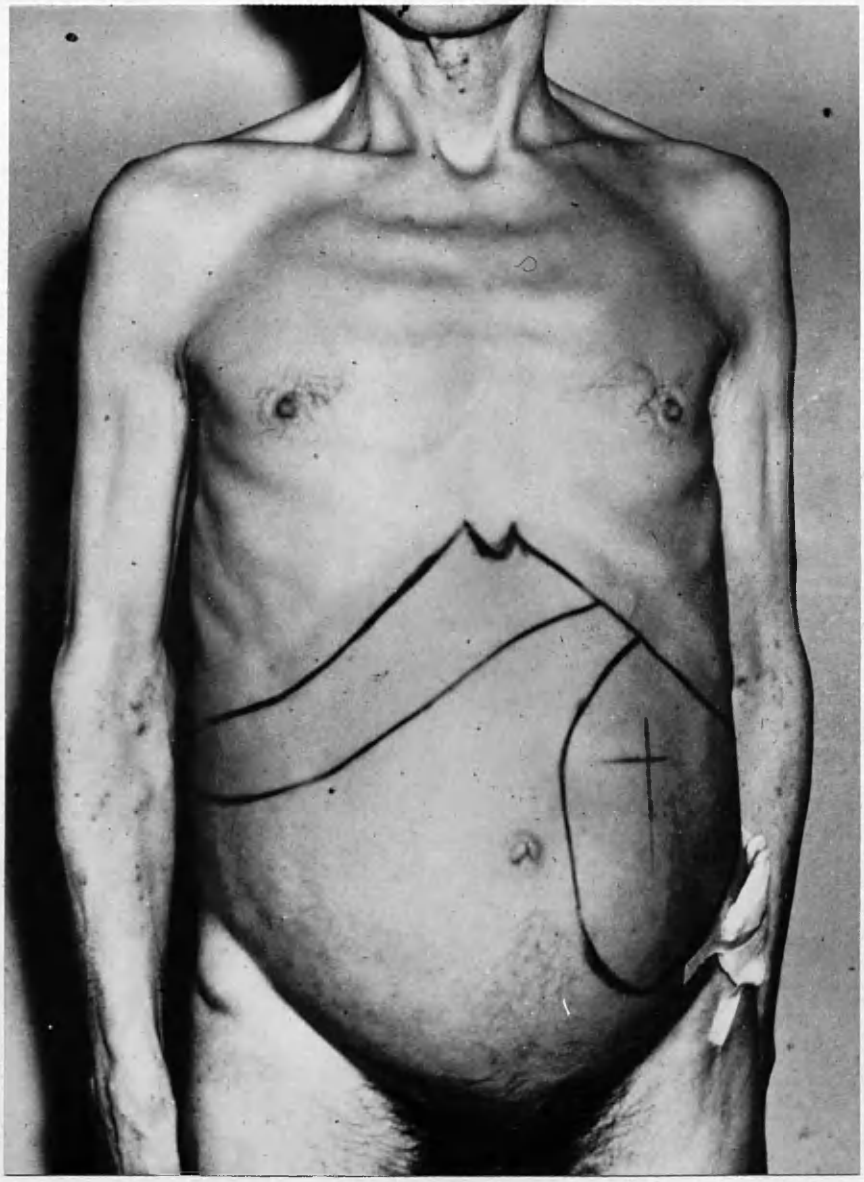

Fig. 47 Hepatomegaly and grade II splenomegaly in patient W.M.

Dameshek (1951), in supporting the theory of the existence of an aetiological relationship of the myeloproliferative disorders, emphasises the occurrence of many 'transition' forms. By this, he implies that while each disease may have a specific pattern or syndrome whereby it can be identified, there may be such variation in the manifestations of the disease that many cases cannot be satisfactorily classified. Leonard, Israels and Wilkinson (1957) oppose this view and state that if 'transition' forms occur at all commonly then constant patterns for each accepted disease would not be expected. This opinion is based mainly on the finding of constant biochemical patterns in each disease but this, of course, is only one aspect of the problem. If transition forms do occur, some of their features should be identical with those of one recognised disease and some with those of another. An answer to the question of relationship of the myeloproliferative disorders may well depend on the existence of these "linking" forms, whose importance, therefore,

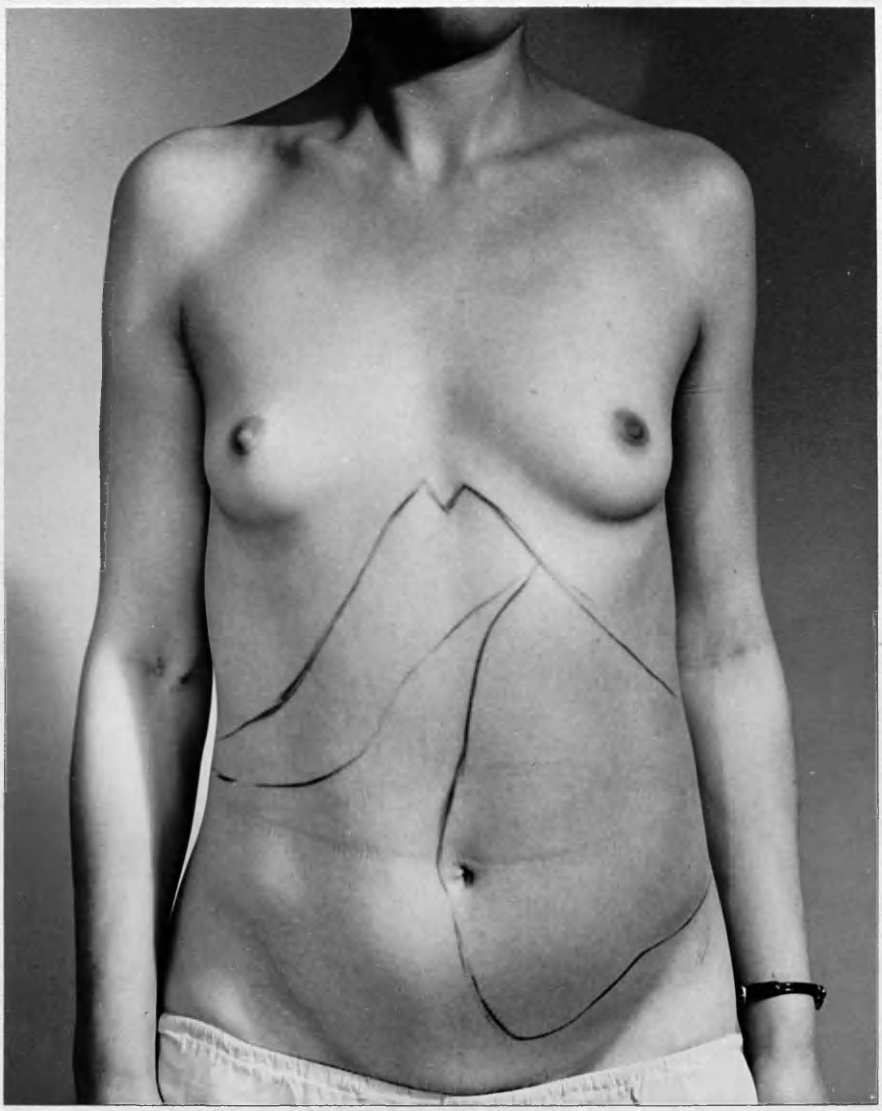

Fig. 48 Hepatomegaly and grade III splenomegaly in patient M.B.

/cannot be overemphasized.

The clinical, haematological and radio-isotopic comparisons of chronic myeloid leukaemia and myelofibrosis have been made and two patients apparently 'intermediate' between these two diseases will now be discussed under similar headings.

Clinical Picture

One patient, W.M., was a male of 57 years of age and the other, M.B., a female of 33 years of age, at the time of diagnosis.

Patient W.M. when first seen complained of breathlessness on exertion, ankle swelling, abdominal discomfort and tiredness of about four to five months duration. He had observed also some weight loss and increasing pallor. A few small lymph nodes were palpable and he had hepatomegaly and grade 2 splenomegaly (fig. 47). Some brown pigmentation of the backs of his hands and around his ankles was also noted.

Patient M.B., presented with breathlessness on exertion, ankle swelling and abdominal discomfort of two to three months duration. She

Patient	Hb G %	W.B.C. per c.mm.	Pl per c.mm.	Retics %	Marrow puncture	Marrow trephine
W.M.	5.0	140,000	445,000	6	dry tap	myeloid hyperplasia reticulum + no fibrosis
M.B.	8.1	196,000	350,000	3	dry tap	myeloid hyperplasia megakaryocytes + reticulum + no fibrosis

Table 10 The principal haematological findings of two 'Intermediate' cases.

/had noted also excessive and severe menstrual loss for the previous year and on examination was found to have a few palpable lymph nodes, hepatomegaly and grade 3 splenomegaly (fig.48).

Thus the clinical features of these patients are consistent with a diagnosis of either chronic myeloid leukaemia or myelofibrosis.

Haematological Investigations

Both patients were anaemic, one with associated iron deficiency, and both had elevated white cell counts. It is of interest that the counts of 140,000 cells per c.mm. and 196,000 cells per c.mm. are well above the usual range for myelofibrosis although such high counts have been reported (Korst et al, 1956). They are not however, of the very high levels characteristically associated with chronic myeloid leukaemia. The blood films of W.M. showed no abnormality of the mature red cells but those of M.B. showed some anisocytosis and poikilocytosis, and typical 'tear drop' cells were not uncommon. Platelet counts in

Patient	W.B.C. per c.mm.	Neutrophils %	Eosinophils %	Basophils %	Lymphocytes %	Monocytes %	Myeloblasts %	Myelocytes %	Metamyelocytes %	Nucleated Reds per 100 W.B.C.
W.M.	140,000	25	7.5	10	6	2	10.5	24.5	14.5	11
M.B.	196,000	63	0	12	2	2	1	9	11	4

Table 11 Differential white cell counts in 2 'Intermediate' cases

/both patients were higher than normal. The principal haematological findings are summarised in table 10 and the differential white cell counts in table 11. In one patient (W.M.) neutrophil polymorphonuclear leucocytes accounted for only 25 per cent of the total white cells but eosinophil and basophil leucocytes were both increased and immature white cells totalled 49.5 per cent including 10.5 per cent 'blast' cells. Nucleated red cells were present in the proportion of 11 per 100 white cells. The other patient (M.B.) had 21 per cent primitive white cells but only 1 per cent 'blast' cells; nucleated red cells were seen in the proportion of 4 per 100 white cells.

Thus, in one patient there is a blood picture rather like chronic myeloid leukaemia, apart from the high proportion of immature red cells and the only moderately raised white cell count, and in the other a picture compatible with myelofibrosis except for the very high white cell count.

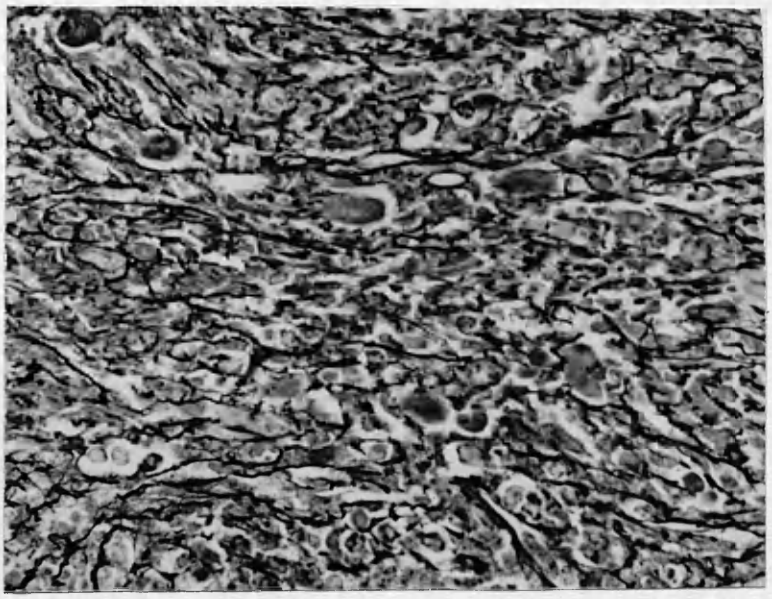

Fig. 49 Iliac crest marrow showing an increase of reticulin in 'intermediate' disease (patient W.M.). (x 400).

/ Marrow puncture on each patient, despite repeated attempts at various sites, yielded, like myelofibrosis, a 'dry tap'. Marrow trephine biopsy was therefore carried out and in each case showed a marked degree of myeloid hyperplasia and a definite increase in reticulum (fig.49) but no fibrosis. One patient (W.M.) also had some increase in megakaryocytes in the marrow.

Thus, these two patients had anaemia and elevated platelet counts compatible with either diagnosis. The white cell counts were much higher than is usual in myelofibrosis but in one patient the differential white cell count was more in keeping with the diagnosis of myelofibrosis yet this was the patient with the higher white cell count (196,000 per c.mm.). Repeated marrow punctures, as in myelofibrosis, yielded 'dry taps' but marrow trephine showed myeloid hyperplasia but a marked increase in reticulum.

It seems that these two patients have some features of chronic myeloid leukaemia and others

Patient	^{59}Fe uptakes		^{59}Fe utilisation (%)	$T\frac{1}{2}$ (^{51}Cr) (days)	^{51}Cr Spleen/Liver
	Marrow	Spleen			
W.M.	0	+ +	16	20	2.3/1
M.B.	0	+ +	97	12	-

Table 12 Summary of results of radioisotopic investigations in 2 'Intermediate' cases.

/of myelofibrosis; as judged by the criteria used for defining chronic myeloid leukaemia and myelofibrosis, they must be classed as 'intermediate' as each set of criteria is satisfied only partially.

The radioactive isotope investigations previously described were carried out on these two patients and are summarised in table 12.

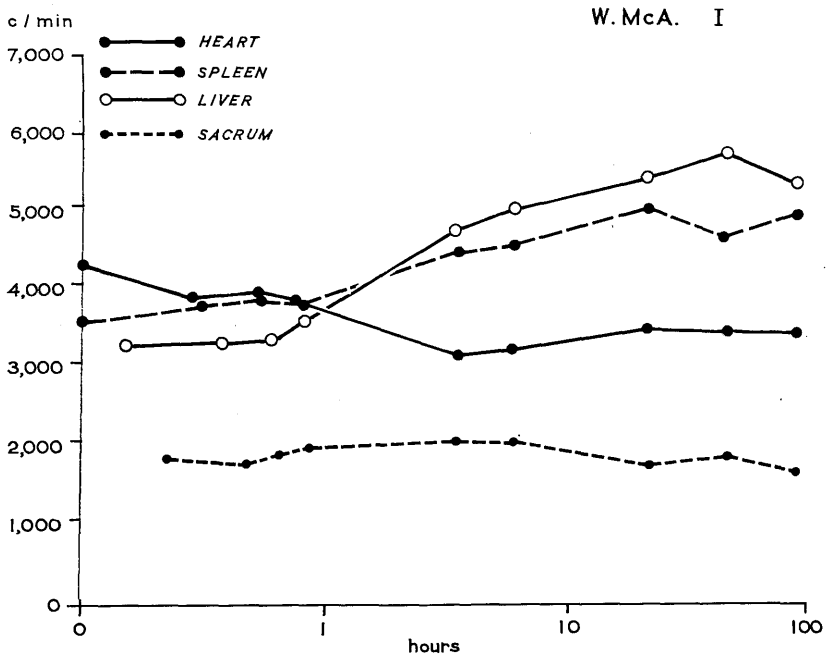

Fig. 50 Radioiron profile in patient W.M. ('intermediate' disease)

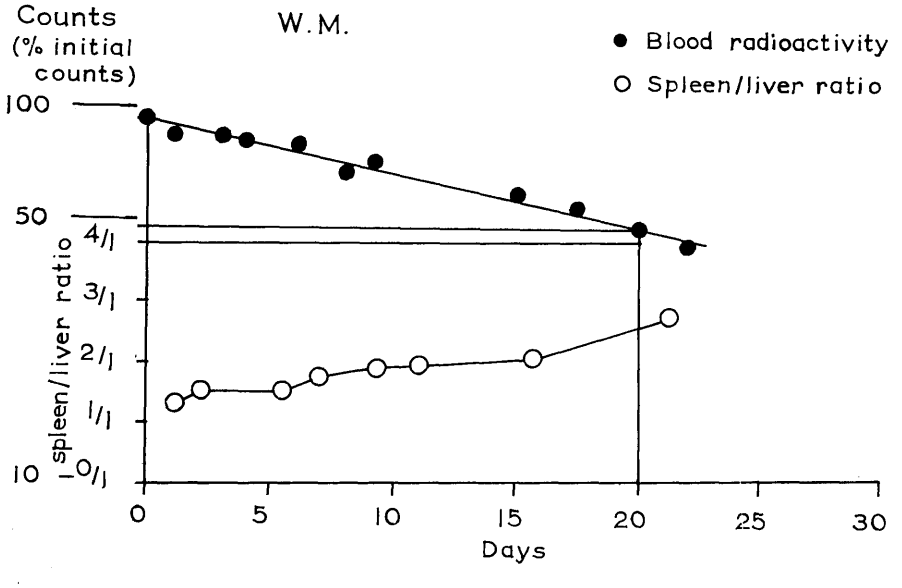

Fig. 51 Sequestration studies and red cell survival by the radiochromium technique in patient W.M.

W.M. - 'intermediate' disease

The radioiron profile is seen in fig.50. In this patient the marrow radioactivity does not rise significantly but there is a considerable increase in the counts over the spleen indicating a moderate degree of splenic erythropoiesis. The iron utilisation is low, only 16 per cent, and there must be, therefore, a greatly reduced total red cell production. The red cell survival time is reduced to 20 days, indicating an increase in the rate of haemolysis, and the spleen/liver ratio at this time is 2.3/1 (fig.51) which does not indicate significant sequestration of erythrocytes.

Thus the anaemia of this patient is due to greatly reduced red cell production combined with an increased rate of haemolysis. The spleen has an erythropoietic function and appears not to sequester red cells.

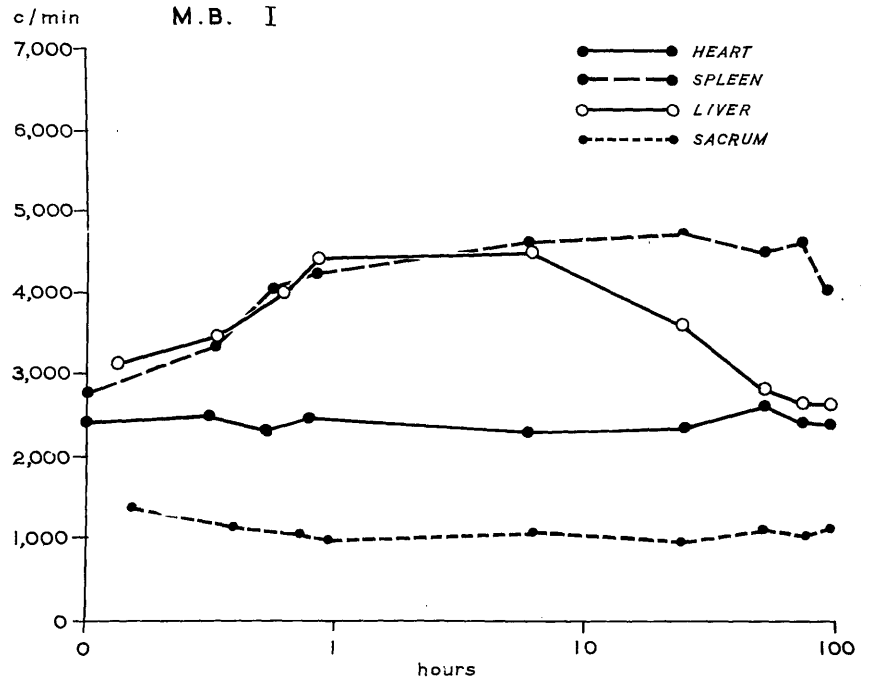

Fig. 52 Radioiron profile in patient M.B. ('intermediate' disease)

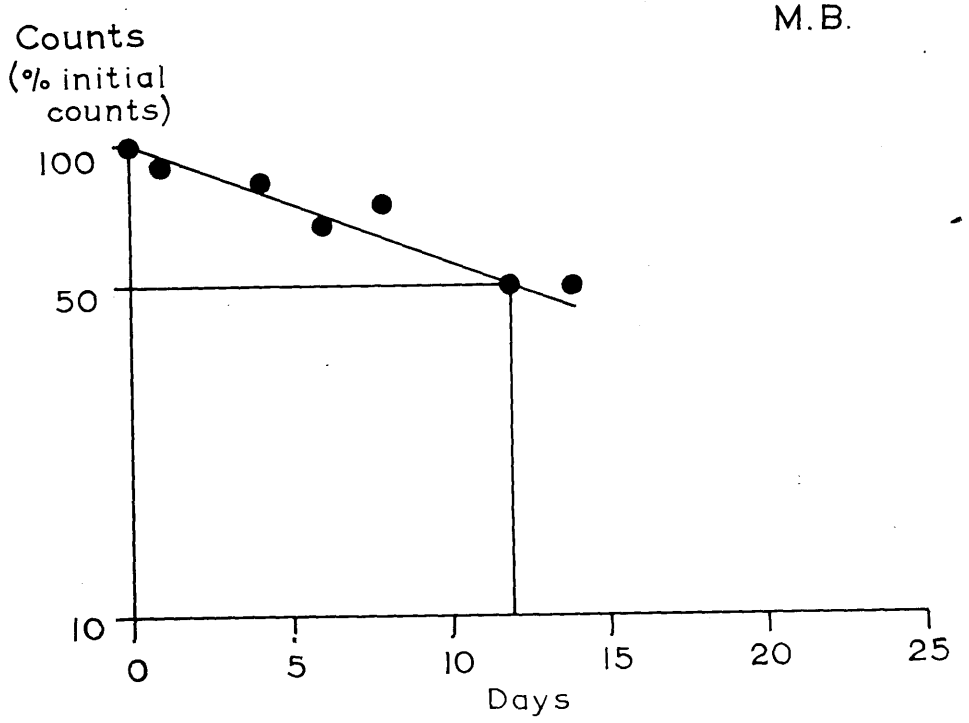

Fig. 53 Red cell survival by the radiochromium technique in patient M.B.

M.B. - 'Intermediate' disease

The radioiron profile for this patient is seen in fig. 52, and again shows no significant increase in marrow radioactivity but a moderate increase in splenic activity. The utilisation of the injected radioactive iron is 97 per cent and this high figure is due to iron deficiency (haemoglobin 9.1 g%, M.C.H.C. 26%, serum iron 70ug % and total iron binding capacity 270ug %). The red cell survival time is greatly reduced to 12 days (fig.53) and indicates a markedly increased rate of haemolysis. Sequestration studies were not carried out.

Thus, there is again marrow hypoplasia and increased haemolysis and the spleen is the site of red cell formation.

/ It is, therefore, easily seen from these investigations that these two patients occupy a very interesting position in the study of chronic myeloid leukaemia and myelofibrosis. The clinical pictures show nothing to distinguish either from these diseases and the blood findings are of no real help. The white cell counts are more in the range of chronic myeloid leukaemia but are not diagnostic and in one patient the differential white cell count resembles that of myelofibrosis much more than that of chronic myeloid leukaemia. Repeated marrow puncture at several sites yielded, in both patients, a 'dry tap', the characteristic result in myelofibrosis. The histology of marrow trephine biopsies, however, demonstrated myeloid hyperplasia, almost of the degree typical of chronic myeloid leukaemia but there was a marked increase in reticulum in each patient to the degree seen in myelofibrosis.

Thus, on the criteria used for definition of chronic myeloid leukaemia and myelofibrosis, there is no doubt that these two patients lie

/between the two, resembling myelofibrosis in some ways and chronic myeloid leukaemia in others, and can be classed as truly 'intermediate'.

The radioisotope studies are of considerable interest for here again is seen the pattern of anaemia produced by combined hypoplasia and haemolysis and in both cases the spleen shows evidence of active erythropoiesis. Thus, the radioisotopic investigations, too, show no specific pattern and are compatible with either diagnosis. Marrow erythropoietic hypoplasia can therefore occur without the gross myeloid hyperplasia seen in chronic myeloid leukaemia and yet without the fibrosis and marrow obliteration of myelofibrosis. Splenomegaly is not as great as in these other diseases and the presence of the enlarged spleen is seen to be beneficial to each patient in that it is the seat of erythropoiesis.

While there may be varying opinions on the relationship of the myeloproliferative group of disorders, the demonstration of these

/'intermediate' cases is strong evidence in favour of a relationship between chronic myeloid leukaemia and myelofibrosis.

Summary

Two patients whose clinical features are those of chronic myeloid leukaemia or myelofibrosis are described.

The blood and marrow findings are not consistent with either disease but lie between the two and the cases are therefore classed as 'Intermediate'.

The radioisotopic studies show that the anaemia of these patients is due to combined marrow hypoplasia and an increased rate of haemolysis.

The occurrence of 'Intermediate' cases is considered evidence in favour of a relationship between chronic myeloid leukaemia and myelofibrosis.

SUMMARY OF

SECTION II

A study is carried out of five patients with chronic myeloid leukaemia and of nine patients with myelofibrosis.

The clinical pictures of these diseases are very similar and the symptoms and signs are usually related to asthenia, anaemia or splenomegaly. Weight loss is often prominent and splenomegaly is striking. The haematological investigations demonstrate differences in the blood pictures of these diseases which usually suggest the diagnosis. Marrow trephine biopsy is necessary to substantiate the diagnosis of myelofibrosis and the histological features of the marrow suggest that myelosclerosis is in fact a complication of some cases of myelofibrosis. Splenic puncture is helpful in making the diagnosis but splenic erythropoiesis may occur in either condition. The radioisotopic investigations show that the anaemia in each disease is usually due to marrow hypoplasia and increased haemolysis. Splenic erythropoiesis may compensate partially for marrow hypoplasia

/but is not secondary to marrow involvement.

While chronic myeloid leukaemia and myelofibrosis are distinct diseases with certain characteristic features of diagnostic importance, they do possess features in common and the basic mechanisms of the anaemia appear to be the same in each disease.

The demonstration of two patients who appear to lie between the two diseases and are classified after investigation as 'Intermediate' or 'linking forms' is taken as strong evidence in favour of relationship between chronic myeloid leukaemia and myelofibrosis. It is concluded that if the other myeloproliferative diseases have similar features and basic abnormalities and if transition forms are found, then relationship of the whole group of disorders is most likely.

SECTION III

OTHER MYELOPROLIFERATIVE DISEASES

Chapter 7

POLYCYTHAEMIA VERA

Since the original articles by Vaquez (1892) and Osler (1903) many further publications on various aspects of Polycythaemia Vera have appeared in the literature. Perhaps the most comprehensive study of a large number of patients with this disease is that of Lawrence, Berlin and Huff (1953) who review the previous literature and discuss the clinical, physiological and therapeutic aspects of 207 patients. Despite this extensive review, which reveals many interesting aspects of the disease, there are still many problems in relation to Polycythaemia Vera, not least the relationship to other myeloproliferative disorders.

Five patients with Polycythaemia Vera have been studied and the results are discussed under the headings of 'clinical picture', 'haematological findings' and 'radioisotopic investigations'.

Clinical Picture

Polycythaemia Vera is a disease of middle life and is slightly more common in males than in females (Wintrobe, 1956). Three males

/and two females are described in this study and the ages range from 37 to 70 years at the time of onset of symptoms. The onset is usually insidious and the most common complaints are of headaches, limb pains, asthenia, attacks of mental depression, dyspnoea on exertion, tinnitus and lightheadedness. Of the five patients, two complained of lack of energy, two had lightheadedness, one dyspnoea, one limb pains and one abdominal discomfort. It is of interest that two patients had symptoms of intermittent claudication, despite satisfactory haemoglobin levels and this is almost certainly due to peripheral vascular disease, which is a known complication of Polycythaemia Vera (Brown and Giffin, 1930). One patient was so troubled with attacks of mental depression that when first seen she was an inmate of a Mental Hospital. This underlines the importance of the recognition of even severe mental depression as a possible symptom of Polycythaemia Vera. The occurrence of gout in two patients is no doubt the result of increased uric acid

/metabolism and high blood uric acid levels.

The spleen is said to be palpable in about 66 per cent of cases and the liver in 25 per cent of cases (Tinney et al, 1943). A palpable spleen was found in four of the five patients and a palpable liver in two. Considerable variation was noted in splenic size and whereas in one patient the spleen was impalpable, in another the spleen extended below the level of the umbilicus but in no case did it reach the dimensions found in some cases of chronic myeloid leukaemia or myelofibrosis.

One of the characteristic features of patients with polycythaemia vera is the dusky red appearance of the face. This was present in four patients but in the other the skin and mucous membranes were pale as a result of a recent severe gastro-intestinal haemorrhage and one other patient gave a history of previous melaena. The occurrence of haemorrhage in polycythaemia vera is well recognised but so also is the liability to thrombosis and it is stated that vascular disasters, either

/haemorrhage or thrombosis, occur in about one third of all cases. (Whitby and Britton, 1957). Thus, the coexistent tendencies to bleed and to thrombose, as seen in chronic myeloid leukaemia and myelofibrosis, again occur. Gastro-intestinal bleeding is often associated with peptic ulcer, as in the above patients, and this association occurs more commonly than can be attributed to chance. Tinney et al (1943a) report peptic ulcer in 7 per cent of patients with polycythaemia vera and Rosenthal and Bassen (1938) in 10 per cent.

The occurrence of hypertension is noted in 50 per cent of patients by Lawrence et al (1953) who consider a systolic pressure of 150 mm. Hg. or more and/or a diastolic pressure of 90 mm. Hg. or more to be evidence of hypertension. Using these criteria, hypertension is present in four of the five patients described here but in fact only one of these had a diastolic pressure of over 100 mm.Hg. Gaisbock (1922) describes a group of patients with polycythaemia who have hypertension but in

/whom the spleen is impalpable, and so patient F.N. who has a blood pressure of 190/100 mm. Hg. and in whom the spleen is not palpable may well be considered to have Gaisbock's disease. Such patients, however, are sometimes observed to develop splenomegaly at a later date (Harrop and Wintrobe, 1938) and clearly do not constitute a separate disease syndrome.

Haematological Findings

The essential haematological feature of polycythaemia vera is the occurrence of red cells in greater than normal numbers in the blood. This feature was found in the current series in all patients and counts from 8.00 to 10.32 million red cells per c.mm. were noted at one time or another for each patient. These high numbers of red cells were not constantly present especially in the two patients with major bleeding episodes and it is of interest that two patients, including one of the above, actually presented with anaemia. All five patients, however, had evidence of iron deficiency as determined by

Name	Hb (g%)	M.C.H.C. (%)	W.B.C. (per c.mm)	Pl. (per c.mm)	Reticulocytes (%)
A.McA.	8.0	24	17,600	590,000	2
M.S.	23.1	28.7	8,700	283,000	1
F.N.	17.7	29.5	6,500	205,000	<2
J.G.	11.8	24	40,000	500,000	<2
J.C.	23.1	29.75	18,700	330,000	<2

Table 13 Summary of haematological findings in five patients with polycythaemia vera.

/M.C.H.C. estimations of below 30 per cent. Thus despite the usually high haemoglobin levels and erythrocyte counts the red cells are often hypochromic and this is probably related to large or repeated bleedings either spontaneous or therapeutic in origin (Wintrobe, 1956). The principal haematological features found at the first attendance are summarised in table 13.

Emphasis has been laid on the involvement of white cells and platelets in about three quarters of all patients with polycythaemia vera and the white cell counts are often in the region of 10,000 to 25,000 per c.mm. but occasionally counts of over 50,000 cells per c.mm. have been recorded (Rosenthal and Basson, 1938). Elevated platelet counts of over 300,000 per c.mm. are seen in 65 per cent of patients (Lawrence et al, 1953) but it must be remembered that these figures are modified by recent therapy which lowers both the white cell and platelet counts; in untreated patients the counts are in all probability somewhat

Patient	white cell count per c.mm.	neutrophils %	eosinophils %	basophils %	lymphocytes %	monocytes %	primitive cells %	'smudge' cells %	Nucleated red cells per 100 W.B.C.
A.McA.	17,600	80	0	0	12	8	0	0	0
M.S.	8,700	77	1	0	20	2	0	0	0
F.N.	6,500	70	3	3	21	3	0	0	0
J.G.	40,000	92	1	1	5	1	0	0	0
J.C.	18,700	85	1	1	10	5	0	0	0

Table 14 Differential white cell counts in five patients with polycythaemia vera.

/higher.

Of the five patients investigated, three had white cell counts of over 10,000 cells per c.mm. and the highest was 40,000 per c.mm. These three also had platelet counts over 300,000 per c.mm. but the platelet levels were normal in the other two patients. The predominant white cell is the polymorphonuclear leucocyte though some immature cells may be seen; no primitive white cells were present in these patients but polymorphonuclear leucocytes constituted 70 to 92 per cent of the total white cells (table 14.).

The histological features of the bone marrow are those of hyperplasia of the erythropoietic and leucopoietic tissues and of the megakaryocytes. It is this increase of megakaryocytes and white cell precursors that Britton and Neumark (1949) consider the principal feature of differentiation from secondary erythrocytosis. These findings were present in all five patients and the typical

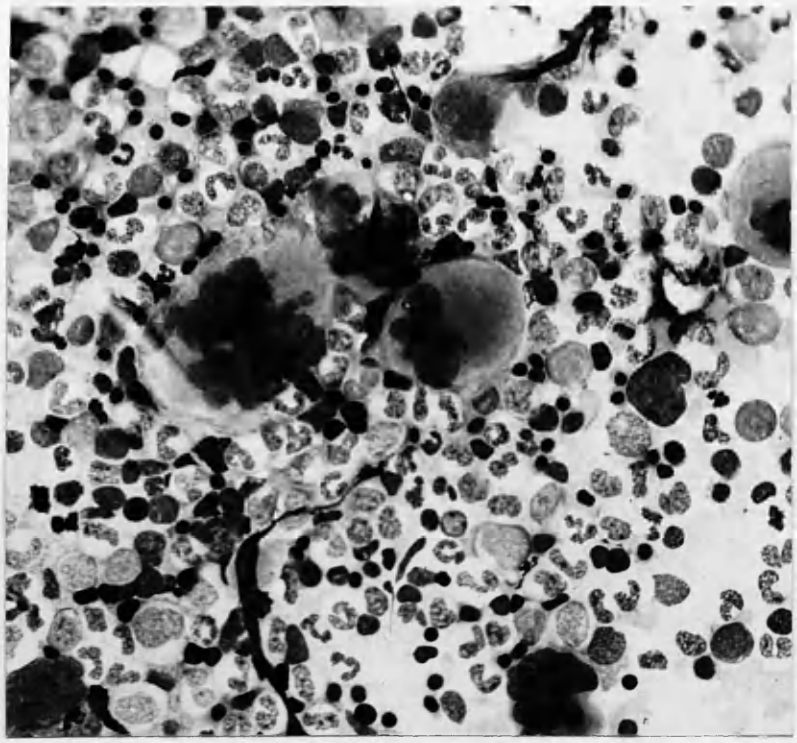

Fig. 54 Marrow histology in polycythaemia vera (patient A.McA.) (May-Grunwald-Giemsa x 400).

/marrow appearance is illustrated in fig.54 which is taken from patient A.McA. The hyperplasia is easily seen and in particular the abundance of megakaryocytes is observed.

Radioisotope Investigations

The iron uptake patterns and iron utilisation were estimated in all five patients and the red cell survival time in four. The results of these investigations are considered separately for each patient. There was no evidence of blood loss at the time of these studies.

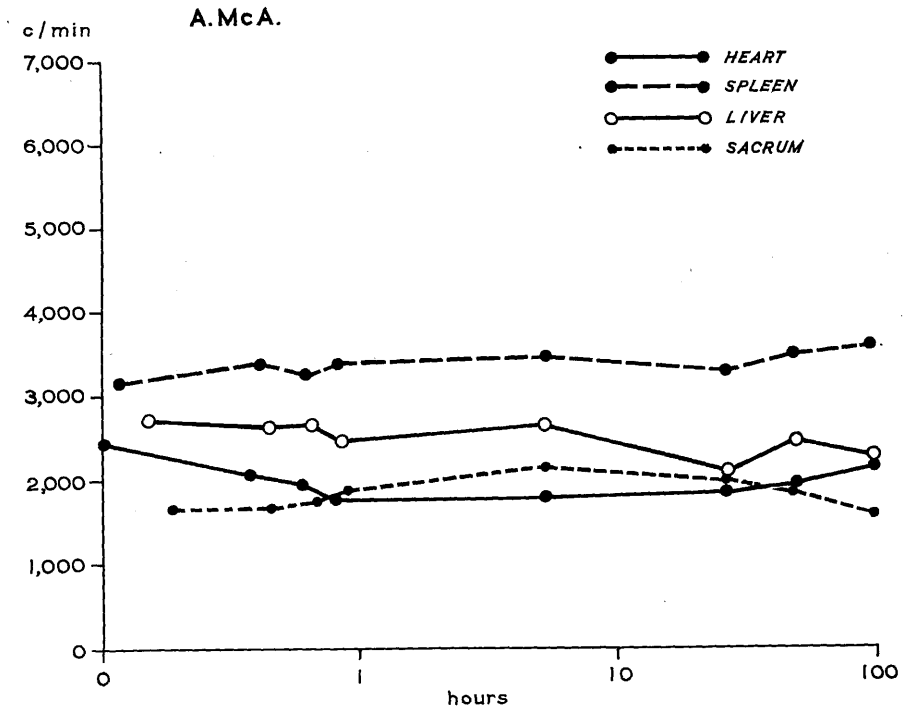

Fig. 55 Radioiron profile in patient A.McA. (polycythaemia vera)

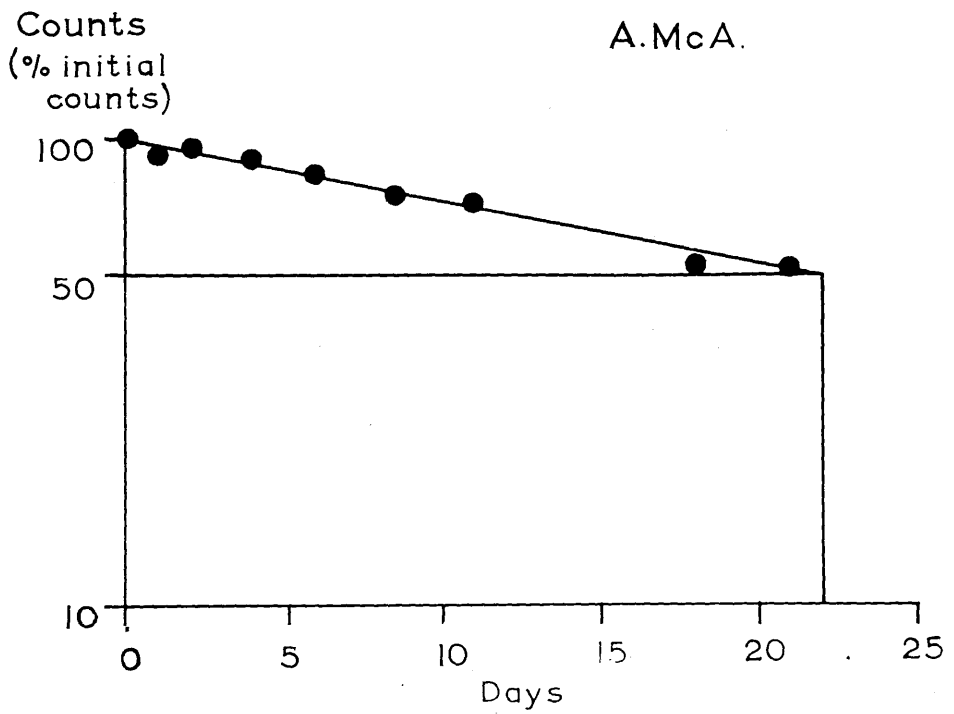

Fig. 56 Red cell survival by the radiochromium technique in patient A.McA.

A.McA. - Polycythaemia Vera

The iron uptake profile is seen in fig.55. There is evidence of activity in the bone marrow but not in the spleen. The iron utilisation is 94 per cent which is high and the red cell survival (^{51}Cr) is 22 days (fig.56).

These results are interpreted as showing continuing marrow red cell production and no splenic erythropoiesis. The high iron utilisation was obtained at a time when the patient was not anaemic, but was iron deficient (haemoglobin 16.9 g%, M.C.H.C. 29%, serum iron 35ug %, total iron binding capacity 345ug %) and is probably partly due to iron deficiency. ^{51}Cr $T\frac{1}{2}$ of 22 days indicates a minor, if any, increase in the rate of haemolysis.

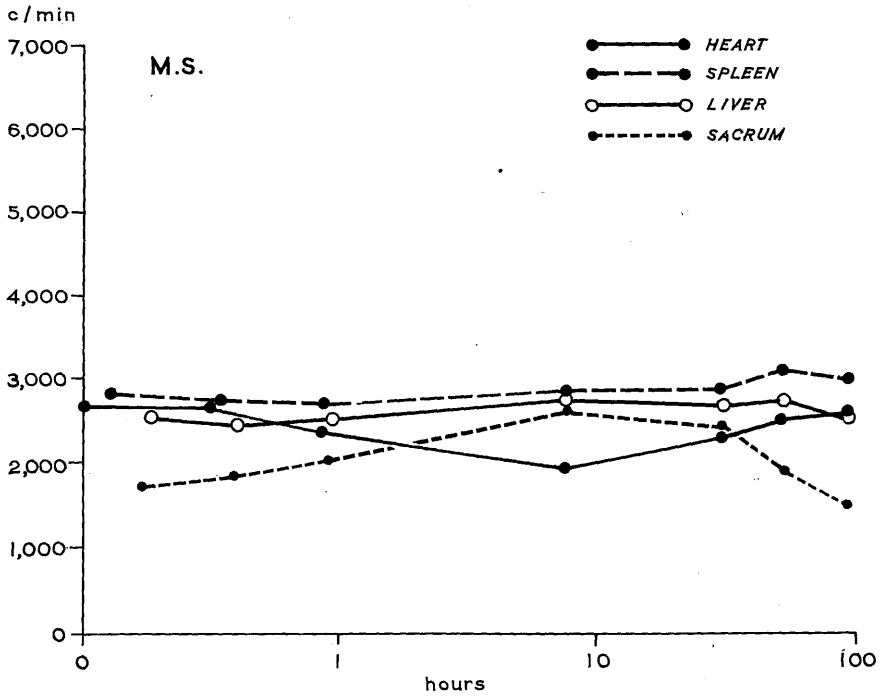

Fig. 57 Radioiron profile in patient M.S. (polycythaemia vera)

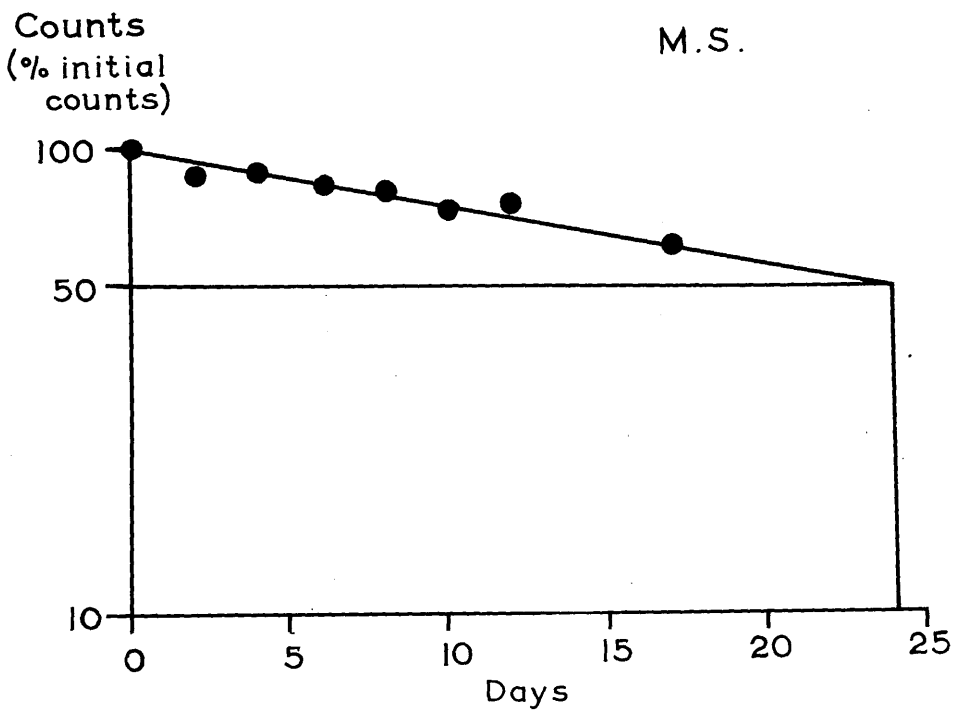

Fig. 58 Red cell survival by the radiochromium technique in patient M.S.

M.S. - Polycythaemia Vera

The radioiron profile is seen in fig.57. There is an increase of marrow radioactivity but no significant alteration in the splenic level. The iron utilisation is 88 per cent of the injected dose and $T\frac{1}{2}$ is 24 days (fig.58).

Thus, marrow production continues and there is no splenic erythropoiesis. There is no significant increase in the rate of red cell destruction.

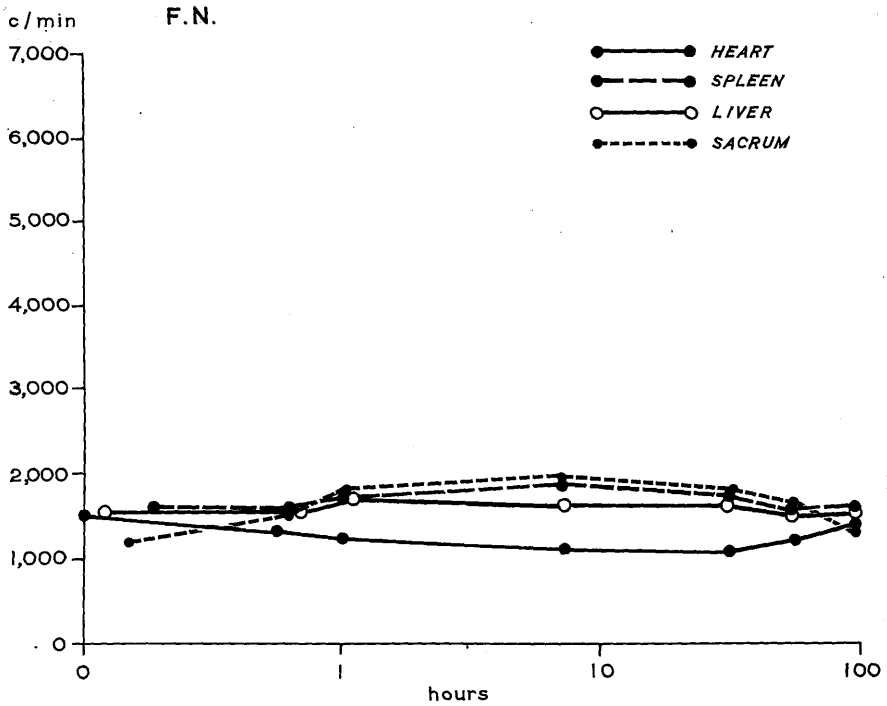

Fig. 59 Radioiron profile in patient F.N. (polycythaemia vera)

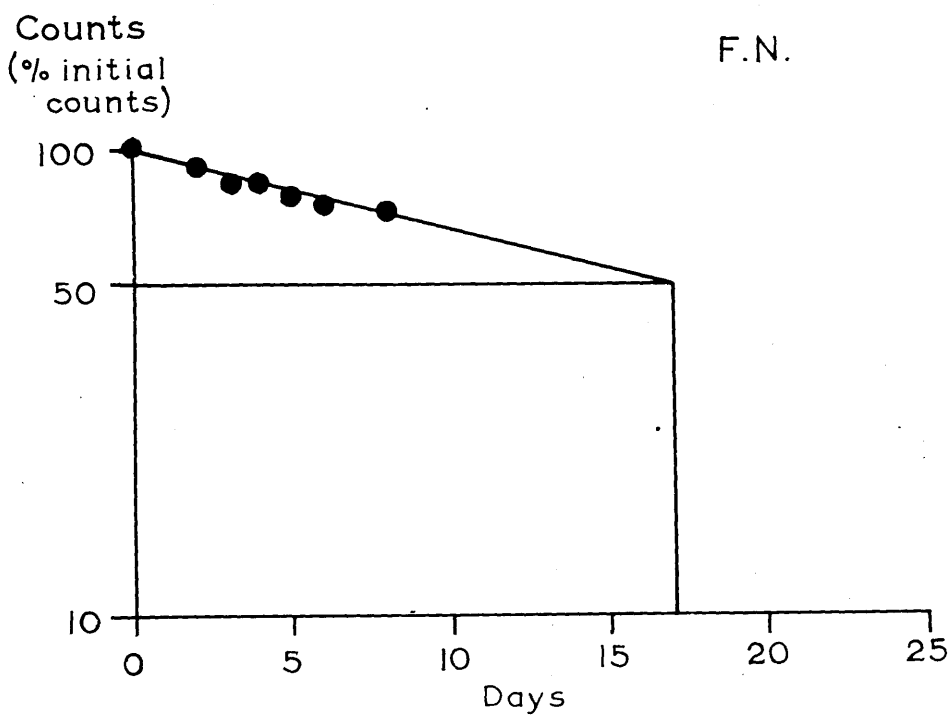

Fig. 60 Red cell survival by the radiochromium technique in patient F.N.

F.N. - Polycythaemia Vera

The radioiron uptake patterns are indicated in fig.59. There is again some evidence of marrow activity but none of splenic erythropoiesis. The iron utilisation is normal (74%) but there is a distinctly increased rate of haemolysis ($T\frac{1}{2}$ = 17 days) (fig.60). An interesting feature is the presence of excessive haemolysis in this one patient.

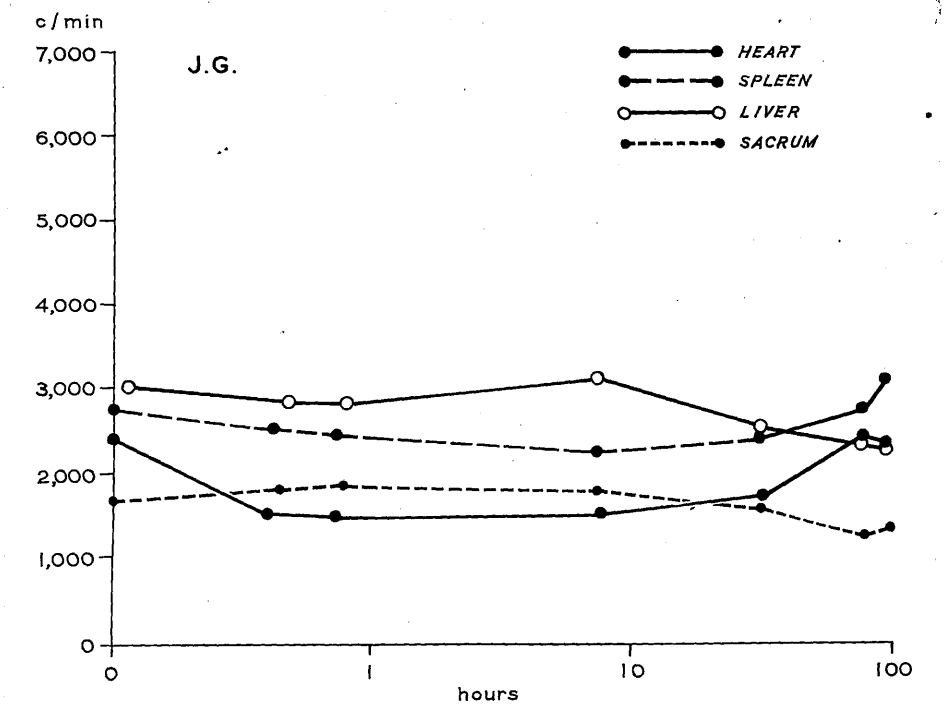

Fig. 61 Radioiron profile in patient J.G.

(polycythaemia vera)

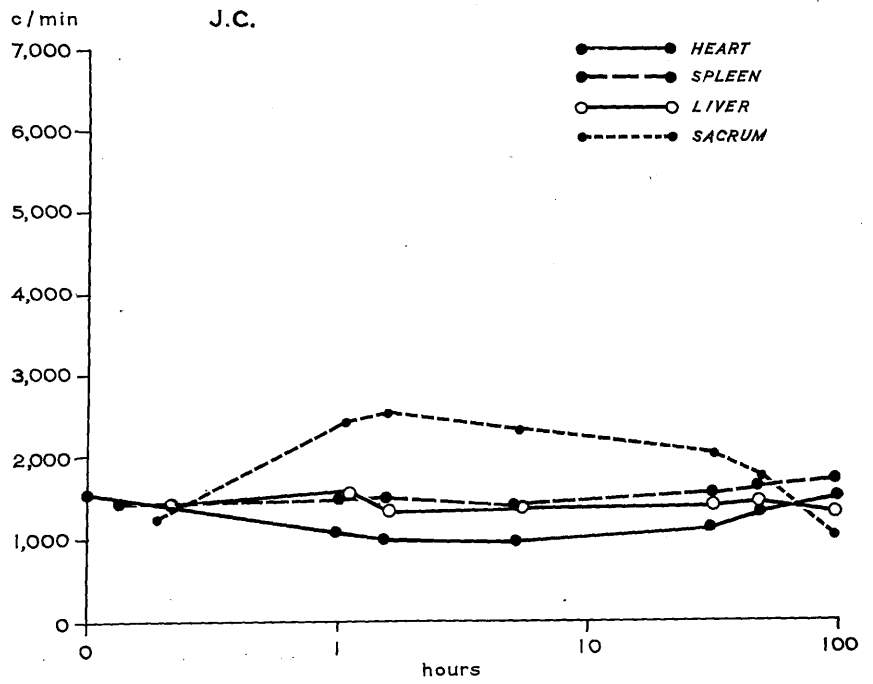

Fig. 62 Radioiron profile in patient J.C. (polycythaemia vera)

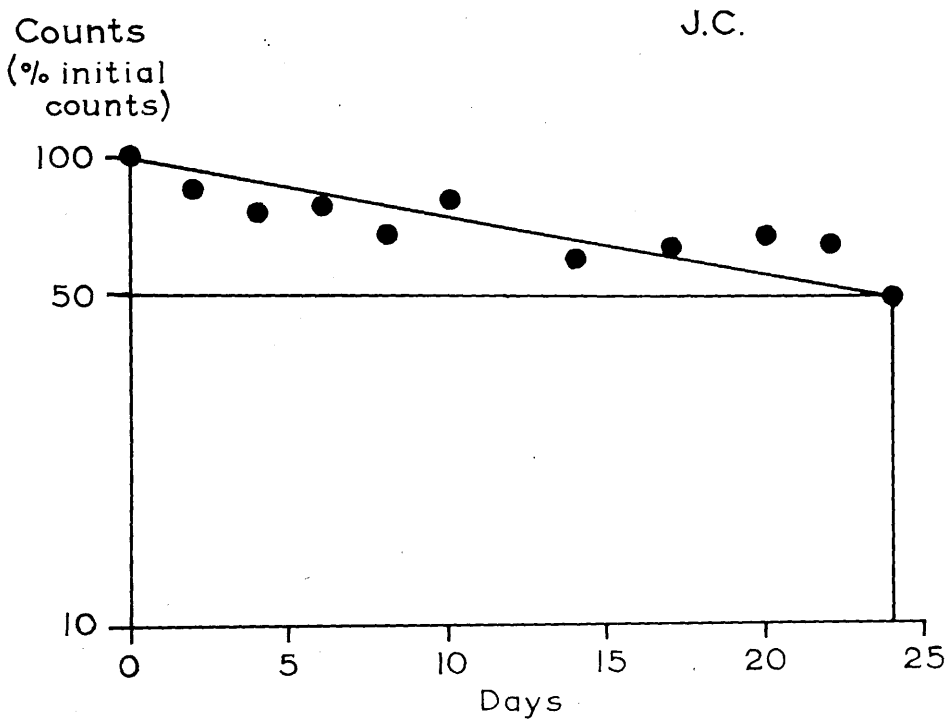

Fig. 63 Red cell survival by the radiochromium technique in patient J.C.

J.C. - Polycythaemia Vera

The radioiron profile is seen in fig.62 and shows a moderate increase in marrow radioactivity but no significant alteration in the splenic level. The iron utilisation is within the normal range (83%) and $T\frac{1}{2}$ is 24 days (fig.63).

Thus, there is definite marrow activity, no splenic erythropoiesis and no significant increase in the rate of haemolysis.

Discussion

The five patients investigated appear to form a group representative of polycythaemia vera as determined by the clinical pictures and haematological findings. The splenomegaly associated with the condition is seen to vary in degree from that of an impalpable spleen to that of a fairly large spleen but never seems to reach the size often attained in chronic myeloid leukaemia and myelofibrosis. In common with these diseases, however, occur other features such as asthenia, dyspnoea, abdominal discomfort, bone pains and gout. It is also noteworthy that in polycythaemia vera there is often a bleeding tendency, such as seen in the two patients with severe gastro-intestinal haemorrhage, despite the normal or high platelet levels, and yet there is also an increased liability to thrombotic complications. The elevated leucocyte and platelet counts are seen also in chronic myeloid leukaemia and myelofibrosis and megakaryocytic increase is a characteristic feature of the bone marrow in

Patient	Iron uptake pattern (^{59}Fe)		Iron (^{59}Fe) utilisation (% of injected dose)	^{51}Cr $T\frac{1}{2}$ (days)
	Marrow	Spleen		
A.McA.	+	0	94	22
M.S.	+	0	88	24
F.N.	+	0	74	17
J.G.	0	0	51	-
J.C.	++	0	83	24

Table 15 Summary of results of radioiron and radiochromium studies in five patients with polycythaemia vera.

/myelofibrosis. Thus, from the clinical and haematological aspects, polycythaemia vera has much in common with chronic myeloid leukaemia and myelofibrosis.

Patients with polycythaemia vera usually are not anaemic unless there has been recent blood loss but the mechanism of blood production and destruction is of considerable interest. The results of the radioactive isotope studies are summarised in table 15. Although the iron uptake profiles can be interpreted only quantitatively and cannot be used as a precise quantitative estimation, it is seen that in only one of the five patients is there much increase in marrow radioactivity, in the others there is little increase and in one no significant alteration. This seems surprising in view of the appearance of marked hyperplasia seen on histological examination of the marrow. It would be expected that there should be a high uptake of iron for use of the proliferating red cells and this can only be explained by a failure of maturation

/or a failure of the release mechanism. A similar iron uptake pattern is reported by Finch et al (1956) in cases of untreated pernicious anaemia where again there is gross erythroid hyperplasia but low marrow iron uptakes. This, combined with the low iron utilisation, illustrates what Finch et al have termed "ineffective" erythropoiesis. The marrow hyperplasia of polycythaemia vera usually extends the full length of the bones and as the marrow can expand to 6 to 8 times the normal amount (Crosby and Ackeroyd, 1952) it is of importance to note that the red cell mass is seldom more than twice normal even in the absence of significantly increased haemolysis (Vide infra). Thus, although the total red cell production is increased and the iron utilisation normal or high, this is not proportionate to the increased marrow cellularity and so favours the presence of 'ineffective' erythropoiesis. As a result the 'effective' erythropoiesis is 'diluted' over a much wider area than normal and this

/may account for the apparently low or normal marrow iron uptake patterns in most patients.

Lawrence et al (1953) describe splenic erythropoiesis, as determined by radioiron studies, in a few patients with polycythaemia vera. In such patients, however, they observe that the marrow is usually hypoplastic, aplastic or fibrotic and fails to take up radioactive iron. Furthermore, these patients usually have very large spleens and markedly elevated white cell counts. It seems probably that they are, in fact, early cases of myelofibrosis and this passage from one disorder to the other is very suggestive of an aetiological relationship.

Berlin et al (1951) describe two red cell populations in polycythaemia, one with a shortened life span and one with a normal life span, but other workers (London et al, 1949, Szur et al, 1959) have found normal cell survival. Thus the overall picture may be that of a normal or slightly increased rate of haemolysis but in the five patients studied here a significant degree of haemolysis was

/noted in only one and this suggests that increased haemolysis is only of minor importance in polycythaemia vera.

The radioisotopic results demonstrate the relatively low marrow iron uptake yet normal or high utilisation. In one patient there is an absence of significant uptake in the marrow and spleen and a low iron utilisation. This patient also has the largest spleen and the highest white cell count and the history dates back 24 years to the time of diagnosis of polycythaemia. Although the bone marrow is still histologically hyperplastic and mature white cells only are present in the blood the radioiron patterns suggest that the disease is at a different phase in development than in the other patients. The iron studies show changes in the fundamental pattern of erythropoiesis and resemble those of some patients with chronic myeloid leukaemia or myelofibrosis. Thus, the disease is probably in a 'transitional' phase and, although splenic erythropoiesis has not yet developed, is one

/of the type described by Lawrence et al (1953) destined to develop myelofibrosis. This is quite in keeping with the results of the investigations as the radioiron studies are a sensitive index of cellular activity and illustrate changes which are not yet reflected in the cruder histological features of the marrow.

This association of polycythaemia vera and myelofibrosis or myelosclerosis is well known and there have been several reports of patients with myelosclerosis who presented initially with polycythaemia vera (Stone and Woodman, 1938; Vaughan and Harrison, 1939; Marson and Meynell, 1949; Hutt, 1950). It has, of course, been suggested that the development of fibrosis could be related to therapy but Wasserman (1954) found no good evidence to substantiate this. Korst et al (1956) stressed the association of these two disorders and found that myelofibrosis could be present during the polycythaemic phase as well as in the advanced or 'spent' phase.

/Furthermore, some well authenticated cases have been reported in which leukaemia has developed in untreated patients (Dameshek, 1950).

All this evidence of transition from one disease to the other, the similarities of clinical picture and haematological findings, and the alteration of patterns of erythropoiesis points to a close relationship between polycythaemia vera and other myeloproliferative disorders.

Summary

Five cases of polycythaemia vera are described and the investigations recorded. The patients appear to form a group representative of the condition.

The clinical and haematological features show considerable resemblance to the other myeloproliferative states already described.

The radioactive iron uptake patterns suggest diminished marrow production, despite the high blood counts, and this is explained on the basis of 'ineffective' erythropoiesis.

Haemolysis appears to play little or no part in the final determination of the blood counts.

A case of 24 years duration is described and the radioiron studies indicate an impending 'transitional' state. This patient seems to be one of the type likely to develop myelofibrosis or chronic myeloid leukaemia.

The evidence is discussed and is considered to support the relationship of polycythaemia vera to other myeloproliferative disorders.

Chapter 8

DI GUGLIELMO'S DISEASE

Investigation	Result
Haemoglobin (g%)	7.4
P.C.V. (%)	23
M.C.H.C. (%)	32
W.B.C. (per c.mm.)	2,300
Platelets (per c.mm.)	25,000
Reticulocytes (%)	4

Table 16 The principal findings on examination of the blood in a case of Di Guglielmo's disease.

Di Guglielmo (1923) described the rare and invariably fatal condition which now bears his name or alternatively is known as 'erythraemic myelosis'. The disease is characterised by an abnormal proliferation of the red cell precursors in the bone marrow and yet, despite this hyperplasia, an anaemia which may be severe is always present.

During the period of study of myeloproliferative disorders one patient with this rare condition was seen and investigated with the following results.

Clinical Picture

The patient, a 77 year old man, was admitted to hospital because of breathlessness on exertion, tiredness and loss of weight over the previous four months. On examination, the mucous membranes and palmar creases were found to be pale and a few petechiae were seen on his legs. The liver and spleen were not palpable and no other physical abnormalities of note were detected.

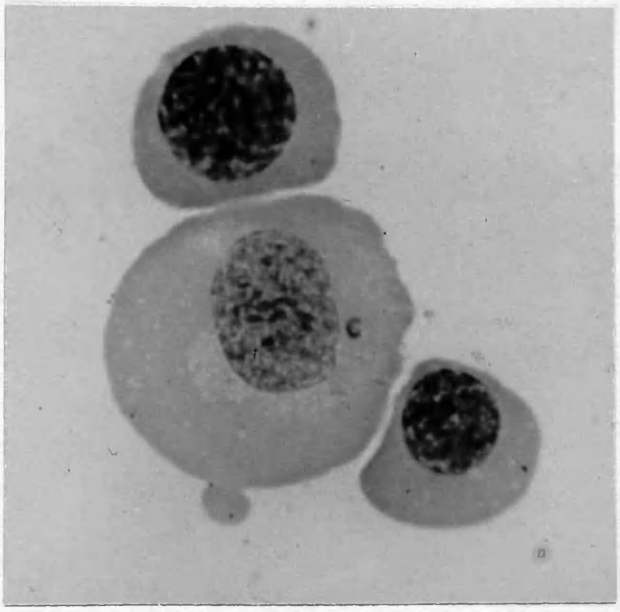

Fig. 64 Megaloblastic erythropoiesis in patient G.McF. (Di Guglielmo's disease). (May-Grunwald-Giemsa x 1500). (reproduced by permission from the "Scottish Medical Journal')

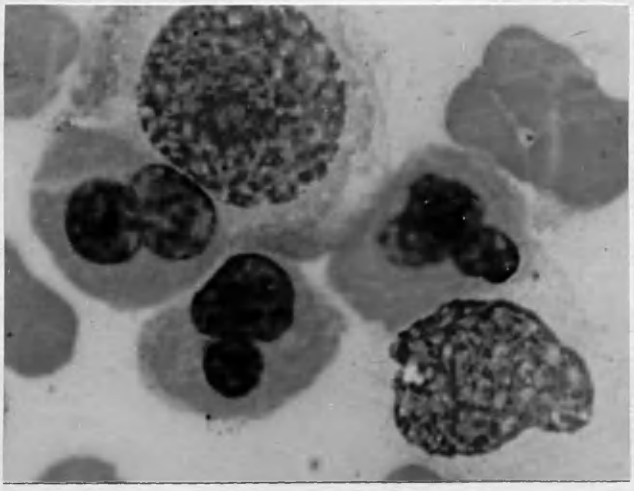

Fig. 65 Multinucleated well haemoglobinised red cells in patient G.McF. (Di Guglielmo's disease). (May-Grunwald-Giemsa x 1500). (reproduced by permission from the 'Scottish Medical Journal')

/Haematological Investigations

The principal features of the blood examination are recorded in Table 16. There was a normocytic normochromic anaemia. Blood films revealed anisocytosis and poikilocytosis and occasional nucleated red cells were seen. The white cell count was 2,300 cells per c.mm. and a differential count showed the cell types to be present in normal proportions. Few platelets were seen on examination of the blood films and the platelet count was only 25,000 per c.mm.

Sternal marrow puncture was carried out and marrow tissue was easily obtained; smears and sections showed erythroid hyperplasia. Erythropoiesis was predominantly megaloblastic (fig.64) and many bizarre forms were seen. A striking feature was the characteristic presence of well haemoglobinised multinucleated red cells (fig.65). Megakaryocytes and cells of the myeloid series appeared reduced in numbers but otherwise normal. Stainable iron was present in the marrow

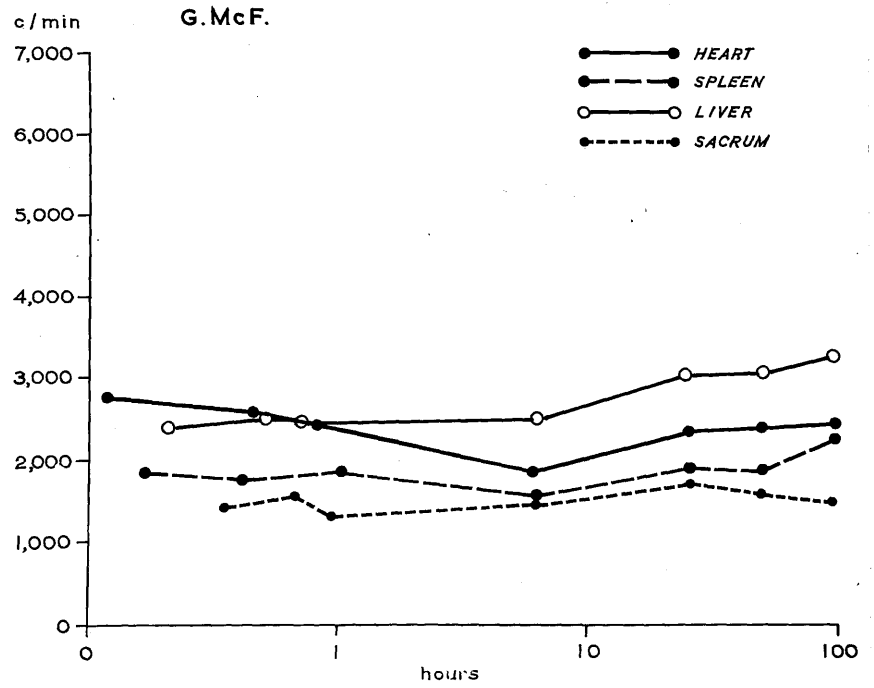

Fig. 66 Radioiron profile in patient G.McF. (Di Guglielmo's disease)

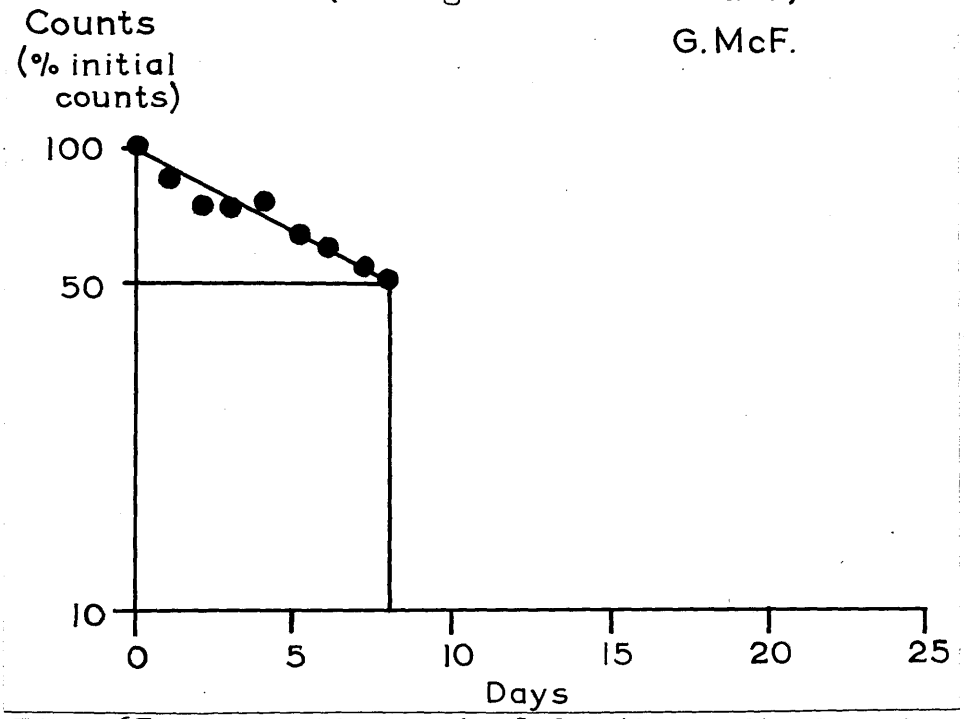

Fig. 67 Red cell survival by the radiochromium technique in patient G.McF.

Radioisotopic investigations

The radioiron profile is seen in fig.66 and the absence of a significant rise in marrow counts is indicative of diminished marrow uptake. There is no evidence of splenic erythropoiesis. The low utilisation of the injected radioactive iron (38%) in the absence of iron deficiency (haemoglobin 7.4 g%, M.C.H.C. 32%, serum iron 115ug, total iron binding capacity 155ug, stainable iron present in the marrow) confirms grossly deficient red cell production. Red cell half-life, by the ^{51}Cr technique, of 8 days was greatly diminished and indicative of a marked increase in the rate of haemolysis (fig.67). Thus, the anaemia is characterised by anisopoikilocytosis, the presence of occasional nucleated red cells in the blood and a low platelet count. Megaloblastic erythropoiesis is seen on marrow examination, many bizarre forms occur and many well haemoglobinised, multinucleated red cells are present. The anaemia appears to be due to marrow hypoplasia

/and an increased rate of haemolysis.

One of the striking features is the occurrence of anaemia in the presence of a histologically hyperplastic marrow. This is similar to the findings in untreated pernicious anaemia, where also there is the apparent anomaly of diminished red cell production despite erythroid hyperplasia in the bone marrow. This suggests that although proliferation of the red cell precursors is taking place, the cells are not released from the marrow; thus erythropoiesis may be 'apparent' or 'effective'. The difference between these two is what Finch et al (1956) term 'ineffective' erythropoiesis and this has been confirmed, as in this case of Di Guglielmo's disease, by radioiron studies. Two further points of resemblance to pernicious anaemia are present: firstly, increased haemolysis is present in both conditions and secondly, erythropoiesis in each disease is predominantly megaloblastic. Here, however, the similarity ends for the defect causing

/megaloblastic erythropoiesis in pernicious anaemia is deficiency of vitamin B_{12}. Serum vitamin B_{12} levels have been recorded in only four cases of Di Guglielmo's disease and in three of these were high (Baldini et al, 1959) and in the other normal (Adams and Seaton, 1960). Thus, the causation of megaloblastic change is by a different mechanism and this will be discussed in a later chapter.

Baldini et al (1959) in a study of 11 cases of Di Guglielmo's disease found no evidence of an immunological mechanism to account for the increased haemolysis and concluded that the defect, leading to the rapid rate of erythrocyte destruction, was at a cellular level. Such a defect could originate in the existence of excessively fragile red cells and, indeed, it was suggested by Di Guglielmo (1942) that such cells were the result of the abnormal erythroblastic proliferation. As the marrow is normally able to expand to a degree

/sufficient to cope with haemolysis six times as rapid as normal (Crosby and Ackeroyd, 1952) the increased rate of haemolysis in this case must be only one factor in the production of anaemia and inadequate marrow production must be another. This impression is confirmed by the reticulocyte count which, though usually slightly raised, is not elevated in proportion to the degree of anaemia or haemolysis. The reticulocytes and nucleated red cells present in the blood may, in fact, be the consequence of abnormal erythropoiesis and resultant failure of the normal mechanism to regulate the release of solely mature cells into the circulation. All this evidence points to defective red cell production as the major factor in the production of anaemia and to the increased rate of haemolysis as a secondary or contributory factor.

This form of 'pure' erythroblastic involvement is rare and it is more common to find mixed forms such as erythroleukaemia, (Di Guglielmo, 1917) where the leucopoietic

/tissue is involved in the proliferation as well as the erythropoietic tissue. It is of considerable interest and importance that some patients with 'pure' erythraemic myelosis have developed erythroleukaemia with the passage of time (Schwartz and Critchlow, 1952; Hindmarch and Wickham, 1955) and some have terminated as acute myeloblastic leukaemia (Discombe and Nichol, 1954). The description of such cases which pass from one phase to another has led Baldini et al (1959) to refer to the whole group as the Di Guglielmo syndrome and is strong evidence in favour of a relationship between erythraemic myelosis and other members of the myeloproliferative group.

Summary

A case of Di Guglielmo's disease (erythraemic myelosis) is described. The anaemia is due to a combination of defective red cell productions and an increased rate of haemolysis. There is no evidence of splenic erythropoiesis.

It is considered that the apparent marrow hyperplasia is due mostly to 'ineffective' erythropoiesis.

The development of erythroleukaemia and sometimes with myeloblastic leukaemia is considered evidence of relationship of these diseases.

Chapter 9

IDIOPATHIC MEGAKARYOCYTIC THROMBOCYTHAEMIA

Thrombocythaemia is the term applied to the presence of a persistently raised platelet count. This occurs secondarily in some cases of chronic myeloid leukaemia and some of polycythaemia vera but the occurrence of primary or idiopathic megakaryocytic thrombocythaemia (haemorrhagic thrombocythaemia) is much more rare. Wintrobe (1956) records one case and other patients with this disease are reported by Fanger et al (1954) Woodrow and Cope (1955), and Fountain (1958). A review of the condition is made by Hardisty and Wolff (1955).

One case of this disease has been investigated and is reported here.

Clinical Picture

Idiopathic thrombocythaemia is not a hereditary disease and is said to occur mainly in elderly people (Whitby and Britton, 1957). The principal features are splenomegaly, a liability to bleed from mucous membranes and a tendency to venous thrombosis. This patient, at the time of her first attendance

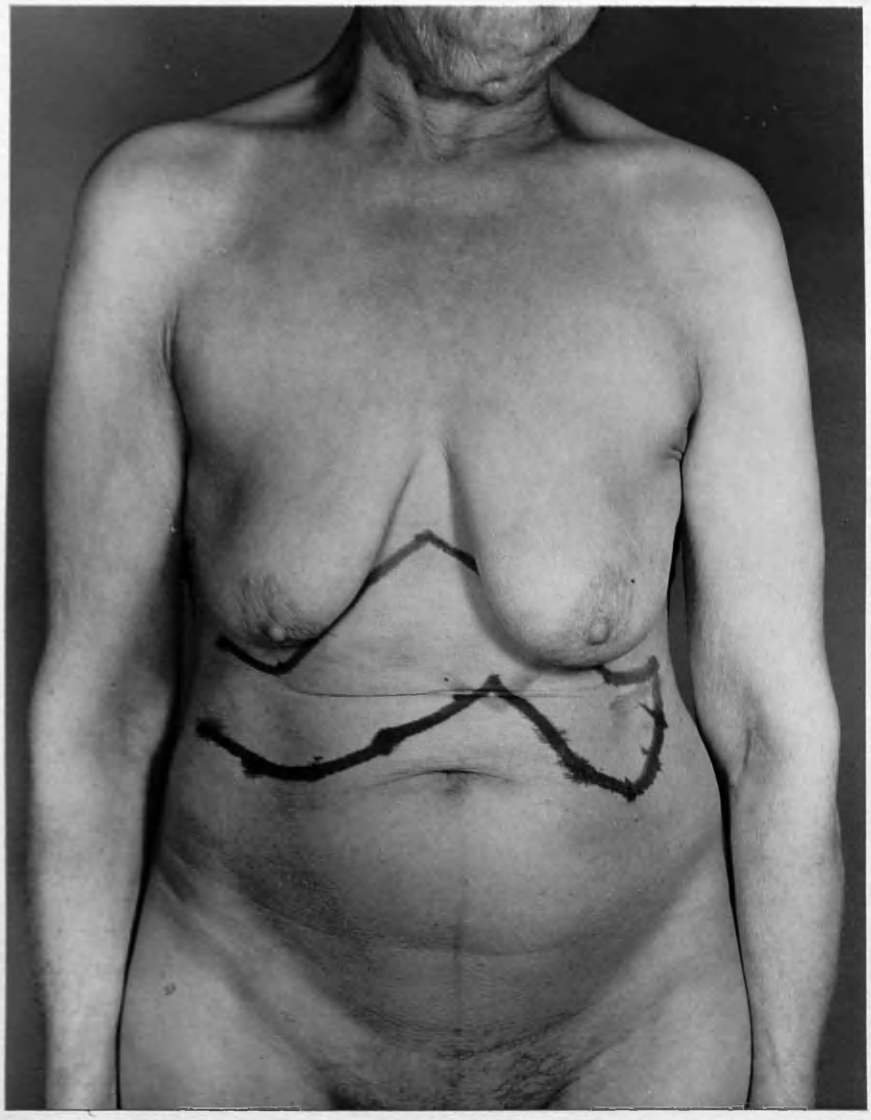

Fig. 68 Hepatomegaly and splenomegaly in patient J.D. with megakaryocytic thrombocythaemia.

/at hospital in 1956 had noted spontaneous bruising for four months and had seen both fresh and altered blood in her stools on several occasions. She was seen to have many bruises and spleen was found to be palpable two finger breadths below the costal margin; by 1959 the spleen was rather larger and hepatomegaly also was present (fig.68). At this time also she had further severe haematemeses which necessitated the transfusion of blood.

Haematological Findings

The disease may be associated with polycythaemia (Uotila, 1938) when there has been no recent bleeding, or with anaemia after recent blood loss (Whitby and Britton, 1957). Leucocytosis is common, bizarre platelets are often seen in the blood and the platelet counts usually exceed one million per c.mm. At the time of first attendance the figures recorded on patient J.D. were as follows: haemoglobin 12.5 g per cent, white cell count, 35,200

Patient	White cell count per c.mm.	Neutrophils %	Eosinophils %	Basophils %	Lymphocytes %	Monocytes %	Immature white cells %	Nucleated red cells per 100 white cells.
J.D.	43,000	88	2	1	8	1	0	0

Table 17 Differential white cell count in a case of idiopathic megakaryocytic thrombocythaemia.

/per c.mm., and platelet count, 1.35 million per c.mm. The differential white cell count is shown in table 17. Over the next three years, however, fluctuation in these levels was noted and the haemoglobin was seen to range from 10.4 g per cent to 17.6 g per cent, the white cell count from 25,000 to 50,000 cells per c.mm. and the platelet count rose to the high reading of 2.14 million per c.mm. Histological examination of the bone marrow revealed normoblastic erythropoiesis with apparent reduction in the numbers of red cell precursors. There was, however, some myeloid hyperplasia and a marked increase in the number of megakaryocytes present (fig.69) which is typical of idiopathic thrombocythaemia.

It has been suggested that the bleeding tendency is due to a qualitative defect in the platelets as determined by the thromboplastin generation test (Hardisty and Wolff, 1955). A slight defect of this nature was demonstrated in this case when the platelets were reduced to 250,000 - 500,000 per c.mm.

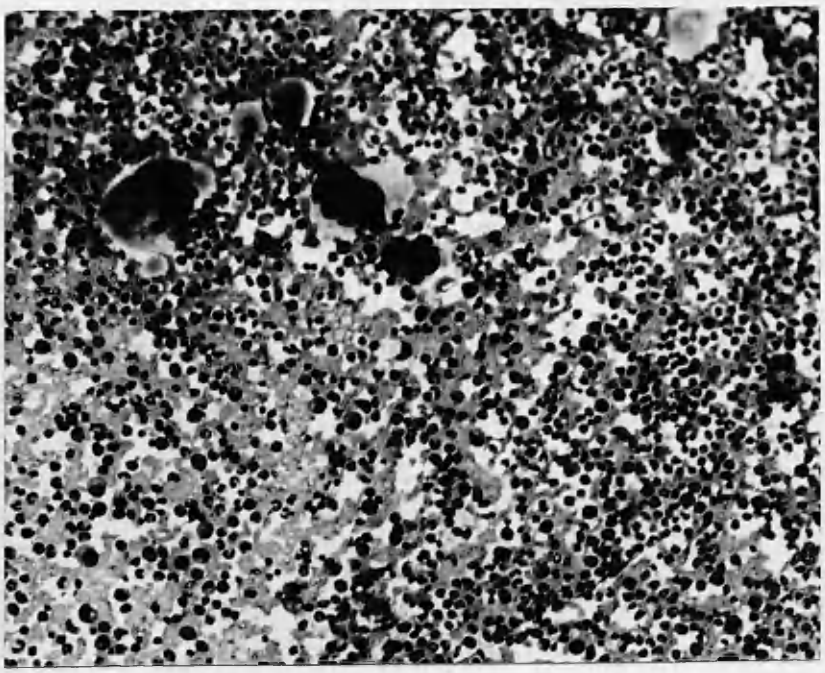

Fig. 69 Marrow histology in patient J.D. (megakaryocytic thrombocythaemia) (Leishman x 150).

/(Cook, 1960). High concentrations of these platelets, however, produced an inhibitory effect on thromboplastin formation as reported previously by Spaet et al (1956). It seems possible that in conditions of venous stasis, which would normally favour thrombosis, very high platelet levels may have an anti-thromboplastic effect (Cook, 1960). On this basis treatment should aim at reducing platelet levels and radiophosphorus would, therefore, appear to be the treatment of choice.

Radioisotope Investigations

The radioiron profile is seen in fig.70. There is a definite increase in marrow activity but no alteration in splenic activity. Thus the uptake pattern is normal but the iron utilisation is only 22.6 per cent of the injected dose. The radiochromium studies indicate a normal red cell survival of 27 days and the spleen/liver ratio at this time is 1.3/1 (fig. 71).

Thus the pattern is probably due to iron deficiency (haemoglobin 16 g%, M.C.H.C. 28%,

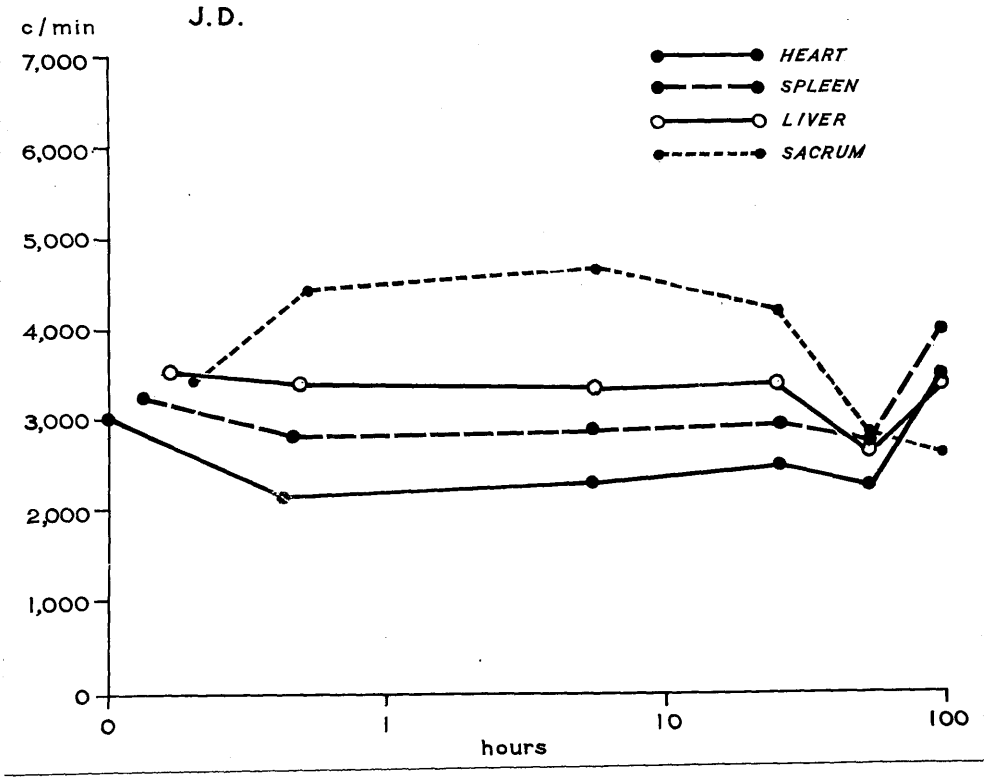

Fig. 70 Radioiron profile in patient J.D. (megakaryocytic thrombocythaemia)

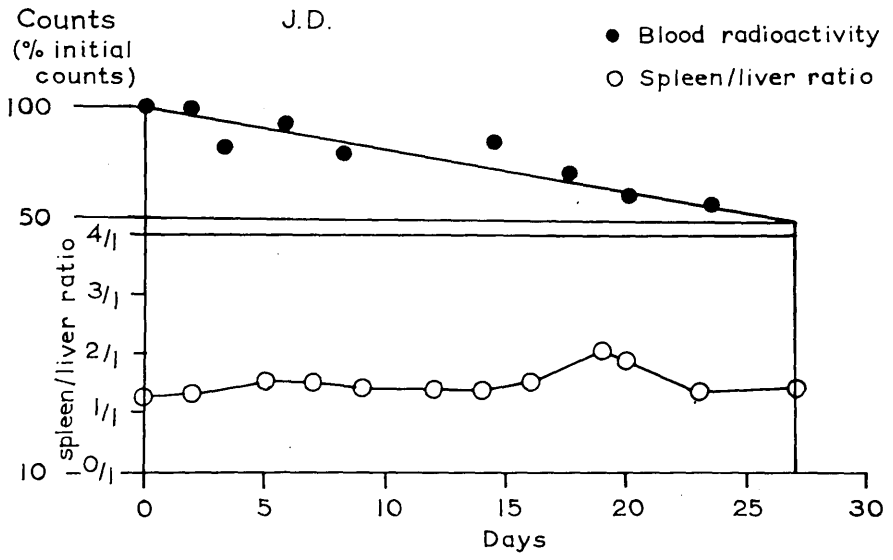

Fig. 71 Sequestration studies and red cell survival by the radiochromium technique in patient J.D.

/serum iron 20ug per 100 ml, total iron binding capacity 320ug per 100 ml) resulting in marked uptake of iron by the marrow; the low utilisation of the iron in the presence of a high haemoglobin and erythrocytosis (7.99 million per c.mm.) suggests that there is a defect of incorporation of iron into the red cells. There is no increased rate of destruction of the red cells and the spleen shows no evidence of either erythropoiesis or sequestration of the red cells.

This interesting patient appears to fulfil the criteria required for the diagnosis of idiopathic megakaryocytic thrombocythaemia (Reid, 1940). She has splenomegaly, hepatomegaly and a bleeding tendency. There was in the initial stages an anaemia and later a polycythaemia but this fluctuation is characteristic. The myeloid hyperplasia and leucocytosis are usually found but the striking feature is the greatly elevated platelet count and the presence of a marked increase in megakaryocytes in the marrow. Furthermore,

/the coagulation defect described previously in idiopathic thrombocythaemia has been demonstrated in this case.

The radioisotopic findings, carried out during a polycythaemic phase are of interest. In contrast to the relatively low marrow uptake of radioactive iron seen in polycythaemia vera there is a very definite marrow uptake in this case. This is a significant finding as it occurs in the presence of reduced numbers of red cell precursors in the marrow and not, as in polycythaemia vera, in the presence of increased numbers. This, and the low iron utilisation despite an elevated red cell count, suggest that the defect is probably in incorporation of iron whereas in polycythaemia vera it appears to be a failure of the release mechanism which results in 'ineffective' erythropoiesis. This shows that idiopathic megakaryocytic thrombocythaemia is, even in the polycythaemic phase, a separate entity and not merely a form of polycythaemia vera.

/ There are, however, many resemblances to polycythaemia vera and some to chronic myeloid leukaemia and the tendencies to bleed and yet to thrombose are features common to most myeloproliferative diseases. On this basis it seems likely that idiopathic megakaryocytic thrombocythaemia is a disease in its own right but that it is related to the other myeloproliferative disorders.

Summary

A case of idiopathic megakaryocytic thrombocythaemia is described and seems typical of this condition.

The clinical and haematological features are discussed and found to resemble those of other myeloproliferative disorders.

The radioisotopic investigations demonstrate a pattern distinct from that of polycythaemia vera which is resembled closely in other ways.

Idiopathic megakaryocytic thrombocythaemia is considered to be a separate entity but one closely related to other myeloproliferative disorders.

Chapter 10

'TRANSITION'

MYELOPROLIFERATIVE STATES

The myeloproliferative group of disorders comprises chronic myeloid leukaemia, myelofibrosis, and myelosclerosis, polycythaemia vera, megakaryocytic thrombocythaemia and erythraemic myelosis. These conditions have been studied and reported as have cases intermediate between chronic myeloid leukaemia and myelofibrosis. There remains the controversial question of other transitional disorders. The existence of such is supported by Rosenthal (1950) and Dameshek (1951) but opposed by Wintrobe (1951) and Leonard et al (1957).

Four patients who could not be given a precise diagnosis are now presented and discussed.

Patient - J.N.

Clinical Picture

This patient, a 54 year old male, presented first with joint pains of several months duration. He also complained of abdominal discomfort and was found to have a grossly enlarged spleen (grade 3) over

White cell count per c.mm.	Neutrophils %	Eosinophils %	Basophils %	Lymphocytes %	Monocytes %	Myeloblasts %	Myelocytes %	Metamyelocytes %	Smudge cells %	Nucleated red cells per 100 white cells
20,500	80	3.5	2.0	12	1.5	0	1	0	0	0

Table 18 J.N. - Differential white cell count.

/which friction was audible. The liver was palpable two finger breadths below the costal margin and a few small axillary nodes were detected. The patient did not appear to be anaemic and no other findings of significance were noted.

Haematological Findings

The haemoglobin was 13.4 g per cent but the M.C.H.C. was only 27.25 per cent which indicated iron deficiency even at this scarcely anaemic level. The white cell count was 20,500 per c.mm. of which 80 per cent were neutrophil polymorphonuclear leucocytes, though occasional myelocytes were seen. The differential white cell count is seen in table 18. The platelet count was normal (210,000 per c.mm). Blood films revealed some hypochromia of the red cells but the presence of a few myelocytes and a few nucleated red cells was noted. Ample marrow was easily obtained on sternal puncture and histological examination demonstrated hyperplasia of myeloid

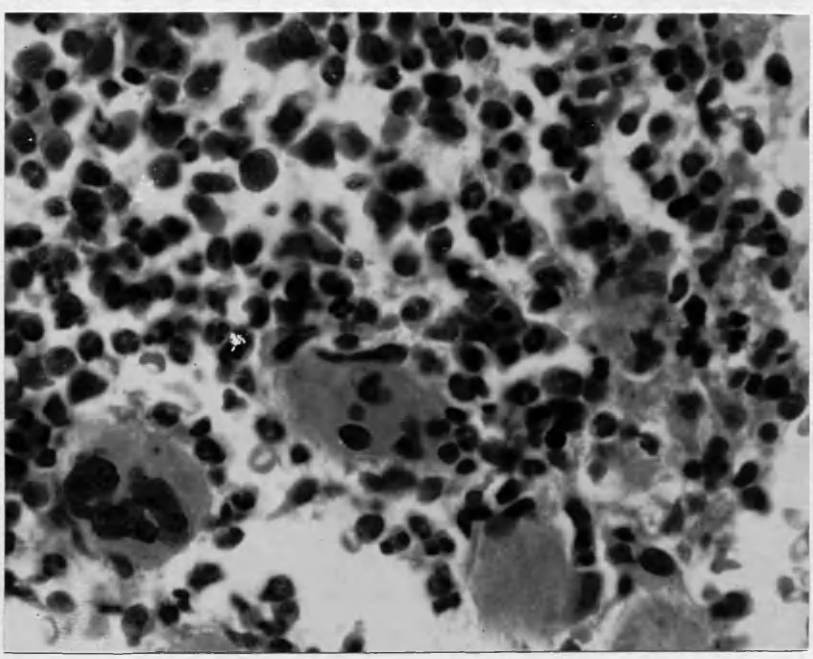

Fig. 72 Marrow histology in patient J.N.

(May-Grunwald-Giemsa x 660).

/and erythroid tissues and a marked increase in the number of megakaryocytes present (fig.72).

Radioisotopic investigations

The radioiron profile is seen in fig.73. There is no significant uptake of radioactive iron by the marrow but there is a definite increase in the level of splenic radioactivity. The iron utilisation, despite iron deficiency, is only 43 per cent and the red cell half life is normal at 25 days. At this time the spleen/liver ratio is 2.1/1 (fig.74).

Thus the picture is that of marrow hypoplasia with splenic erythropoiesis. The effective marrow output is low, as indicated by the iron utilisation, there is no increase in haemolysis and the spleen does not appear to sequester red cells.

Comment

The histology of the marrow is characteristic of polycythaemia vera. The leucocytosis and peripheral blood findings are compatible also with this diagnosis. The

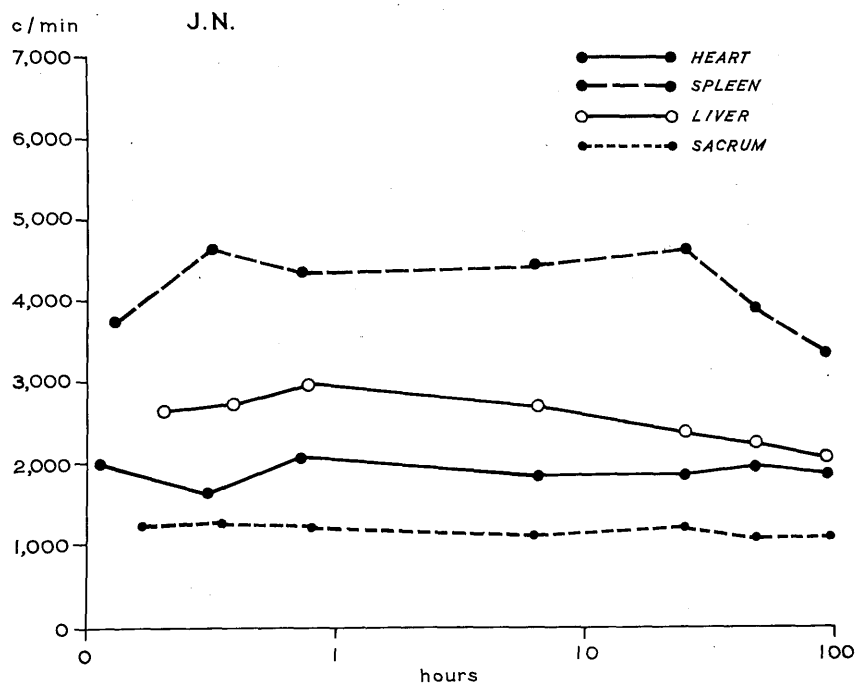

Fig. 73 Radioiron profile in patient J.N. ('transition' disease)

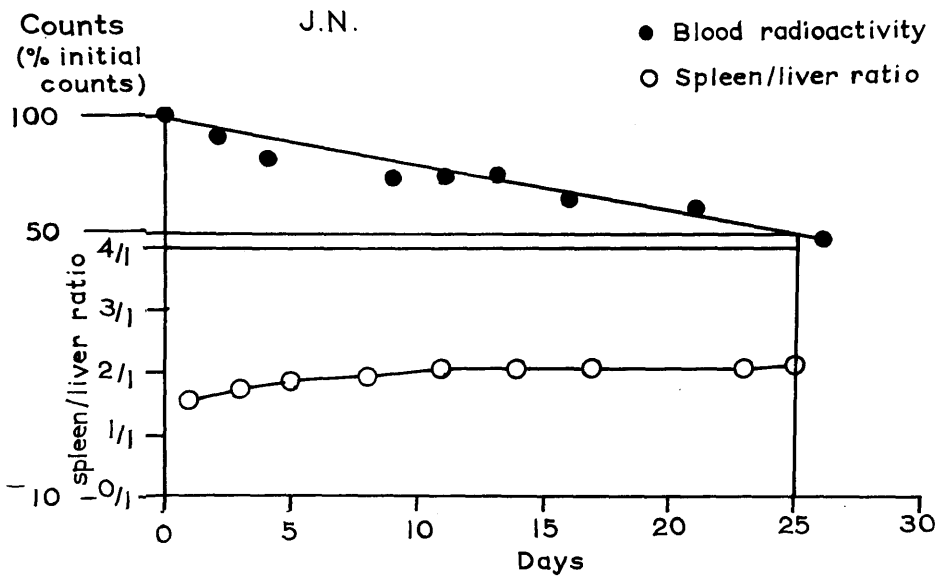

Fig. 74 Sequestration studies and red cell survival by the radiochromium technique in patient J.N.

/gross splenomegaly, however, is greater than usually is seen in polycythaemia vera and is an indication that the case may not be classical. The low marrow iron uptake and low utilisation of iron in the presence of apparent erythroid hyperplasia suggests 'ineffective' erythropoiesis as occurs in polycythaemia vera; the low utilisation of iron, however, is not typical of polycythaemia vera. The presence of nucleated red cells and myelocytes in the blood and the occurrence of splenic erythropoiesis as indicated by the radioactive iron studies place this case in the category described by Lawrence et al (1953) as destined to develop myelofibrosis.

Thus, in this case the disease appears to be in a state of transition from polycythaemia vera to myelofibrosis.

Patient - J.McG.

Clinical Picture

This patient presented with exertional dyspnoea of one year's duration and following dental extraction about that time he had bled

White cell count per c.mm.	Neutrophils %	Eosinophils %	Basophils %	Lymphocytes %	Monocytes %	Myeloblasts %	Myelocytes %	Metamyelocytes %	Smudge cells %	Nucleated red cells per 100 white cells
11,000	70	3	0	23	3	0	0	1	1	0

Table 19 J.McG. - Differential white cell count

/considerably for five or six hours. Since then he had noticed a ready tendency to bleed or bruise. Pallor had been observed for four months and tiredness and lethargy for three months. On examination the mucous membranes were seen to be pale and the liver was found to be two finger breadths enlarged below the costal margin. The spleen was not palpably enlarged and no other findings of significance were detected.

Haematological Findings

The patient was very anaemic and the haemoglobin was only 3.6 g per cent. There was no evidence of iron deficiency but the red cells showed anisocytosis, poikilocytosis and some degree of macrocytosis. The white cell count was 11,000 per c.mm. and the platelet count 975,000 per c.mm. The differential white cell count was normal and the proportions of cells are seen in table 19. Marrow examination revealed a marked increase in megakaryocytes (fig.75) but the surprising feature was a myeloid hyperplasia to a degree

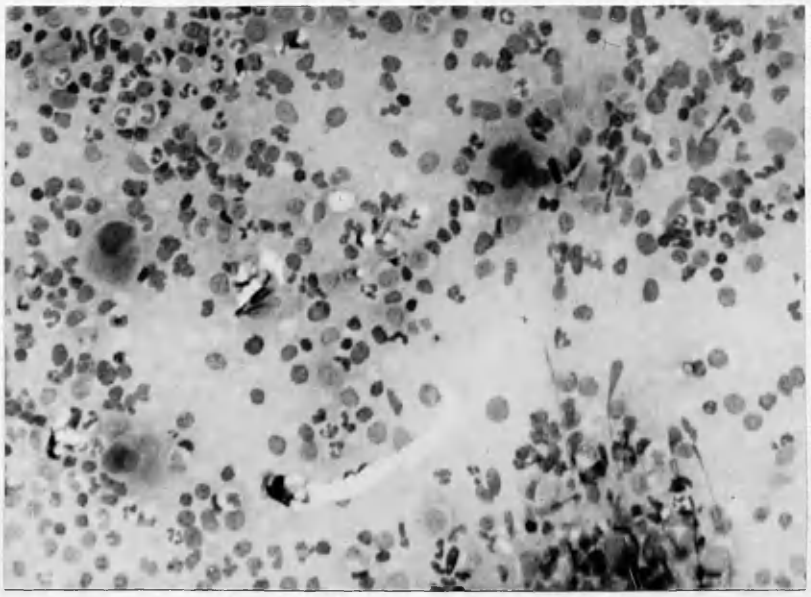

Fig. 75 Marrow histology in patient J.McG. showing megakaryocytic proliferation. (May-Grunwald-Giemsa x 210)

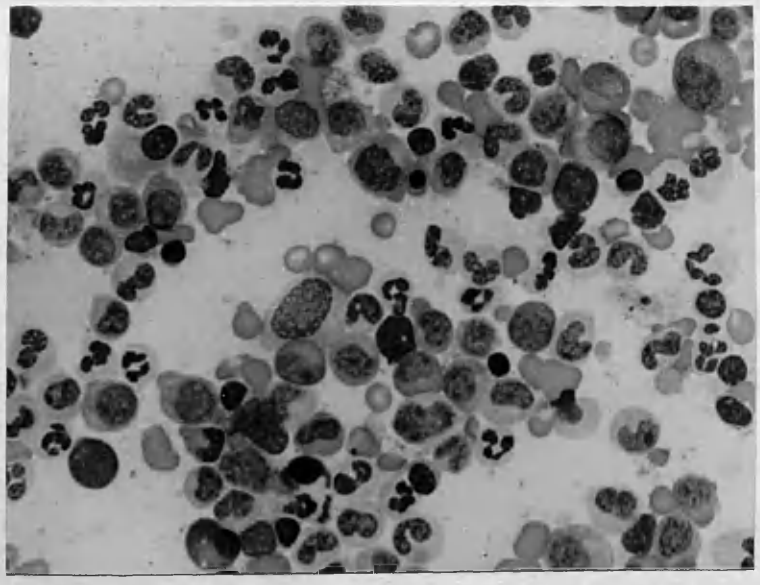

Fig. 76 Marrow histology in patient J.McG. showing myeloid hyperplasia. (May-Grunwald-Giemsa x 375)

/compatible with the diagnosis of chronic myeloid leukaemia (fig.76). There was no maturation arrest and white cells were seen at all stages of development but erythropoietic tissue was scanty.

Radioisotopic Investigations

The radioiron profile is seen in fig.77 and demonstrates a typical aplastic pattern with no evidence of splenic erythropoiesis. The iron utilisation is only 8 per cent and the red cell survival time is more or less normal with $T\frac{1}{2}$ of 24 days (fig.78).

Thus the anaemia is due to pure deficiency of red cell production and there is no significant increase of haemolysis.

Comment This patient presents a most interesting picture. The duration of the disease from the time of the first symptoms to the fatal outcome was only 18 months. The spleen was never palpable yet the histological appearance of the marrow was akin to that of chronic myeloid leukaemia. The white cell count however, was only

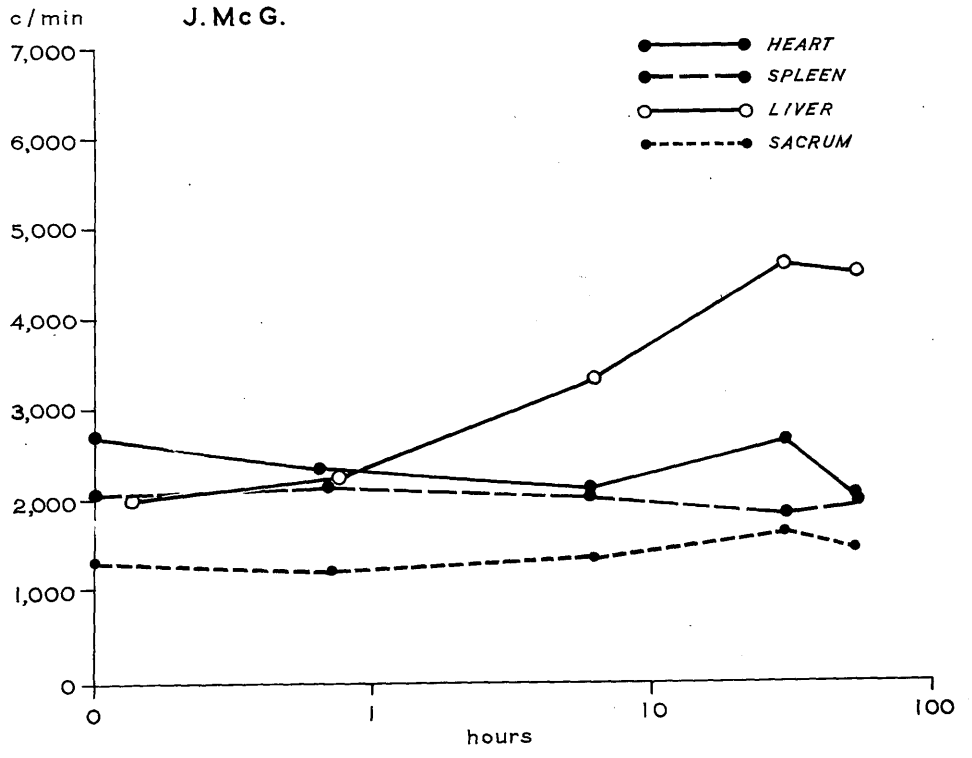

Fig. 77 Radioiron profile in patient J.McG. ('transition' disease)

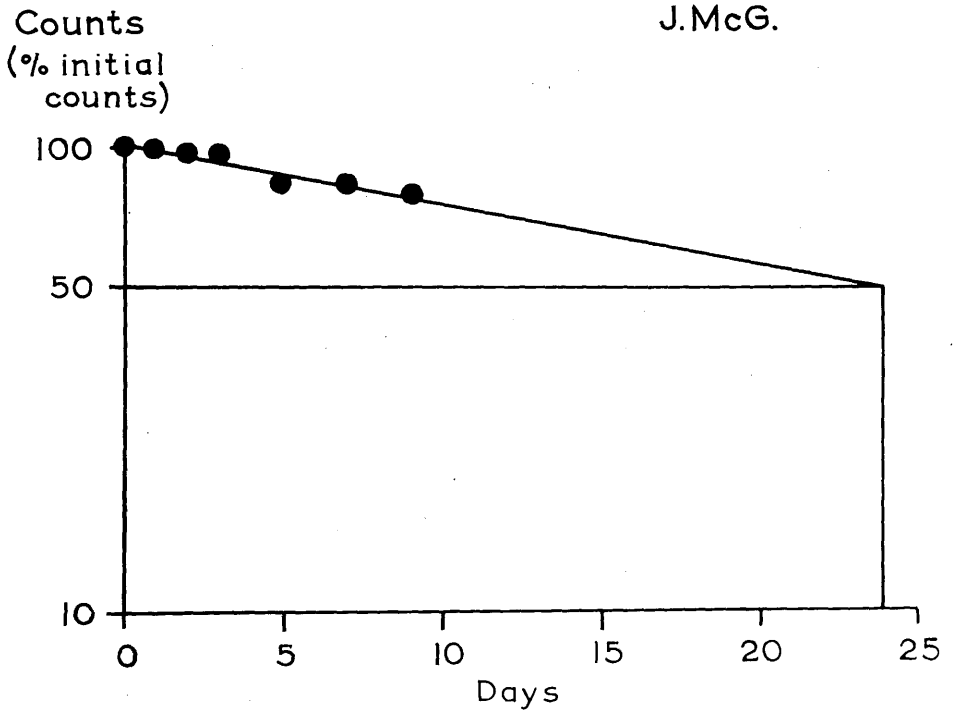

Fig. 78 Red cell survival by the radiochromium technique in patient J.McG.

/11,000 per c.mm. and the proportions of the various cells were normal. There is nothing to suggest hypersplenism to account for the normal white cell count; there is no increase in haemolysis of the red cells and the platelet count is in fact very high. It would be distinctly unusual to get a selective leucocytic destruction resulting in a normal differential white cell count in a case of true chronic myeloid leukaemia and it seems more likely that this is an aleukaemic or incipient chronic myeloid leukaemia.

The platelet count in chronic myeloid leukaemia may well be high but seldom reaches a level of one million per c.mm. and probably never reaches this level in the absence of a marked leucocytosis.

Thus the myeloproliferative process appears mainly to affect the megakaryocytes with a resultant high platelet count. The myeloid tissue also is affected but this proliferation has not yet resulted in any

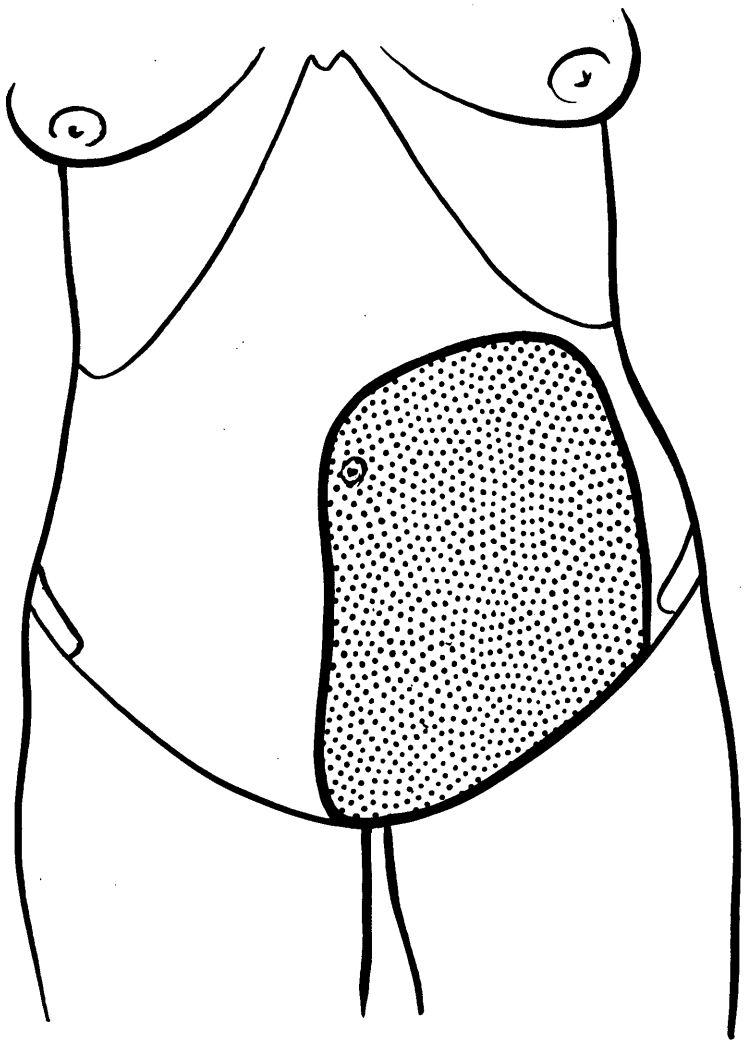

Fig. 79 Illustration of abdominal mass found on palpation in patient J.McD.

/abnormality of the white cells in the peripheral blood.

This disease, could, therefore, be classified as a transition state between megakaryocytic thrombocythaemia and chronic myeloid leukaemia.

Patient J.McD.

Clinical Picture

In 1956 this patient first presented with weakness and lassitude and was found to have marked hepatosplenomegaly. In 1959 she attended again with weakness, lassitude, breathlessness on exertion and swelling of the ankles. At this time, the spleen and liver were still palpable but the spleen appeared to be smaller than in 1956 and there was present below the spleen a large firm mass extending to the pelvis (fig.79). This mass appeared to be quite distinct from the spleen but no such mass had been noted in 1956. Great difficulty was found in deciding the nature of this mass which was considered to be either pelvic tumour or ectopic spleen.

White cell count per c.mm.	Neutrophils %	Eosinophils %	Basophils %	Lymphocytes %	Monocytes %	Myeloblasts %	Myelocytes %	Metamyelocytes %	Smudge cells %	Nucleated red cells per 100 white cells.
3,500	74	3	1	12	1	3	2	4	0	4

Table 20 J.McD - Differential white cell count.

/Haematological Findings

In 1956 the haemoglobin was reported to be 14.1 g per cent and the white cell count 16,000 per c.mm. but when the patient was seen for investigation in 1959 the haemoglobin had dropped to 7.3 g per cent and the M.C.H.C. to 28.1 per cent. There was then anisocytosis and poikilocytosis and some hypochromia of the red cells. The white cell count was 3,500 per c.mm. and the differential count is demonstrated in table 20; four nucleated red cells were seen per 100 white cells. The platelet count was low at 70,000 per c.mm. Marrow puncture yielded an almost 'dry tap' but histological examination of the aspirate showed about 50 per cent of the cells to be myeloblasts.

Radioisotopic Investigations

The radioiron profile is seen in fig.80. This shows some increase in the level of radioactivity of the marrow and the abdominal mass over the period of 100 hours but no significant alteration of the level of splenic radioactivity. Thus the abdominal mass

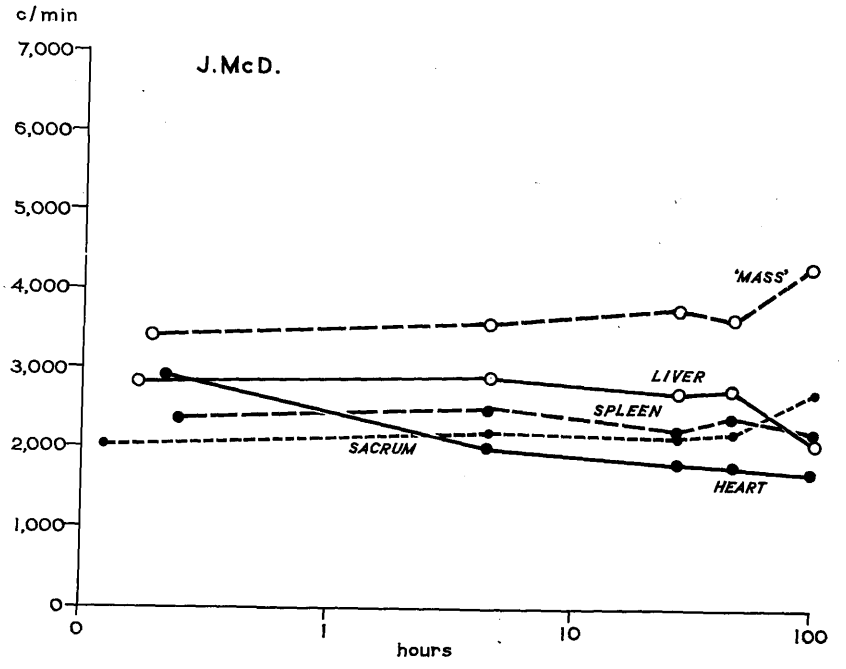

Fig. 80 Radioiron profile in patient J.McD. ('transition' disease)

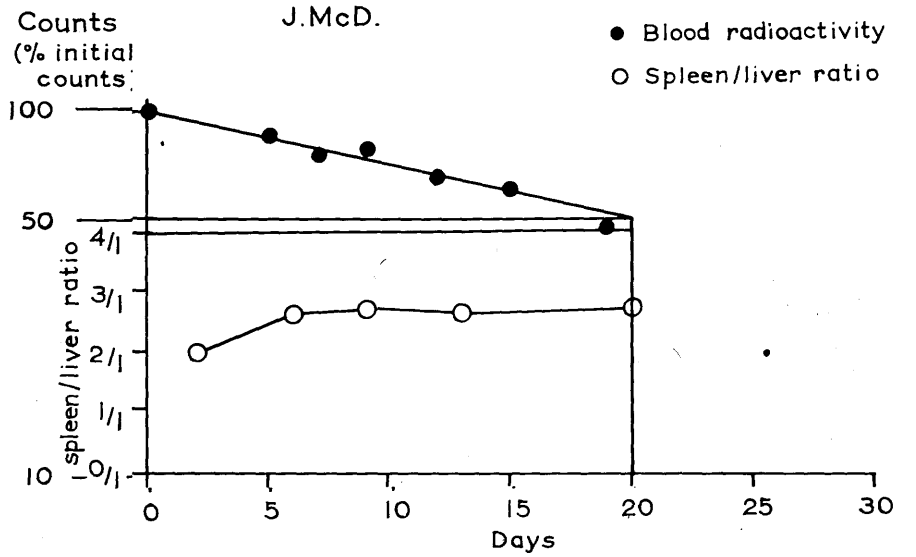

Fig. 81 Sequestration studies and red cell survival by the radiochromium technique in patient J.McD.

/appears to contain erythropoietic tissue and is in all probability ectopic spleen. Red cell survival time, $T\frac{1}{2}$, is 20 days and the mass/liver ratio at this time is 2.7/1 (fig.81). This confirms the impression that the mass is in fact spleen, and shows that in addition to producing red cells it sequesters and destroys red cells.

Thus the marrow is productive of red cells but the total red cell formation is diminished. There is increased haemolysis and the spleen, including the ectopic part, plays a part both in erythrocyte production and destruction. The anaemia appears to be due to combined hypoplasia and excessive haemolysis.

Comment

This interesting patient has possibly been a case of polycythaemia vera in 1956 and has now developed anaemia with leucopenia, thrombocytopenia and nucleated red cells in the peripheral blood. The abdominal mass is shown to be spleen (confirmed later at laparotomy (fig.82)) and is very large and

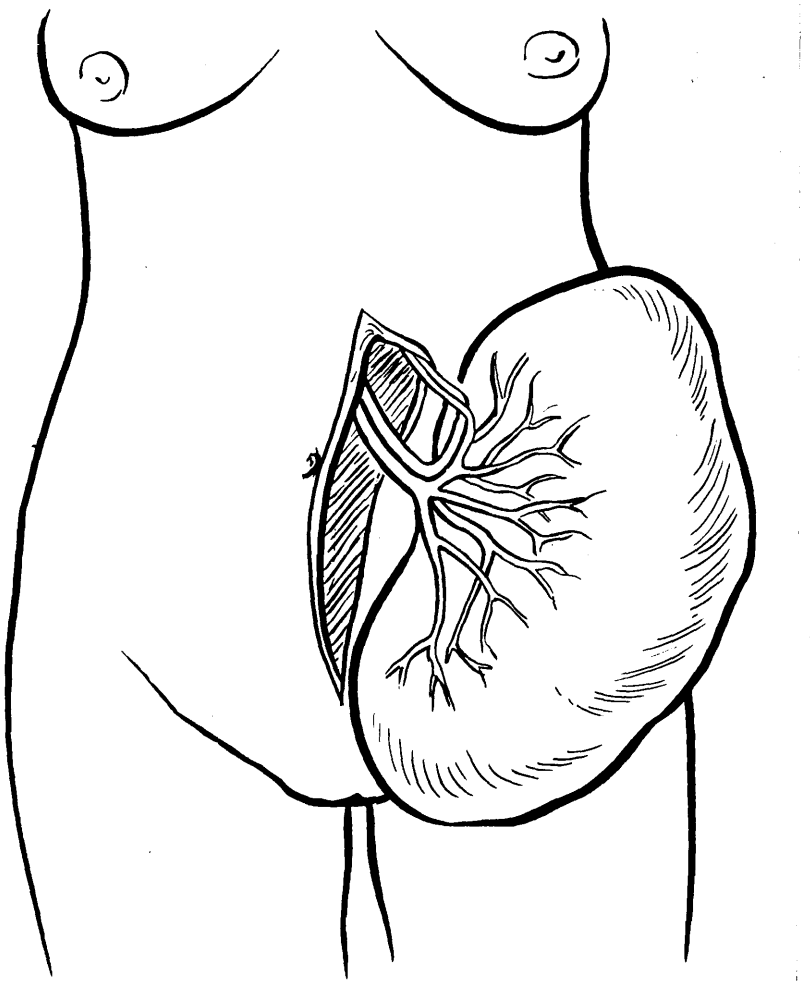

Fig. 82 Illustration of the findings at laparotomy in patient J.McD.

/this indicates that the patient is probably one of the type destined to develop marrow hypoplasia which is now confirmed by radioiron studies. This is not the whole picture, however, as the marrow contains many myeloblasts; it seems likely that the patient is in fact developing a leukaemic process. While there is excessive haemolysis and sequestration, marrow hypoplasia seems to be the major factor in the production of anaemia but whether the low white cell and platelet counts are related to diminished marrow activity or to excessive destruction is not known. In view of the red cell sequestration it appears probable that 'hypersplenism' is the cause.

Thus the patient appears to be a case of polycythaemia vera who has developed hypersplenism and marrow hypoplasia and is in the process of developing leukaemia.

Patient A.McG.

Clinical Picture

In 1946 this patient presented with pain and swelling of the legs and she had

/also noted excessive bleeding after dental extractions about this time. She was found to have a thrombophlebitis of the left lower leg. In 1950 she again attended and was then seen to be of ruddy complexion and the spleen was three fingerbreadths palpable below the left costal margin. Her condition was diagnosed as polycythaemia vera and she was thereafter treated by removal of one pint of blood at intervals over the next six years. By 1956 she had pain and swelling of her joints and small yellow-white deposits were seen at some joints. She complained also of weakness and tiredness. Physical examination revealed gout and a thrombophlebitis and both liver and spleen were three to four finger-breadths palpable below the costal margin. When seen for investigation in 1959 she had hypertension (blood pressure 190/100 mm.Hg) gout, and splenomegaly.

Haematological Findings

When the patient first presented in 1946 the haemoglobin was 17.1 g per cent

White cell count per c.mm.	Neutrophils %	Eosinophils %	Basophils %	Lymphocytes %	Monocytes %	Myeloblasts %	Myelocytes %	Metamyelocytes %	Smudge cells %	Nucleated red cells per 100 white cells
9,200	81	1	1	13	3	0	0	0	1	0

Table 21 A.McG. - Differential white cell count

/and the white cell count 12,200 per c.mm. By 1950 the haemoglobin had risen to 19.4 g per cent but in 1956 the patient was anaemic and the level was then only 11.1 g per cent and the white cell count 7,000 per c.mm. Platelets were noted to be abundant on examination of a blood film.

At the time of investigation in 1959 the haemoglobin was 10.0 g per cent and the M.C.H.C. 27.25 per cent. The white cell count was 9,200 per c.mm. and the differential count is seen in table 21. The platelet count was elevated to as much as 600,000 per c.mm. Bone marrow histology showed the appearances of some red cell hyperplasia, marked white cell hyperplasia and gross megakaryocytic proliferation.

Radioisotope investigations

The radioiron profile is seen in fig.83. There is only slight increase in the radioactivity of the marrow and none of the spleen. The iron utilisation is very low, only 17 per cent of the injected dose. Red

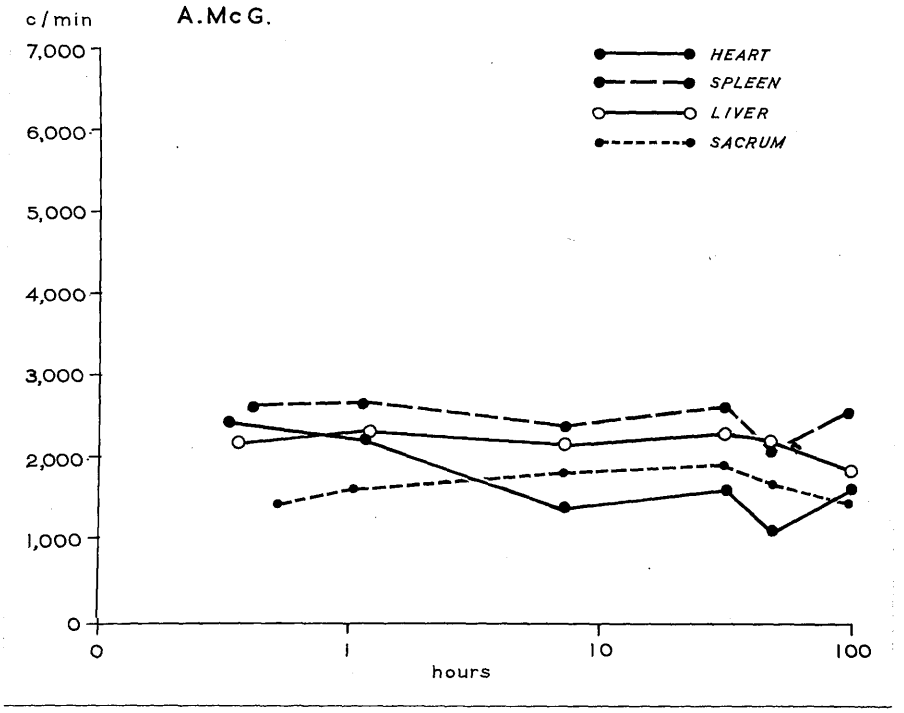

Fig. 83 Radioiron profile in patient A.McG. ('transition' disease)

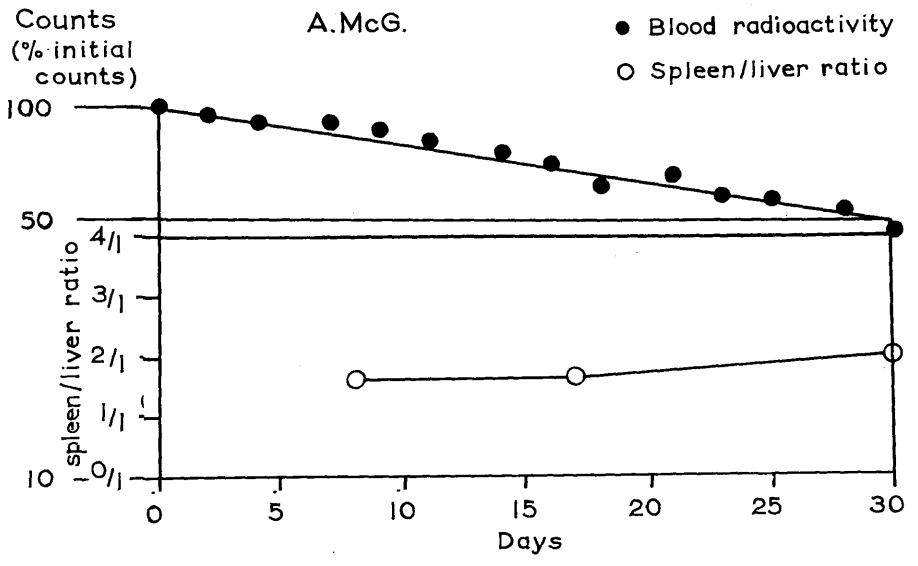

Fig. 84 Sequestration studies and red cell survival by the radiochromium technique in patient A.McG.

/cell survival $T\frac{1}{2}$ is 30 days and there is no significant sequestration of red cells by the spleen as the spleen/liver ratio at $T\frac{1}{2}$ is 1.9/1 (fig.84).

Thus the anaemia is due to diminished erythrocyte production and the spleen plays no significant role in either production or sequestration of red cells.

Comment

This patient appears to have been a case of polycythaemia vera and she has now entered an anaemic phase due to the development of functional hypoplasia of the marrow. Although the precursors of all three cellular elements of the blood are involved in the proliferative process in the marrow, the megakaryocytes principally are affected. As there are no immature cells in the blood and no splenic erythropoiesis the case is not as yet one of the type described by Lawrence (1953) but seems to be progressing towards this state. Furthermore, the marked megakaryocytic increase and the elevated platelet count suggest that thrombocythaemia may well be developing.

/ Thus, it seems likely that the disorder in this patient lies between megakaryocytic thrombocythaemia and 'spent' polycythaemia vera.

Discussion

Many of the so called 'myeloproliferative' diseases can be given a precise diagnosis. These diseases, therefore, differ in many ways and a different set of criteria is demanded for each disease. There is however, a considerable overlap of features and this has been the basis for the hypothesis of relationship of the myeloproliferative diseases. If this hypothesis is to be substantiated there must be more than a mere demonstration of similarity of disorders: if relationship is intimate then there should occur not uncommonly forms which possess features of myeloproliferative disease but do not satisfy the criteria for any one diagnosis. These 'transitional' forms should resemble closely two of the recognised diseases and yet apparently lie between the two. Four such cases have been described and

/comment has been made on each. One appears to lie between polycythaemia vera and leukaemia, one between polycythaemia vera and myelofibrosis, one between megakaryocytic thrombocythaemia and chronic myeloid leukaemia and one between spent polycythaemia vera and megakaryocytic thrombocythaemia. Furthermore, description has already been made of two cases which lie between chronic myeloid leukaemia and myelofibrosis.

Thus, of twenty seven cases investigated, six are transitional forms of myeloproliferative disease and represent a fairly high incidence of 'impure' forms. The not uncommon occurrence of such 'linking' forms is considered strong evidence in favour of relationship of the myeloproliferative disorders.

Summary

Four cases are described which do not conform to the criteria for any precise diagnosis. These cases resemble more than one disease and apparently lie between these diseases. Such cases appear to be 'transition' forms as described by Dameshek (1951). The occurrence of such 'linking' forms is considered important evidence in favour of the relationship of the various myeloproliferative disorders.

SUMMARY OF

SECTION III

Cases of polycythaemia vera, erythraemic myelosis and megakaryocytic thrombocythaemia are described and the results of the investigations discussed.

There are many clinical and haematological features in common but a precise diagnosis can be determined by careful study of these features.

The radioisotopic patterns show that there is no pattern of erythrocyte production and destruction which is specific for any of these diseases though markedly increased haemolysis is not common in polycythaemia vera. 'Ineffective' erythropoiesis is a feature of note in polycythaemia vera and in erythraemic myelosis.

Four cases are discussed which appear to be transition forms, resembling one disease in some ways and another disease in other ways. No precise diagnosis can be made in these cases.

The results of the investigations and the not uncommon occurrence of 'transition'

/forms is considered strong evidence in favour of relationship of the whole group of myeloproliferative disorders.

THE MYELOPROLIFERATIVE DISORDERS: AN INVESTIGATION INTO THEIR NATURAL HISTORY AND AETIOLOGY.

by

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VOLUME II

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'V O L U M E II'

SECTION IV

STUDIES IN MYELOPROLIFERATIVE DISEASE

Chapter 11

MEGALOBLASTIC ERYTHROPOIESIS

IN

MYELOPROLIFERATIVE DISEASE

The anaemia of myeloproliferative disease has been shown to be due mainly to diminished red cell production or to an increased rate of haemolysis or to a combination of these mechanisms. In some states, however, comment has been made on the occurrence of a histologically hyperplastic marrow in association with a diminished effective cellular production and this has been compared with the findings in pernicious anaemia. There are several possible explanations for this but the most likely mechanism appears to be a failure of release of the cells from the marrow cavity. The resemblance to pernicious anaemia could be carried a step further in the presence of megaloblastic erythropoiesis and this finding is reported in Di Guglielmo's disease (Baldini et al, 1959, Adams and Seaton, 1960) and in other forms of myeloproliferative disease (Wellington and Whitcomb, 1960). The occurrence of megaloblastic erythropoiesis suggests a defect most probably in the

/absorption or metabolism of vitamin B_{12} or of folic acid. If this defect is in the absorption of vitamin B_{12} or folic acid then a satisfactory response should be obtained by the parenteral administration of the deficient substance. Some workers on myelofibrosis (Hutt, 1950, Hickling, 1953) record no response to injections of liver extract which suggests that the defect is more likely to be in the metabolism of one or other or both of these substances. On the other hand Croft (1956) records a patient with a megaloblastic anaemia who responded on two occasions to injections of liver extract. Thus, knowledge of megaloblastic erythropoiesis in myeloproliferative disease is limited and information on other cases with megaloblastic erythropoiesis may provide some further insight into this interesting state.

Three cases with suspected abnormality of vitamin B_{12} or folic acid metabolism are described.

/Case Report 1 - Myelofibrosis

Patient M.C. first attended hospital in 1949 because of lethargy and abdominal swelling. The spleen was markedly enlarged, sternal puncture yielded a 'dry tap' and there was gastric achlorhydria. Blood films revealed a macrocytic picture. She was treated at that time by thyroid extract without benefit. Three months later she was readmitted and examination of the blood then revealed a haemoglobin of 65 per cent, a red cell count of 1.8 million per c.mm., a colour index of 1.8, a white cell count of 5,200 per c.mm. and a reticulocyte count of 1 per cent. Blood films again showed macrocytosis and poikilocytosis. She was then treated with 'Anahaemin' and the reticulocytes rose to 12 per cent on the seventh day after the first injection. Within two months the haemoglobin was recorded as 100 per cent but the blood film remained macrocytic. Thus, she responded to 'Anahaemin' at an early stage in the development of

/myeloproliferative disease.

The reason for this response remains obscure but it seems likely that the patient was at that time suffering from a deficiency of vitamin B_{12} or folic acid. In the more advanced stages of myelofibrosis, however, when anaemia returned there was no response to vitamin B_{12} and no further evidence of vitamin B_{12} or folic acid deficiency. Thus, there may be a stage early in the development of myelofibrosis, where deficiency of vitamin B_{12} or folic acid occurs.

Case report 2 - myelofibrosis

Patient M.G.B. first attended hospital in 1956 with abdominal discomfort and lethargy. The spleen was markedly enlarged. Examination of the blood revealed a haemoglobin of 5.1 g per cent, a red cell count of 1.3 million per c.mm., a colour index of 1.3, a haematocrit of 15 per cent, a mean corpuscular volume of 115 cu and a reticulocyte count of 1 per cent. Marrow puncture yielded a dry tap but examination of the peripheral

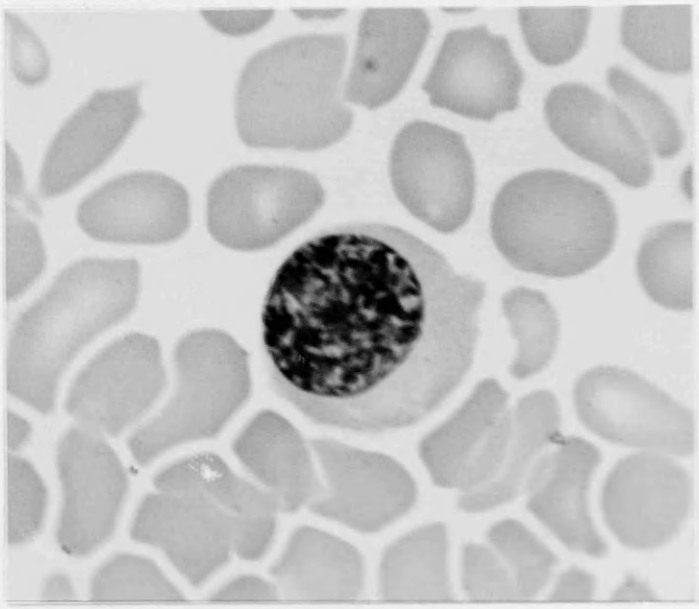


Fig. 85 Megaloblast in the peripheral blood in patient M.G.B. (Leishman x 1500).

/blood films revealed the presence of some megaloblasts (fig.85). Free acid was found in the gastric secretion. The serum vitamin B_{12} level (Hutner et al, 1956) was 25 uug/ml. Following administration of vitamin B_{12} the reticulocyte count rose to a maximum of only 6 per cent by the tenth day. The absorption of radioactive vitamin B_{12} (^{58}Co) was estimated by the urinary excretion method (Schilling, 1953) as described by Adams and Seaton (1961). This gave normal results both with and without intrinsic factor, 23.8 per cent and 23.2 per cent respectively of the dose given being excreted in the urine within 24 hours.

The low serum vitamin B_{12} level is therefore not attributable to malabsorption and is an unusual finding in myeloproliferative disease as Mollin and Ross (1955) have described very high levels in cases of chronic myeloid leukaemia and moderately high levels in a few cases of myelofibrosis. The low serum B_{12} level, however, and the presence

Examination	Result (uug/ml.)
Total serum vitamin B_{12}	150
Free serum vitamin B_{12}	25
Bound serum vitamin B_{12}	125
Serum vitamin B_{12} binding capacity.	845

Table 22 Results of serum vitamin B_{12} investigation by microbiological assay in patient G.McF.

/of megaloblasts in the peripheral blood before but not after administration of vitamin B_{12} suggest an abnormal metabolism or excessive utilisation of vitamin B_{12}. The cause of this is probably related to the basic myeloproliferative process but the mechanism of its production is not clear.

Case Report 3 - Di Guglielmo's disease

Patient G. McF. first attended hospital because of symptoms of anaemia. The haemoglobin level was 7.4 g per cent, haematocrit 23 per cent and the reticulocyte count 4 per cent. Sternal marrow examination revealed predominantly megaloblastic erythropoiesis and free acid was present in the gastric juice. Serum vitamin B_{12} levels were normal (table 22) and radioactive vitamin B_{12} (^{58}Co) absorption (Schilling test) also appeared normal with a 24 hour urinary excretion of 18.4 per cent of the dose administered. Folic acid clearance also was normal (table 23).

Thus there was no demonstrable deficiency

Time after injection (min).	Result (m ug/ml).
3	208
15	70
30	50

Table 23 Folic acid clearance in patient G.McF. (after intravenous injection of 0.9 mgm. folic acid).

/of vitamin B_{12} or of folic acid and the administration of these substances had no effect.

There is little information on megaloblastic erythropoiesis in Di Guglielmo's disease but Baldini et al (1959) record megaloblastic erythropoiesis in 11 cases. Dameshek and his co-workers (Dameshek, 1958; Dameshek and Baldini, 1958; Baldini et al, 1959) stress the importance of megaloblastosis in relation to the aetiology of the disease. It is generally accepted that megaloblastic erythropoiesis represents a deficiency or deficient utilisation of vitamin B_{12} of folic acid or their derivatives at some stage in the development of the red cells. Serum vitamin B_{12} levels appear to have been estimated in only 3 other cases (Baldini et al, 1959) and were high whereas in this case the result was normal.

In addition there was no evidence of abnormality in the binding of vitamin B_{12} to the serum protein. The folic acid clearance

/was normal thus excluding folic acid deficiency (Chanarin et al, 1958).

There were therefore none of the usual abnormalities of vitamin B_{12} or folic acid metabolism but defective utilisation is not excluded. In this way the megaloblastosis of Di Guglielmo's disease appears to resemble that caused by anticonvulsant drugs (Girdwood and Lenman, 1956; Chanarin et al, 1958a) although these anaemias respond to folic acid or occasionally to vitamin B_{12}. Thus the megaloblastosis of Di Guglielmo's disease may be due to a defect in the metabolism of vitamin B_{12} or folic acid at a cellular level.

Summary

Three cases with evidence of abnormality of vitamin B_{12} or folic acid metabolism are described. Two of them were patients with myelofibrosis and the other had Di Guglielmo's disease.

In myelofibrosis the abnormality appears to be due to vitamin B_{12} deficiency or metabolism.

In Di Guglielmo's disease it seems likely that the defect is at a cellular level and may concern either vitamin B_{12} or folic acid metabolism.

Chapter 12

INFECTION

AND

HYPOGAMMAGLOBULINAEMIA

It has for many years been apparent that patients with leukaemia are particularly susceptible to infection. The reason for this is still not clear; the increased incidence of infection has been attributed to poor state of health of these patients especially in the later stages of the disease. The high white cell counts of chronic myeloid and chronic lymphatic leukaemia afford no protection to these patients and this presumably is due to the fact that the great majority of the cells are abnormal.

It has been noted in recent years that hypogammaglobulinaemia, whether primary or secondary, leads to increased susceptibility to infection. Jim and Reinhard (1956) reported one patient with chronic lymphatic leukaemia and agammaglobulinaemia. Since then several reports of secondary hypogammaglobulinaemia in the leukaemias and in myeloproliferative disease have been published. (Jim, 1957; Hudson and Wilson, 1957; Teitelbaum et al, 1959). In view of the possibility of this

No	Age (yrs)	Sex	Albumin (g/100ml)	Globulin (g/100ml)	Alpha 1 globulin (mgm/100ml)	Alpha 2 globulin (mgm/100ml)	beta globulin (mgm/100ml)	gamma globulin (mgm/100ml)
1	36	M	5.5	1.7	168	447	536	549
2	21	F	5.6	1.9	151	484	539	726
3	23	F	5.0	1.4	148	325	445	482
4	50	F	5.0	1.5	110	351	458	581
5	23	F	5.5	2.3	217	487	595	1001
6	27	M	5.0	2.1	177	434	668	821
7	36	M	4.8	2.0	128	384	574	914
8	32	M	5.6	2.2	163	451	662	924
9	29	M	5.3	1.9	154	391	595	760
10	48	M	5.0	2.2	241	502	694	763
11	30	M	5.4	1.5	121	359	495	525
12	31	M	4.9	2.0	122	428	616	834

Table 24 Serum protein components in 12 normal control subjects.

/occurrence in myeloproliferative disease and its probable relation to infection, a study of the serum proteins was carried out on 12 normal controls and on 10 patients with myeloproliferative disease.

Results

Control subjects

The results in the 12 healthy normal adults aged 21 years to 50 years, who acted as controls are seen in table 24. The serum gammaglobulin in normal subjects is over 500 mgm. per 100 ml. (Cohn et al, 1944; Hawk et al, 1947) and the figures obtained in this series are in agreement. Only one control subject had a gammaglobulin level of less than 500 mgm. per cent with an estimated 482 mgm. per cent and this slight difference is within the error of the method.

Myeloproliferative disease

The results in the 10 patients with myeloproliferative disease are seen in table 25. Two patients with polycythaemia vera, one patient with 'transition' myeloproliferative disease and one patient with chronic myeloid

Patient	Diagnosis	Age	Sex	Albumin (g/100ml)	Total globulin (g/100ml)	Alpha 1 globulin (mgm/100ml)	Alpha 2 globulin (mgm/100ml)	beta globulin (mgm/100ml)	gamma globulin (mgm/100ml)
C.L.	C.M.L.	47	F	4.3	2.2	301	416	518	965
J.C.	Mf.	45	F	4.7	1.3	199	250	384	467
T.Q.	Mf.	56	F	3.0	1.4	217	276	350	557
M.C.	Mf.	71	F	2.9	1.0	185	231	298	286
A.S.	Mf.	49	F	4.0	1.4	181	305	381	533
M.G.B.	Mf.	63	F	3.5	1.7	263	301	408	728
M.B.	Int.	34	F	3.3	1.6	516	397	397	290
A.McA.	P.V.	70	M	4.0	1.8	219	272	454	855
J.C.	P.V.	50	M	4.5	2.2	190	380	600	1030
J.McD.	M.P.	64	F	2.9	2.8	389	325	468	1618

Table 25 Serum protein components in 10 patients with myeloproliferative disease. (C.M.L.=chronic myeloid leukaemia; Mf.= myelofibrosis; Int.= 'Intermediate' disease; P.V.= polycythaemia vera; M.P.= transition myeloproliferative disease).

/leukaemia had normal levels of gammaglobulin. One patient with 'intermediate' disease was examined and found to have hypogammaglobulinaemia with a level of 290 mgm. per cent. Of 5 patients suffering from myelofibrosis, 2 had hypogammaglobulinaemia with levels of 467 mgm. per cent and 286 mgm. per cent.

Thus, hypogammaglobulinaemia was found in 3 out of 10 patients with myeloproliferative disease.

There were only two instances of bacterial infection during the period of observation of these 10 patients and both infections occurred in patients with hypogammaglobulinaemia.

Case reports of patients with bacterial infections

1. Patient M.B., with 'intermediate' disease, as shown on examination of peripheral blood and bone marrow trephine, had had a large spleen for at least 3 years and developed a haemolytic anaemia which required frequent blood transfusion. As splenic irradiation and steroid therapy failed to control the haemolytic process splenectomy was carried out. Following this operation the patient improved

/initially but later developed pyrexia, cough and a purulent sputum. The operation wound became infected and culture of the pus gave a heavy growth of coagulase positive staphylococci. She then developed an abscess in the left subcostal region and pus aspirated from this site gave a similar growth. At this time her serum albumin was 3.3 g per cent, serum globulin 1.6 g per cent and the gammaglobulin level was 290 mgm. per cent. Despite antibiotic therapy her condition deteriorated steadily and she died 2 months after splenectomy.

2. Patient M.C., with myelofibrosis, had been known to have gross splenomegaly for 10 years. She had been in hospital on several occasions for treatment of her myelofibrosis. In 1959 she was readmitted because of nausea, vomiting, lassitude, pyrexia ($104^{\circ}F$) and rigors. A growth of E. Coli was obtained from urine and blood cultures on several occasions. At this time her serum albumin was 2.9 g per cent,

/serum globulin 1.0 g per cent and the gammaglobulin level was 286 mgm. per cent. She was treated with chloramphenicol and her symptoms subsided and the temperature returned to normal. She remained well for a further 3 months before eventually dying in severe shock after splenectomy.

Discussion

The increased incidence of bacterial infections in the leukaemias is well known and although this could be related to abnormalities of the leucocytes it seems likely that low gammaglobulin also plays an important part in some cases. The occurrence of hypogammaglobulinaemia in association with the only two infective episodes in the 10 patients examined is much in favour of a direct relationship. It is possible that infection and toxaemia could suppress gammaglobulin formation but this seems improbable for two reasons; firstly, infection is the usual stimulus to increased production of gammaglobulin and secondly, in primary hypogammaglobulinaemia the low levels persist after eradication of

/infection and this has been observed also in hypogammaglobulinaemia secondary to leukaemia and reticuloses (Teitelbaum et al, 1959). It seems more probable that the hypogammaglobulinaemia is a result of the disease and when it occurs, leads to an increase in the individual susceptibility to infection.

A striking feature is the severity of the infections encountered. One patient died as a result of bacterial infection but the other patient survived despite a severe urinary tract infection and septicaemia. It is said that hypogammaglobulinaemia occurs late in the disease (Ultmann et al, 1959) and this is in agreement with the 3 examples found in this series. All three patients are now dead. One had myelofibrosis for almost 11 years and the other for at least 3 years and the patient with 'intermediate' disease had attended hospital for over 3 years. All three died at a stage when treatment had little or no effect on the primary process and it does appear that they were all advanced cases.

/ In view of the not uncommon occurrence of hypogammaglobulinaemia in myeloproliferative disease and of the severity of infection which may ensue, routine investigation of the serum proteins in all cases with bacterial infection or unexplained pyrexia is desirable.

Summary

Serum protein electrophoresis was carried out on 10 cases of myeloproliferative disease and hypogammaglobulinaemia was found in 3 patients; two of these had myelofibrosis and the third was classed as 'intermediate' disease.

The only two instances of bacterial infection encountered in the 10 patients occurred in patients with hypogammaglobulinaemia.

The severity of the infections which may be associated with hypogammaglobulinaemia is stressed and it is thought that this is more likely to occur in advanced cases of myeloproliferative disease.

Chapter 13

URIC ACID STUDIES

AND GOUT

The occurrence of gout in myeloproliferative disease dates back at least to 1889 when Duckworth included a probable case of chronic myeloid leukaemia in his treatise on gout. This association was thought to be coincidental and more recently Forkner (1938) considered the simultaneous occurrence of gout and leukaemia to be so rare that any real relationship was doubtful. Hickling (1937), however, reported cases of non leukaemic myelosis with gouty arthritis and hyperuricaemia; later he wrote of 9 cases with gout, splenomegaly and immature cells in the circulating blood and observed that 5 of these had been polycythaemic at some stage (Hickling, 1953a). Further examples of this association of gout with polycythaemia vera were described by Beattie and Withey (1953). The occurrence of gout in myeloproliferative disease extends also to myelofibrosis and myelosclerosis and Korst et al (1956) found 5 of 14 patients to have blood uric acid levels of over 7 mgm.per cent; 2 of these patients had

Patient	Diagnosis	Gout	Serum uric acid (mgms.%)
M.G.B.	Mf.	-	8.7
J.C.	Mf.	+	10.5
A.McA.	P.V.	+	8.1
F.N.	P.V.	-	9.2
J.C.	P.V.	+	8.5
J.N.	T	-	9.4
A.McG.	T	+	9.0

Table 26 The incidence of gout in the 7 patients with elevated serum uric acid levels.
(Mf. = myelofibrosis; P.V. = polycythaemia vera; T = 'transition' disease).

/gout. Hickling (1953) reported 2 cases of myelosclerosis with gout and it is of interest that one of these patients had attacks of gout after each of 7 courses of splenic irradiation. Leonard et al (1957) record another such patient who developed gout after X-ray treatment for polycythaemia vera.

The occurrence of gout in myeloproliferative disease is related to the increased uric acid metabolism in these patients. The precipitation of attacks of gouty arthritis by X-ray treatment probably is due to the rapid breakdown of cells and their nucleic acids, the excessive release of uric acid and the consequently high blood uric acid levels.

Serum uric acid levels and urinary uric acid excretion were estimated in 24 patients with myeloproliferative disease.

Results

1. Gout and serum uric acid levels

A serum uric acid level of over 7 mgm. per cent was found in 7 of the 24 patients examined and 4 of these 7 had gout (table 26).

Patient	Urinary uric acid excretion	
	mgm./24 hours	mgm./Kg/24 hours
A.McA.	657	11.1
F.N.	413	6.0
M.S.	603	10.0
J.C.	394	4.8
Mean	517	8.0

Table 27 Urinary uric acid excretion in 4 patients with polycythaemia vera

Patient	Urinary uric acid excretion	
	mgm./24 hours	mgm./Kg./24 hours
A.S.	755	14.0
M.G.B.	1500	23.1
R.C.	596	9.6
S.H.	802	14.8
J.G.	983	11.8
J.C.	346	5.2
M.C.	950	17.6
T.Q.	1053	22.4
Mean	876	14.8

Table 28 Urinary uric acid studies in 8 patients with myelofibrosis.

/Thus, a high serum uric acid level did not necessarily indicate gouty arthritis but gouty arthritis always was associated with a high serum uric acid level. Of the 4 patients with gout two had polycythaemia vera, one had myelofibrosis and the other was classified as 'transition' myeloproliferative disease.

Gout and high serum uric acid levels are obviously not related to the level of the peripheral blood white cell count; in chronic myeloid leukaemia where the white cell counts numbered hundreds of thousands there was no case of gout and no raised serum uric acid level; and in polycythaemia vera where the highest white cell count was 40,000 per c.mm. three patients had elevated serum uric acid levels and two of these had gout.

2. Urinary uric acid excretion

Polycythaemia vera

The results are seen in table 27. The mean 24 hour urinary uric acid excretion is 517 mgm. and the mean 24 hour excretion per kilogram of body weight is 80 mgm. These

Patient	Urinary uric acid excretion	
	mgm./24 hours	mgm./Kg/24 hours
A.R.	1115	13.5
C.L.	861	14.8
E.W.	953	19.9
J.L.	1260	17.0
M.M.	1773	37.7
Mean	1192	20.6

Table 29 Urinary uric acid studies in 5 patients with chronic myeloid leukaemia

Patient	Urinary uric acid excretion	
	mgm/24 hours	mgm/Kg/24 hours
M.B.	960	15.5
W.M.	220	3.4
J.McG.	452	8.2
J.N.	1067	18.1
J.McD.	596	9.9
A.McG.	533	7.7
Mean	488	10.5

Table 30 Urinary uric acid studies in 6 patients with 'intermediate' or 'transition' disease

/values are a little above the mean normal levels of 399 mgm. per 24 hours and 8 mgm. per kilogram of body weight per 24 hours. (Sandberg et al, 1956) but there is overlap of the individual levels.

Myelofibrosis

The results are seen in table 28. There is a wide range of individual results from within normal limits to well above normal but the mean 24 hour excretion is 876 mgm. and the mean 24 hour excretion per kilogram of body weight is 14.8 mgm. Thus, both these values are well above the normal and much higher than the levels found in polycythaemia vera.

Chronic myeloid leukaemia

The results are seen in table 29. Again there is a wide range of individual results but the mean results are well above normal. The mean 24 hour excretion is 1192 mgm. and the mean 24 hour excretion per kilogram of body weight is 20.6 mgm.

Thus, the mean excretion in chronic myeloid leukaemia is greater than in either polycythaemia

/vera or myelofibrosis.

'Intermediate' and 'transition' cases

The results are seen in table 30. As might be expected with the wide variety of disorders included in this group the results range from normal to very high. The mean value for 24 hour excretion is 488 mgm. and for 24 hour excretion per kilogram of body weight is 10.5 mgm.

Megakaryocytic Thrombocythaemia

The one patient with megakaryocytic thrombocythaemia had a 24 hour excretion of 625 mgm. i.e. an excretion of 13.3 mgm. per kilogram of body weight per 24 hours. Thus both values are higher than normal.

Discussion

The relationship between gout and myeloproliferative disease is well known but while gout is obviously secondary to elevated blood uric acid levels, although not a necessary accompaniment of them, the problems of uric acid metabolism in these diseases are not yet solved. It is doubtful

/if many conclusions are justified from the results obtained in this small series as many factors influence the findings and affect their interpretation. Firstly, urinary excretion of uric acid will partly depend on renal function which is known to become impaired in some cases of long standing gout with elevated blood uric acid levels; secondly, the contribution to uric acid metabolism by non-haemopoietic tissue is not known; thirdly, the peripheral blood white cell count is not necessarily a true reflection of total white cell mass; and, fourthly, the white cell survival time in these cases is not known. For these reasons, impressions only are gained from studies of this type and additional information is required before these can be substantiated.

The total urinary excretion of uric acid is shown to be very high in chronic myeloid leukaemia, moderately raised in myelofibrosis and only slightly increased in polycythaemia vera. The same order is maintained when the

/figures are corrected for the influence of body weight. This suggests that the white cell count may be related to the urinary uric acid excretion but, as already mentioned, the occurrence of gout is not related to white cell count. Further, the mean serum uric acid level is greatest in polycythaemia vera, next in myelofibrosis and least in chronic myeloid leukaemia i.e. the reverse order; this, however, is the order of the frequency of gout as a complication of these diseases.

It is suggested that the differences shown to occur in uric acid excretion and blood uric acid levels in the disorders studied may be related to fundamental differences in the disease process. However, not only is there some overlap, but the wide scatter of results in the 'intermediate' and 'transition' group of cases gives an impression of some common factors which suggest relationship between the disorders.

Summary

Urinary uric acid excretion and serum uric acid levels were estimated in 24 patients with myeloproliferative disease. The relationship of gout to uric acid metabolism is discussed.

Different patterns are seen to occur in different disorders. There is some overlap which, when considered with the wide scatter of patterns in 'transition' cases, suggests that although each disease may be a separate entity there are probably factors common to all.

Chapter 14

RADIOLOGICAL FEATURES OF MYELOPROLIFERATIVE DISEASE

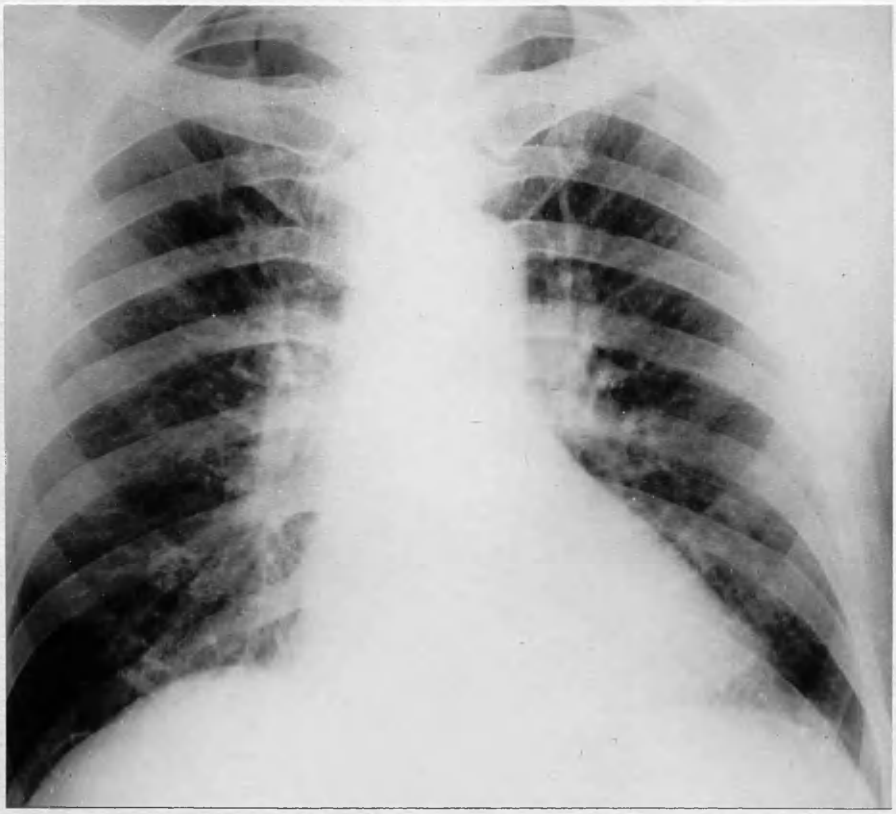


Fig. 86 Chest X-ray in polycythaemia vera.

(Patient J.C.)

There are few radiological features of importance in the myeloproliferative group of diseases with the exception of myelofibrosis. In the other members of this group any radiological features present are usually secondary to the disease rather than part of the disease.

Thus, in polycythaemia vera the increased blood volume may lead to increased pulmonary vascular markings and may also, as a result of the increased cardiac work, produce an enlargement of the cardiac shadow. The cardiac size may sometimes be increased because of systemic hypertension. Of the five patients with polycythaemia vera, cardiomegaly was noted in two, and increased pulmonary vascular markings in three patients. These appearances can be seen in fig. 86. Gout is occasionally seen in polycythaemia vera, secondary to increased uric acid metabolism, and was found clinically and radiologically in two patients. Slight osteoporosis was noted in two patients but is not assumed to be related to the

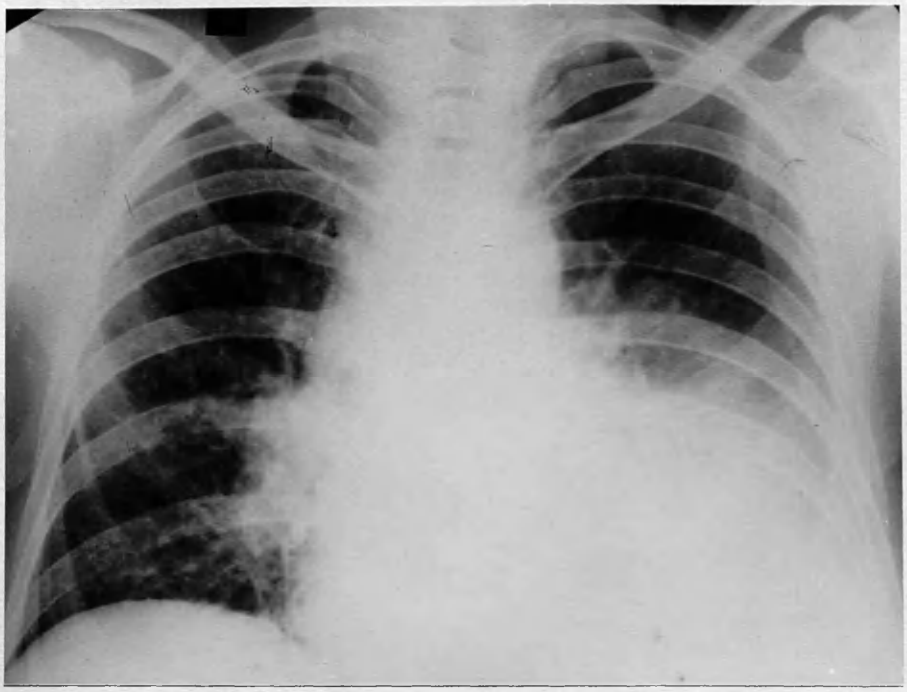


Fig. 87 Chest X-ray in patient M.M. with chronic myeloid leukaemia.

/diagnosis of polycythaemia vera.

No radiological abnormalities were found in the patients with Di Guglielmo's disease and megakaryocytic thrombocythaemia. Skeletal abnormalities are rare in chronic myeloid leukaemia. Craver and Copeland (1935) in a study of 82 cases of myeloid leukaemia found only one patient with radiological changes. This patient, a female aged 59 years, had a white count up to but never exceeding 34,000 per c.mm., myelocytes were a constant finding in the peripheral blood and the X-rays showed mottling at the upper ends of the femur. Thus, even this one patient was probably a case of myelofibrosis. None of the 5 cases in this study had radiological changes in the skeleton although X-ray of the chest in one patient revealed a left sided pleural effusion, mottling of the right lung and an enlarged right hilum (fig.87). These appearances indicated extension of the leukaemic process to the hilar glands and to the pulmonary parenchyma. At this time, of course, the

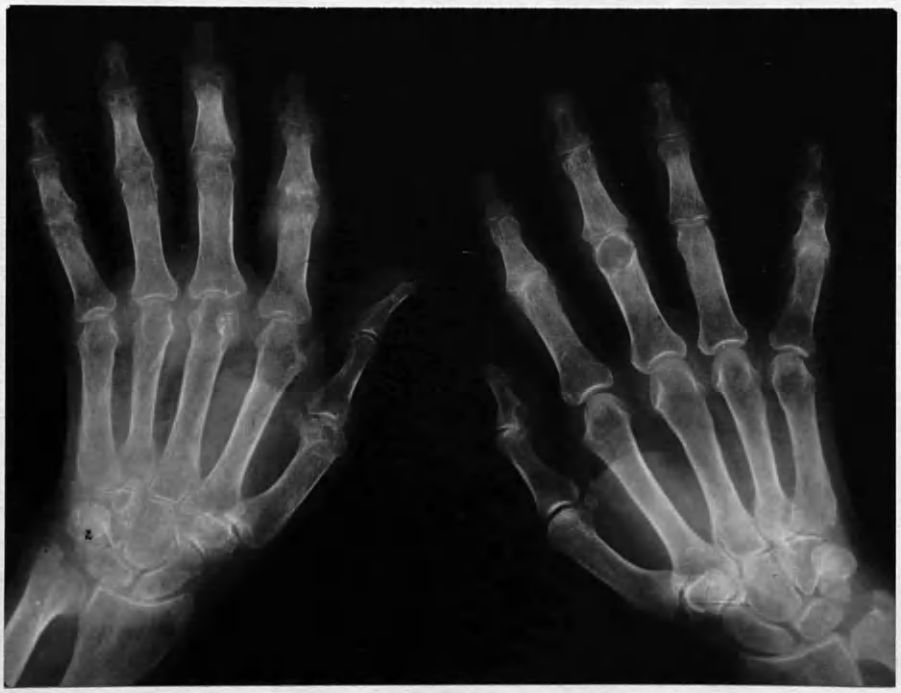


Fig. 88 X-ray appearances of gout in patient A.McG. with 'transition' disease.

/disease was in its terminal stages.

In the radiological study of the 'transition' myeloproliferative diseases no skeletal abnormalities were found apart from the appearances of gout in one patient (fig.88).

The most interesting myeloproliferative disorder from the radiological point of view is, of course, myelofibrosis. Radiological changes are common in this disease and are usually found in one third to one half of the cases examined. Rosenthal and Erf (1943) found radiological changes in 6 of 17 patients, Korst et al (1956) in 11 of 22 patients and Mulcahy (1957) in 9 of 19 patients investigated. The bones most frequently affected are those constituting the central part of the skeleton and changes are commonly seen in the ribs, vertebrae, pelvis, femora and humeri. Abnormalities distal to the elbow and knee joints are much less common but have been recorded (Mulcahy, 1957) and the skull is said to be exempt from changes

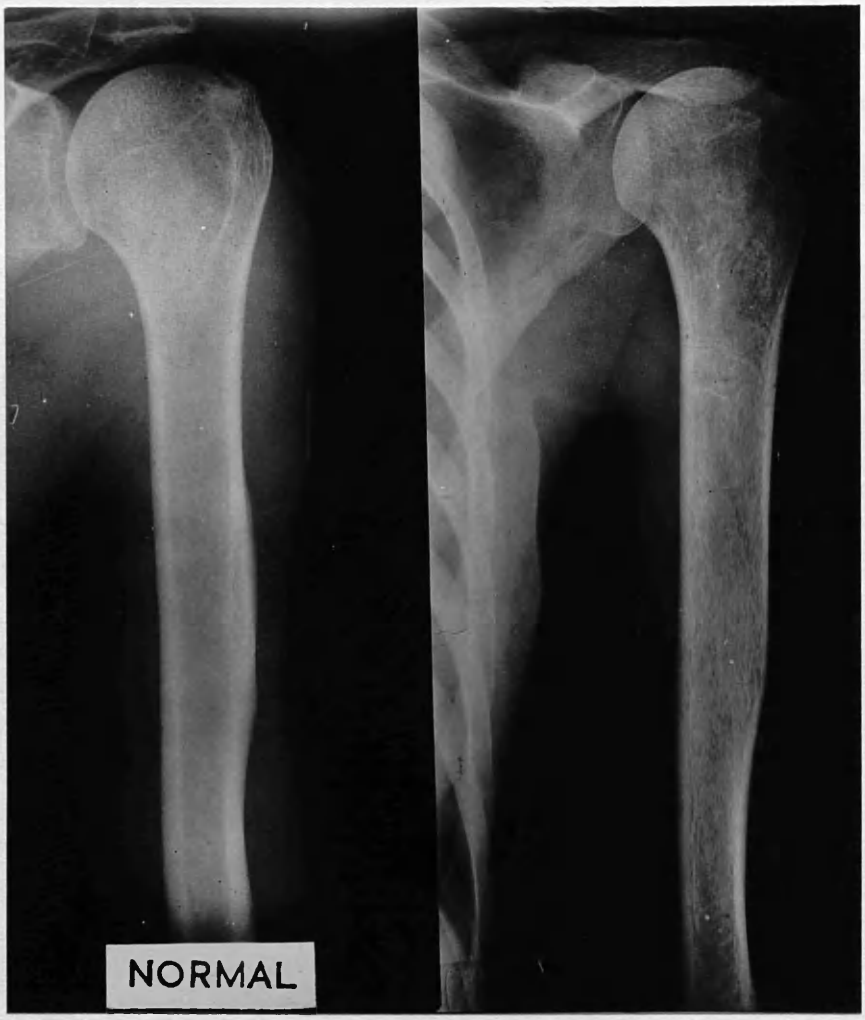


Fig. 89 Comparison of normal humerus and a humerus with the early changes of myelofibrosis (patient T.Q.)

/(Schinz et al, 1951; Leonard et al 1957).

The usual changes are of widening and coarsening of the trabeculae. There are often areas of irregular deposition of bone and patches of osteoporosis which result in a granular or mottled appearance in the medulla of the long bones. Despite the increased density of the bone, the cortex usually is not thickened although the inner aspect may be rarefied and appear to merge with the medulla.

The early radiological changes may prove difficult to detect and Brailsford (1953) recommends comparison with X-rays from normal subjects. Even using this technique, Leonard et al (1957) find difficulty and state that skeletal radiographs are the least useful of all the diagnostic procedures undertaken in their series of patients. There is no doubt, however, that this technique can be of value in detecting early changes in some patients (fig.89). As the radiological changes depend on alteration of the bony structure,

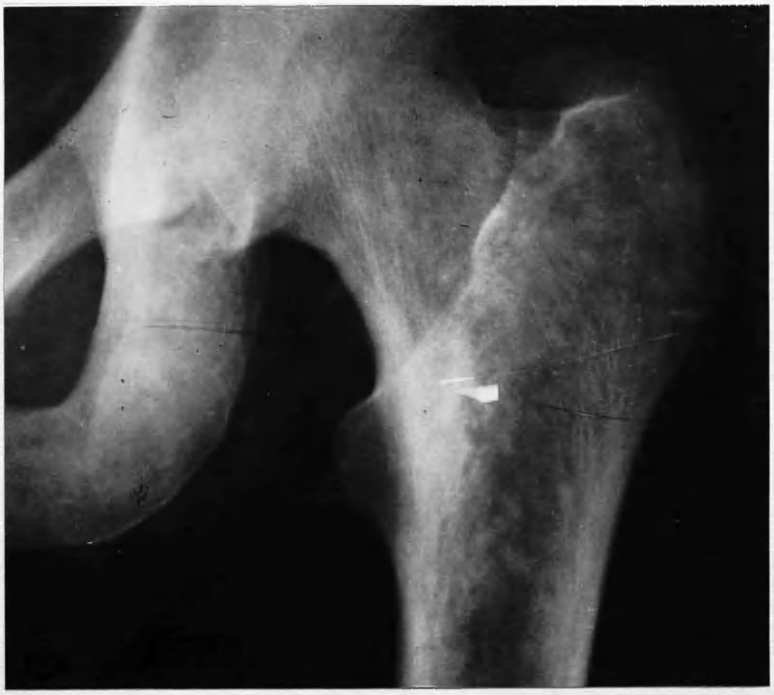


Fig. 90 Changes of myelofibrosis at the upper end of a femur (patient M.G.B.).

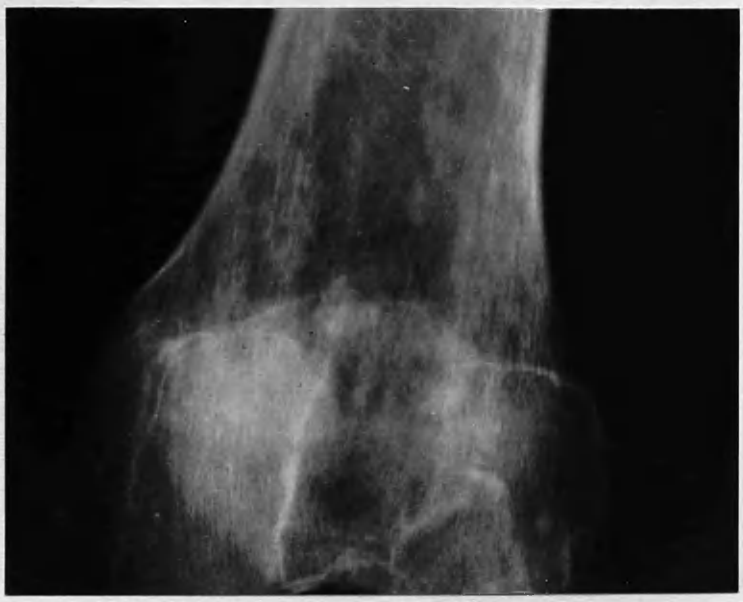


Fig. 91 Changes of myelofibrosis at the lower end of a femur (patient M.G.B.).

/some correlation would be expected with the histological features of the bone marrow. Nelson (1954) found no X-ray changes in one patient in whom the marrow trephine showed increased trabeculae but Richmond and Duncan (1956) noted a close relationship between radiological appearances and the histological features in 8 patients with radiological changes. Leonard et al (1957) classified patients on histological appearances as early, intermediate or late but found an early case with radiological changes and some late cases with no radiological changes.

Skeletal surveys were carried out on nine patients with myelofibrosis and radiological features of the disease were found in six patients. Three of these patients had been histologically classified as early cases, two as intermediate cases and one as a late case. No radiological features were found in the remaining early case, in an intermediate case or in the patient who was not classified as no marrow trephine had been performed. Thus, correlation between the

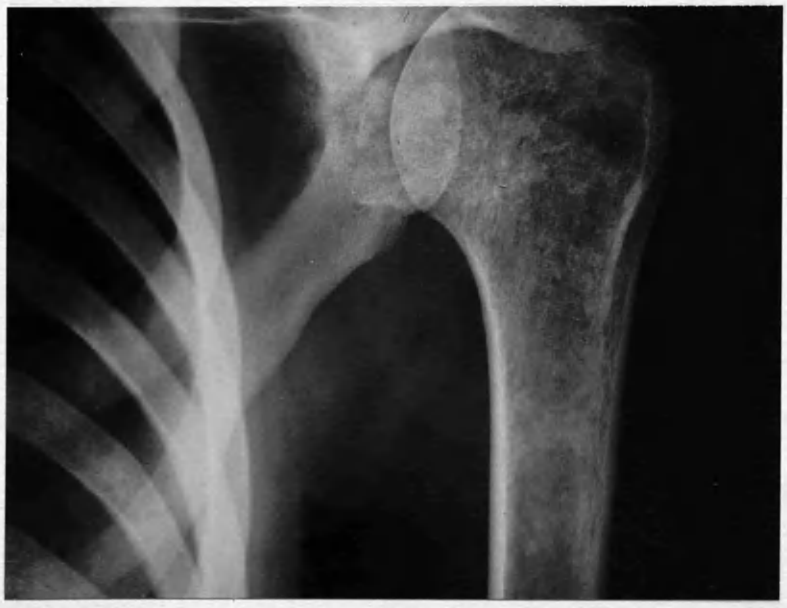


Fig. 92 Changes of myelofibrosis in a humerus (patient A.S.)

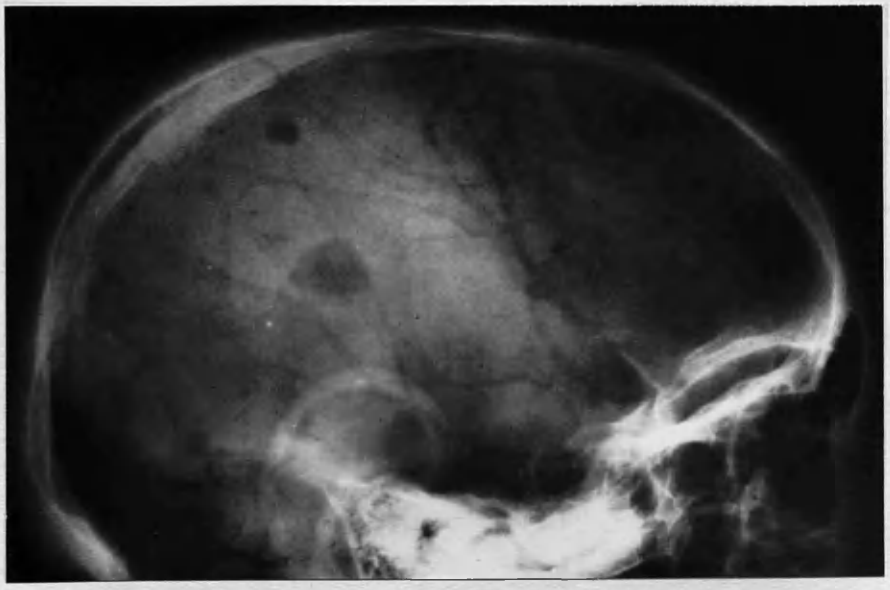


Fig. 93 Osteolytic areas in the skull of a patient with myelofibrosis. (patient M.G.B.).

/histological appearances and the radiological appearances is not established in the patients reported here.

Coarsening of the trabeculae at the upper end of the femur of one patient is illustrated in fig.90, and patchy osteoporosis of the lower end of the femur in fig.91. The humerus was affected in other patients (fig.92) and on occasions even the scapula and lumbar vertebrae were found to have radiological changes typical of the condition. It is of interest that in one patient two areas of bone destruction were seen in the skull (fig.93). These have the appearance of osteoplastic metastases but in other sites would be compatible with a diagnosis of myelofibrosis. No other cause for these lesions was found and it is possible that they were related to myelofibrosis. This patient died six months later in another hospital but unfortunately no autopsy was obtained and the possibility of other causes cannot be excluded completely.

Summary

The radiological appearances of twenty seven cases of myeloproliferative diseases are presented.

The occurrence of gouty arthritis was noted in 2 cases of polycythaemia vera, 1 of 'transition' myeloproliferative disease and one of myelofibrosis. Significant skeletal changes were otherwise seen in myelofibrosis only.

In polycythaemia vera cardiomegaly and increased pulmonary vascular markings were found and X-ray of chest in one patient with late chronic myeloid leukaemia revealed pulmonary involvement.

The cases of Di Guglielmo's disease, megakaryocytic thrombocythaemia and 'transition' myeloproliferative disease had no significant skeletal changes.

The radiological features of myelofibrosis were found in 6 of 9 patients. There appears to be no definite correlation between the radiological changes and the histological

/appearances of the bone marrow.

Chapter 15

BLOOD VOLUME STUDIES

It is well known that blood volume changes occur in polycythaemia and indeed the one essential criterion for this diagnosis is an increased red cell volume. Changes are found also in anaemia and it is to be expected, then, that changes in blood volume will occur in myeloproliferative states.

Wintrobe (1956) gives the normal blood volume as derived from the results of several workers as approximately 66 - 88 ml. per kg. of body weight. Szur et al (1959) define their ranges for red cell volume as 28 - 35 ml. per kg., for plasma volume as 40 - 50 ml. per kg., and for total blood volume as 68 - 85 ml. per kg. of body weight. There is little difference between these figures and the figures of previous workers and so those of Szur et al (1959) being the most recently reported are accepted as the normal range for the purpose of this study.

RESULTS

Polycythaemia vera

Szur et al (1959) in a study of 35 cases

Patient	Blood Volume (ml/kg.body weight).	Plasma Volume (ml/kg. body weight).	Red Cell Volume (ml/kg. body weight).
A.McA.	107	51	56
F.N.	72	35	37
M.S.	90	45	45
J.G.	119	56	63
J.C.	90	35	55

Table 31 Blood volume studies in polycythaemia vera.

Patient	Blood Volume (ml/kg.body weight)	Plasma Volume (ml/kg. body weight)	Red Cell Volume (ml/kg. body weight)
J.D.	129	61	68

Table 32 Blood volume studies in a case of megakaryocytic thrombocythaemia.

/of untreated polycythaemia vera report the red cell volume as increased in 34 patients, the blood volume as increased in 22 patients and the plasma volume as low in 16 patients and high in 3 patients. Similar results are found in the 5 patients studied here (table 31). The blood volume is increased in 4 patients, and the red cell volume in all 5 patients. Plasma volume is increased in 2, normal in 1 and decreased in 2 patients. Thus, the blood volume increase is due mainly to increase in red cell volume and may or may not be accompanied by an increase in plasma volume.

Megakaryocytic thrombocythaemia

This patient was polycythaemic at the time of investigation and not only is the total blood volume increased but both components, the red cell volume and the plasma volume, are also increased (table 32).

Chronic myeloid leukaemia

Four cases of chronic myeloid leukaemia were studied and the results are seen in table 33. The total blood volume is increased in 2 patients

Patient	Blood Volume (ml/kg. body weight).	Plasma Volume (ml/kg. body weight).	Red Cell Volume (ml/kg. body weight).
M.M.	62	47	15
C.L.	71	56	15
E.W.	99	75	24
J.L.	101	82	19

Table 33 Blood volume studies in chronic myeloid leukaemia.

Patient	Blood Volume (ml/kg. body weight).	Plasma Volume (ml/kg. body weight).	Red Cell Volume (ml/kg. body weight).
W.M.	91	73	18
M.B.	110	88	22

Table 34 Blood volume studies in 'intermediate' disease.

/and the plasma volume in 3 patients. In all 4 patients the red cell volume is diminished.

'Intermediate' disease

Both patients show an increase in total blood volume and plasma volume but a diminished red cell volume (table 34).

Myelofibrosis

The results obtained from 9 cases of myelofibrosis are seen in table 35. The total blood volume is increased in 5 patients, normal in 3 patients and diminished in 1 patient. The plasma volume is the component which forms the main contribution to the blood volume increase as it is increased in all 5 patients having an increased blood volume. The plasma volume is also high in one other patient with a normal blood volume. In all but one case the red cell volume is below the normal range and in this one case it is slightly increased and indicates a degree of polycythaemia. This polycythaemia is not apparent from the peripheral blood findings but it is worthy of note that this patient

Patient	Blood Volume (ml/kg. body weight)	Plasma Volume (ml/kg. body weight)	Red Cell Volume (ml/kg. body weight)
S.H.	85	60	25
M.C.	76	50	26
J.C.	103	66	37
J.G.	118	91	27
R.C.	69	49	20
M.G.B.	100	81	19
A.S.	76	55	21
W.P.	67	48	19
T.Q.	124	109	15

Table 35 Blood volume studies in myelofibrosis.

/had the highest white cell count (73,000 per c.mm.) recorded in the group with myelofibrosis. Thus, although this patient was never seen in an obviously polycythaemic phase she could have had polycythaemia vera without severe symptoms until the development of myelofibrosis. When first seen she had gross splenomegaly and thus may have had a myeloproliferative disorder for some time. Further, she presented with gout which is a more common complication of polycythaemia vera than any other myeloproliferative disease.

'Transition' myeloproliferative disease

The results of these four patients are seen in table 36. One patient only shows an increase of total blood volume, and this is due to increase of plasma volume. Plasma volume is increased in one other patient with a normal blood volume. The red cell volume is low in all cases and in one patient all three values are low.

Di Guglielmo's disease

This patient shows a normal blood volume,

Patient	Blood Volume (ml/kg. body weight)	Plasma Volume (ml/kg. body weight)	Red Cell Volume (ml/kg. body weight).
J.N.	60	36	24
J.McD.	99	82	17
A.McG.	46	36	10
J.McG.	73	58	15

Table 36 Blood volume studies in 'transition' myeloproliferative disease.

Patient	Blood Volume (ml/kg. body weight)	Plasma Volume (ml/kg.body weight)	Red Cell Volume (ml/kg. body weight).
G.McF.	72	61	11

Table 37 Blood volume studies in a case of Di Guglielmo's disease.

/an increased plasma volume and a low red cell volume (table 37).

Discussion

Blood volume studies appear to be of diagnostic value in polycythaemia vera. The total red cell volume must, by definition be increased, the total blood volume is usually raised and the plasma volume may be high, normal or even low. The results of this study agree with those of other workers and the cases appear to be representative.

In two other patients an increase of red cell volume is found. One of these is the patient with megakaryocytic thrombocythaemia, a condition often associated with polycythaemia, and the increase affects all three volumes as sometimes seen in polycythaemia vera. The other patient is one of myelofibrosis with gross splenomegaly and the high white cell count of 73,000 per c.mm.; this patient also had gout and in view of these findings the possibility of her being a late case of polycythaemia vera complicated by myelofibrosis

/must be considered.

In all other cases the red cell volume is found to be low, in keeping with the anaemia found on examination of the peripheral blood. It is interesting that the red cell volume is less seriously diminished in myelofibrosis at the time of diagnosis than in the other myeloproliferative diseases and this finding may be related to the rather better prognosis in myelofibrosis.

Despite the low red cell volume usually found in myeloproliferative disease (with the exception of polycythaemia vera) the total blood volume often is increased due to the presumably compensatory increase in plasma volume. This is not in agreement with McMichael et al (1943) who report a decrease in blood volume despite a relative increase in plasma volume in many anaemias. The difference may be attributable to the gross splenomegaly accompanying many cases of myeloproliferative disease and the large amount of blood which may be 'stored' in the

State	Blood Volume (ml/kg. body weight)	Plasma Volume (ml/kg. body weight)	Red Cell Volume (ml/kg. body weight)
after haematemesis	78	48	30
polycythaemic	129	61	68

Table 38 Blood volume studies in patient J.D. with thrombocythaemia after a haematemesis and when polycythaemic.

/spleen.

The blood volume and its components were estimated in the case of megakaryocytic thrombocythaemia 3 months after a large haematemesis and later when she had regained her previous polycythaemic state (table 38). In the polycythaemic state there was increase in total blood volume, red cell volume and plasma volume. After haematemesis, however, there was a fall in all three volumes to normal levels. One might have expected the blood volume to be maintained at a high level by a further increase in total plasma volume; that this did not occur (except possibly in the immediate post-haemorrhagic state) suggests that the increase in total blood volume and plasma volume seen in the polycythaemic state may be the result of efforts to dilute the cells to a more normal concentration. When the red cell volume returns to normal after bleeding the need for excess plasma no longer exists and so the plasma, and consequently the total blood volume returns

Date	Blood Volume (ml/kg. body weight)	Plasma Volume (ml/kg. body weight)	Red Cell Volume (ml/kg. body weight)
March,1957	103	66	37
July,1958	100	69	31
June,1959	93	75	18

Table 39 Blood volume studies at approximately yearly intervals in a case of myelofibrosis (J.C.).

/to normal.

The myelofibrosis patient with slight polycythaemia had blood volume studies carried out on 3 occasions at intervals of approximately one year. The results are seen in table 39. Over the 2 year period the red cell volume fell steadily. The plasma volume increased but not sufficiently to prevent a fall in total blood volume. Thus the patient is observed to leave the polycythaemic phase and enter the more usual myelofibrotic state.

Summary

Blood volume, red cell volume and plasma volume studies are reported in 26 cases of myeloproliferative disease. The red cell volume is increased in polycythaemia vera, and the total blood volume usually is increased also.

In other myeloproliferative states the red cell volume is diminished, but the plasma volume and total blood volume often are increased and this may be related to the gross splenomegaly so frequently present.

The red cell volume is less seriously diminished in myelofibrosis and this is compatible with the slightly better prognosis in this condition.

The blood volume measurements after a haematemesis and in the polycythaemic state in megakaryocytic thrombocythaemia are discussed.

One case of myelofibrosis had repeated studies carried out and was observed to pass from a polycythaemic state to the more usual anaemic state in myelofibrosis.

Chapter 16

THE DIAGNOSIS OF HAEMOLYSIS

The main diagnostic features in a patient with increased haemolysis depend on three factors; firstly, the shortened life span of the red cells; secondly, the alteration of pigment excretion as a result of excessive haemoglobin breakdown; and thirdly, the efforts of the marrow to increase production in an attempt to compensate for the more rapid destruction of the red cells. It is obvious that the first factor is a direct measure of haemolysis whereas the second and third factors are only indirect measurements. The measurement of red cell life span by radiochromium is the most direct measurement, but for the radiochromium $T\frac{1}{2}$ to be used as an index to the true survival time of the red cells, certain corrections require to be made (Jones and Mollison, 1956). Nevertheless, the $T\frac{1}{2}$ is a guide to the degree of haemolysis and is apparently more accurate than any indirect method.

The faecal excretion of urobilinogen has been used as a measure of haemolysis

/by Miller et al (1942). They provide a 'haemolytic index' which takes into account the total mass of circulating haemoglobin and the difference between the actual normal excretion figures and the theoretically expected excretion. The normal range for the haemolytic index is 11 - 21 and an index of over 21 is indicative of increased haemolysis.

The reticulocyte count is a measurement, not of haemolysis, but of the efforts of the erythropoietic tissue to compensate for increased haemolysis. If marrow production is adequate and equilibrium is reached then the level of the reticulocyte count may be an indication of the degree of haemolysis.

When the bone marrow responds to increased haemolysis there are released into the circulation not only reticulocytes but also other immature cells in the form of nucleated red cells. Thus, there may be a relationship between haemolysis and the presence of nucleated red cells in the peripheral blood.

Haemolysis was estimated by these methods

Patient	Diagnosis	Haemolytic Index	$^{51}Cr\ T_{\frac{1}{2}}$ (days)
M.M.	C.M.L.	23	14
C.L.	C.M.L.	16	20
E.W.	C.M.L.	21	16
M.B.	Int.	10	12
W.M.	Int.	53	15
M.C.	Mf.	8	13
J.C.	Mf.	12	22
J.G.	Mf.	34	17
R.C.	Mf.	47	15
T.Q.	Mf.	41	14
M.G.B.	Mf.	114	16
S.H.	Mf.	25	30
A.McA.	P.V.	12	22
M.S.	P.V.	21	24
J.McG.	M.P.	66	24
G.McF.	D.G.	90	8

Table 40 Comparison of haemolytic index and $^{51}Cr\ T_{\frac{1}{2}}$ in 16 patients with myeloproliferative disease.

(C.M.L. = chronic myeloid leukaemia;
Int. = 'intermediate' disease; Mf. = myelofibrosis;
P.V. = polycythaemia vera; D.G. = Di Guglielmo's disease).

/and the results obtained are compared.

The haemolytic index and radiochromium $T\frac{1}{2}$ were determined on 16 patients (table 40). Of these patients, 15 have increased haemolysis as judged by the $T\frac{1}{2}$ but only 9 of these have a haemolytic index over 21 indicating increased haemolysis. One patient had no haemolysis by radiochromium but the haemolytic index is elevated. The radiochromium method is a direct measurement of the destruction of red cells and is therefore more reliable than any indirect method. If the radiochromium result is assumed to be correct then the haemolytic index is wrong in approx. 44 per cent of cases and thus is not a reliable measurement. Furthermore, there appears to be no correlation between the level of the elevated index and the reduction from normal of the radiochromium half life.

The reticulocyte count, in absolute figures, is compared with the radiochromium half life. As reticulocytes may be present in the normal individual up to a figure of

Patient	Diagnosis	Reticulocytes (per c.mm.)	^{51}Cr $T\frac{1}{2}$ (days)
M.M.	C.M.L.	<76,000	14
J.L.	C.M.L.	147,000	10
C.L.	C.M.L.	140,000	20
E.W.	C.M.L.	75,000	16
M.B.	Int.	90,000	12
W.M.	Int.	108,000	15
M.C.	Mf.	196,000	13
J.C.	Mf.	144,000	22
J.G.	Mf.	330,000	17
R.C.	Mf.	82,000	15
T.Q.	Mf.	286,000	14
M.G.B.	Mf.	52,000	16
S.H.	Mf.	< 80,000	30
W.P.	Mf.	704,000	14
A.S.	Mf.	< 84,000	24
J.McG.	M.P.	< 19,800	24
A.McG.	M.P.	85,400	30
J.McD.	M.P.	61,200	20
G.McF.	D.G.	108,000	8

Table 41 Comparison of reticulocyte count and ^{51}Cr $T\frac{1}{2}$ in 19 patients with myeloproliferative disease.

(C.M.L. = chronic myeloid leukaekia;
Int. = 'intermediate' disease; Mf. = myelofibrosis;
M.P. = 'Transition' myeloproliferative disease;
D.G. = Di Guglielmo's disease).

/2 per cent of the total red cell count, a level of 100,000 per c.mm. is taken as the upper limit of normal reticulocyte count. This, of course, can apply only to patients with normal or low red cell counts as 2 per cent of an elevated red cell count will be over 100,000 per c.mm. Thus, all patients with polycythaemia at the time of investigation are excluded from this comparison; this leaves out five cases with polycythaemia vera, one case of megakaryocytic thrombocythaemia and one case of 'transition' myeloproliferative disease. No attempt was made to enumerate the reticulocytes when they constituted less than 2 per cent of the red cell count (i.e. less than 100,000 in a count of 5 million red cells.

The results in 19 patients are compared in table 41. In eleven patients there is agreement; nine results indicate increased haemolysis by reticulocyte count and radiochromium technique, and two indicate a normal rate of haemolysis by each method.

/In the other eight patients haemolysis is indicated by radiochromium study but the reticulocyte count is less than 100,000 per c.mm. in each case.

The reticulocyte count depends upon productive activity and if compensatory hyperplasia does not or cannot occur then the reticulocyte count will not be raised. A low radio iron utilisation indicates low red cell production and thus provides a method for analysis of the figures which do not agree with the radiochromium estimation. In 6 of the 8 cases of disagreement the radioiron utilisation is low and indicates erythropoietic hypoplasia thus accounting for the failure to find an elevated reticulocyte count. In the other two cases there was doubtful haemolysis ($T\frac{1}{2}$ = 24 days) or a just normal reticulocyte count (90,000 per c.mm.). Thus, of the nineteen cases 11 (58%) show complete agreement, and 2 (10%) have borderline results. In the other 6 cases (32%) there is disagreement which appears to be due to erythropoietic

Patient	Diagnosis	Nucleated Red Cells (per c.mm.)	^{51}Cr $T_{\frac{1}{2}}$ (days)
M.M.	C.M.L.	5,250	14
J.L.	C.M.L.	1,700	10
C.L.	C.M.L.	1,500	20
E.W.	C.M.L.	5,400	16
M.B.	Int.	7,840	12
W.M.	Int.	15,400	15
M.C.	Mf.	900	13
J.C.	Mf.	2,555	22
J.G.	Mf.	420	17
R.C.	Mf.	464	15
T.Q.	Mf.	135	14
M.G.B.	Mf.	340	16
S.H.	Mf.	0	30
W.P.	Mf.	357	14
A.S.	Mf.	250	24

Table 42 Comparison of nucleated red cell count and ^{51}Cr $T_{\frac{1}{2}}$ in 15 cases of myeloproliferative disease.

(C.M.L. = chronic myeloid leukaemia;
Int. = 'intermediate' disease; Mf. = Myelofibrosis)

/hypoplasia and consequent failure of the reticulocyte count to rise.

Nucleated red cells were found in the peripheral blood in 14 of the 15 cases of chronic myeloid leukaemia, myelofibrosis and 'intermediate' disease who had radiochromium studies carried out. In all these 14 cases there is increased haemolysis (table 42). In the one case of myelofibrosis with no nucleated red cells there is no increase in the rate of haemolysis. ($T\frac{1}{2}$ = 30 days). It is of interest that nucleated red cells are present in larger numbers in chronic myeloid leukaemia than in myelofibrosis with only one exception.

Discussion:

The most direct measurement of red cell survival is the radiochromium method. The haemolytic index, which is dependent on the faecal excretion of urobilinogen does not appear to be a satisfactory method of estimating haemolysis as compared to the radiochromium method. It is, of course, an indirect

/assessment and must depend upon other factors such as each individual's normal excretion and liver function. For example, one person may normally excrete 50 mgm. of urobilinogen daily in the faeces. If haemoglobin breakdown is increased then this figure may rise to 100 mgm. daily but will still be well within normal limits. Thus, it is not surprising that the haemolytic index is an inaccurate guide.

The reticulocyte count depends upon the response of the erythropoietic tissue to increased haemolysis and in myeloproliferative disease this is often limited. If the absolute reticulocyte count is taken as a measure of haemolysis in myeloproliferative disease then there is about 60 per cent accuracy, but if it is remembered that the erythropoietic tissue cannot respond satisfactorily in many cases, and if this is taken into account, then there are few counts which are in complete disagreement with the radiochromium study. Further, if the reticulocyte count is applied

/only in such cases where the marrow can respond then it becomes a tolerably accurate method of estimating haemolysis.

It might be expected that the number of nucleated red cells in the peripheral blood provide a guide to increased haemolysis when the marrow attempts to compensate by releasing very immature cells. A good correlation is found but it must be remembered that increased haemolysis occurs in most cases of chronic myeloid leukaemia and myelofibrosis as in fact do nucleated red cells in the blood; this is not necessarily a direct relationship but may be merely the coincidental occurrence of two very common findings. Furthermore, the patients studied form a very selective group. It is of interest that whereas nucleated red cells in the peripheral blood are generally accepted as more common in myelofibrosis, the absolute figures indicate that higher numbers occur in chronic myeloid leukaemia. This is no doubt because the accepted figures are read as a percentage of the white count;

/as the count is so much higher in chronic myeloid leukaemia a smaller proportion may represent a much higher absolute figure.

Summary

A comparison is made of the radiochromium estimation of red cell survival with the haemolytic index, the reticulocyte count and the occurrence of nucleated red cells in the peripheral blood.

The haemolytic index does not appear to be a reliable guide to haemolysis in myeloproliferative disease.

The reticulocyte count appears to be much more reliable provided erythropoietic activity can be assessed and taken into account.

Nucleated red cells in the blood seem to occur with increased haemolysis but this association is accepted with reservations.

SUMMARY OF

SECTION IV

A study has been made of various aspects of myeloproliferative disease.

Megaloblastic erythropoiesis occurred in three patients. It is suggested that in Di Guglielmo's disease there may be a defect of vitamin B_{12} or folic acid metabolism at a cellular level. In one case of myelofibrosis it is possible that there was a deficiency of vitamin B_{12} or folic acid in the early stages but in the other case of myelofibrosis there was definite serum vitamin B_{12} deficiency.

Infection was found to be related to hypogammaglobulinaemia in two patients but another patient with hypogammaglobulinaemia had no history of infection. All cases of hypogammaglobulinaemia occurred late in the disease.

Gout was found in four cases of myeloproliferative disease. Uric acid studies suggest that while each disorder may have its own pattern of uric acid metabolism there probably are factors common to all.

The radiological features of myeloproliferative disease are discussed and one case with defects in the skull was noted in the myelofibrosis group.

Blood volume studies were carried out on patients with myeloproliferative disease and the results are discussed. One patient having myelofibrosis is of particular interest as initially she was polycythaemic but later developed more usual volumes of myelofibrosis.

Studies on the diagnosis of haemolysis suggest that provided the bone marrow is capable of response the reticulocyte count is a good index of the degree of increased haemolysis.

SECTION V

THE TREATMENT OF MYELOPROLIFERATIVE DISEASE

Chapter 17

THE GENERAL ASPECTS

OF TREATMENT

All forms of myeloproliferative disease are eventually fatal. Treatment is, therefore, aimed at relieving symptoms and maintaining the general well-being of the patient. This is most successful in the case of polycythaemia vera where the patient may remain well for many years, and least successful in erythraemic myelosis which usually proves fatal within a short time despite any attempt at treatment.

Whereas treatment of polycythaemia vera with radioactive phosphorus (^{32}P) appears to prolong life (Lawrence, 1955) it is less certain that the duration of life is extended in any other myeloproliferative disorder. There is no doubt, however, that life can be made much more comfortable for these patients. This applies particularly to chronic myeloid leukaemia where frequent courses of treatment are often required but the patient may remain tolerably well for a few years.

Although there is a definite place for treatment of myeloproliferative disease, it is essential to realise that in some cases the

/patient may remain well for long periods, sometimes for years, without any form of treatment. Patients J.G. and S.H. with myelofibrosis each remained well for over three years without treatment for myelofibrosis.

Specific Therapy

This is applicable only to polycythaemia vera where the administration of radioactive phosphorus (^{32}P) appears to effect a haematological cure; more than one treatment, however, may be required to produce this.

Supportive Therapy

Treatment is not uncommonly required for cardiovascular complications in myeloproliferative disease and the use of digitalis and diuretics may bring about marked improvement when cardiac failure is present. Leukaemic patients appear to be more susceptible to infection than normal and antibiotic therapy may be indicated on occasions.

Iron Therapy

The administration of iron was found to be indicated in the treatment of ten patients.

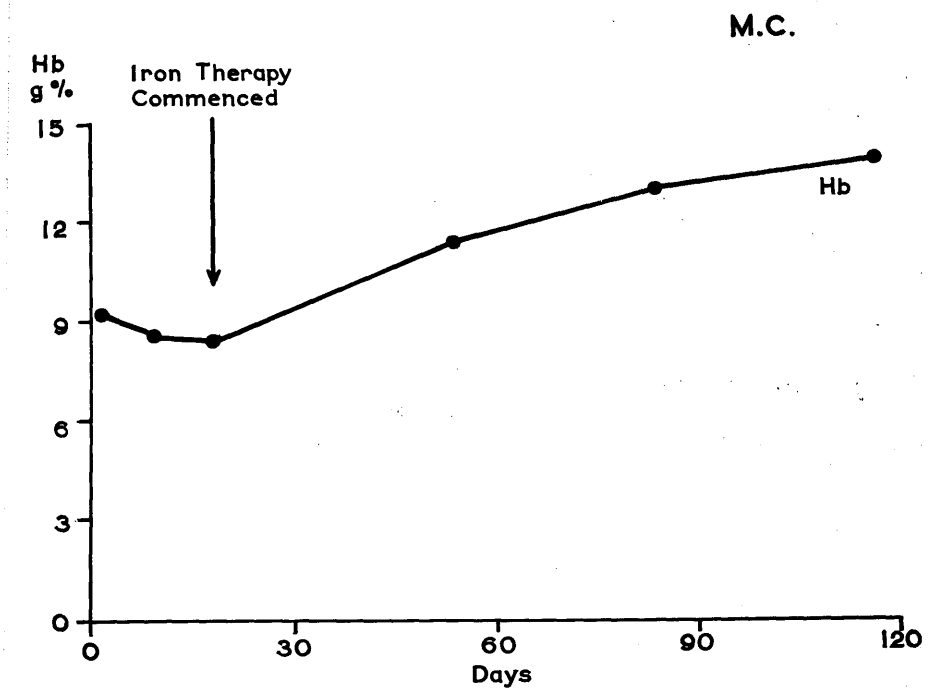


Fig. 94 Response to iron therapy in myelofibrosis. (Patient M.C.)

/The response was variable but sometimes iron proved remarkably effective (fig.94) and obviated the use of other treatment for some time. Although oral iron was found to be effective in most cases parenteral administration occasionally was required.

Blood Transfusion

Blood transfusion was necessary for many patients who did not respond to iron, or who had blood loss, or defective erythropoiesis, or excessive haemolysis; it is obvious that in the latter two the effect of transfusion would be of temporary benefit only but this is often of value in preparation for other treatment.

Blood transfusion was given in the treatment of 15 patients with myeloproliferative disease. In 5 patients this was in preparation for radiotherapy and in 4 patients in preparation for splenectomy. In 3 patients blood transfusion was given for excessive haemolysis with only temporary benefit and

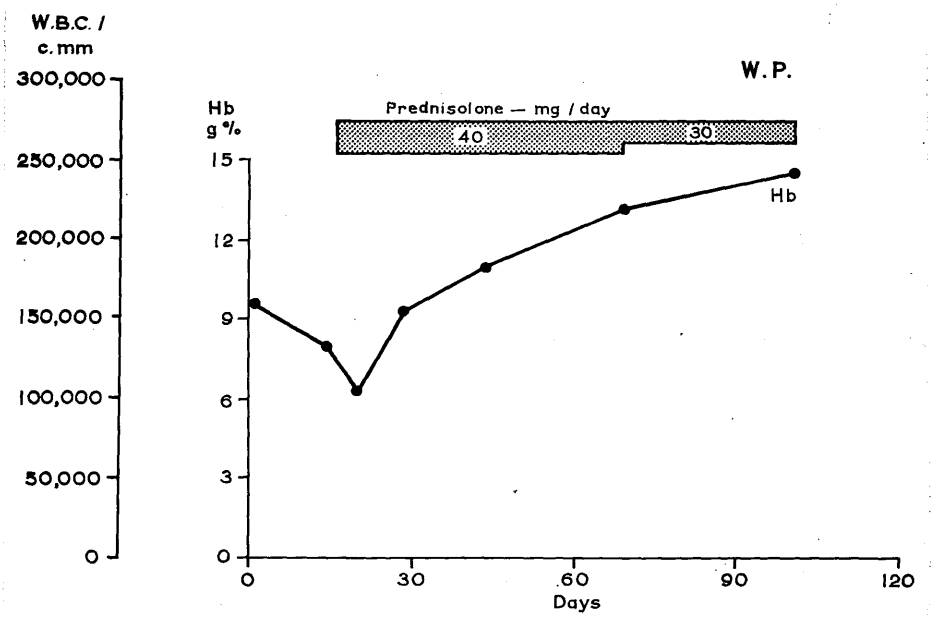


Fig. 95 Response to steroid therapy in myelofibrosis. (Patient W.P.)

/in 2 patients it was used during investigation of the cause of anaemia. One patient with polycythaemia vera required transfusion after an episode of melaena. The effect of blood transfusion was never prolonged but temporary benefit was obtained in many cases.

Antimetabolite drugs

Busulphan was used in the treatment of one patient with chronic myeloid leukaemia and one with 'transition' myeloproliferative disease. In neither of these cases was anything other than temporary benefit derived. Folic acid antagonists are of occasional benefit in myeloblastic crises. Aminopterin was used in one case of terminal chronic myeloid leukaemia with no apparent effect.

Corticosteroids

As myeloproliferative disease is often accompanied by increased haemolysis, corticosteroid therapy may be beneficial by reducing the haemolysis, especially if antibodies are present. With this possibility in mind, 'Prednisolone' was used in the

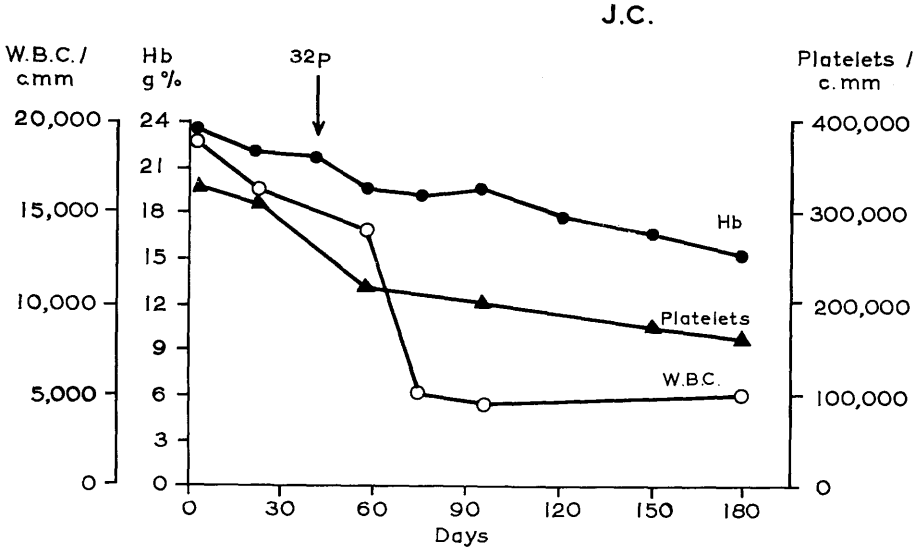


Fig. 96 Response to radiophosphorus in polycythaemia vera. (Patient J.C.)

/treatment of 3 patients. In Di Guglielmo's disease and a case of 'intermediate' disease there was no apparent effect. In one patient with myelofibrosis, however, there was a good response and the haemoglobin level rose steadily with a dosage of 60 mgm. Prednisolone daily (fig.95). Thereafter the dose was reduced to 20 mgm. daily and the patient has remained clinically and haematologically satisfactory since then.

Radioactive Phosphorus

Radioactive phosphorus (^{32}P) was used in all 5 patients with polycythaemia vera with good effect though 3 patients required more than one dose. The satisfactory response of one patient is illustrated in fig.96.

In the case of megakaryocytic thrombocythaemia, radiophosphorus appeared to be the treatment of choice and with this form of therapy the platelet count fell from over 2 million to under 300,000 per c.mm. and the patient to date has had no further haemorrhagic episodes.

One patient with 'transition'

/myeloproliferative disease with an excess number of cells of the myeloid series in the marrow though not in the blood, also was treated with radiophosphorus and again the response was favourable but temporary..

Comment

There are, therefore, many forms of therapy which may be of some value in the treatment of myeloproliferative disease. Radiophosphorus appears to be the treatment of choice in polycythaemia vera and occasionally in other forms of myeloproliferative disease. The other treatments mentioned previously have a temporary effect only but are sometimes of considerable benefit when some major form of therapy, such as splenic irradiation or splenectomy, is contemplated. The usefulness of splenic irradiation and splenectomy in some forms of myeloproliferative disease is controversial and as these two treatments form the basis of effective therapy they are considered in later chapters.

Summary

The general aspects of the treatment of myeloproliferative disease are considered.

Treatment is aimed at improving the patient's condition but in polycythaemia vera the use of radioactive phosphorus may produce prolonged benefit.

In other myeloproliferative disease treatment usually is of temporary value only. Occasional satisfactory results however, may be obtained with radiophosphorus, corticosteroids or antimetabolite drugs.

Chapter 18

SPLENECTOMY

Splenectomy in the treatment of myeloproliferative disease is an operation which is not performed frequently. Probably the first occasion of its use dates back about a hundred years when Bryant (1866) removed the spleen in a patient with chronic myeloid leukaemia. The patient died and the operation then went into disuse. More recently, however, it has again been described in the treatment of patients with chronic myeloid leukaemia. It is sometimes performed when there is severe anaemia, probably mainly haemolytic in origin, in the presence of a white cell count which is too low to permit radiotherapy. In such circumstances the patient may benefit considerably for a temporary period (Scott, 1957).

Splenectomy is rarely considered in other myeloproliferative states with the exception of myelofibrosis. In myelofibrosis there is great controversy regarding its usefulness and it is considered contraindicated by several authorities (Scott, 1949; Wintrobe,

/1951; Learmonth, 1951; Whitby, 1952; Chatterjea et al, 1952). Hickling (1953), in a review of the subject, refers to 14 patients on whom splenectomy was performed with mixed results. Beneficial results, however, are reported by other writers (Engell, 1947; Edwards, 1951; Andersen and Sørensen, 1952; Franks and Richardson, 1952; Merskey and Budtz-Olsen, 1953; Goldberg and Seaton, 1960).

It is clear, then, that splenectomy may have harmful effects and it may even prove fatal within a few hours (Shinton, 1957) but on the other hand it may produce marked improvement for as much as 3 years (Nelson, 1954). Thus, there must be certain factors which determine the value of splenectomy. It has been suggested that splenectomy could be undertaken because of the disability caused by gross enlargement of the spleen (Shinton, 1957). This, however, must be a hazardous procedure as it takes into account none of the possible functions of the spleen.

/Excessive haemolysis is considered an indication for splenectomy by some writers (Korst et al, 1956; Leonard et al, 1957) but this policy ignores the possible erythropoietic activity of the spleen. Edwards (1951) states that the relative proportions of the spleen's favourable and unfavourable activities cannot be assessed except by its removal. This is no longer the case as the radioisotopic studies described in earlier chapters provide accurate information of the productive and destructive functions of the spleen. Splenectomy was carried out on four patients on the basis of these investigations and the results are reported.

Myelofibrosis

Patient T.Q. had a large bulky spleen and ample evidence of hypersplenism. She had a leucopenia, a thrombocytopenia and a haemolytic anaemia which necessitated frequent blood transfusion. The radioiron studies demonstrated some remaining marrow erythropoietic activity

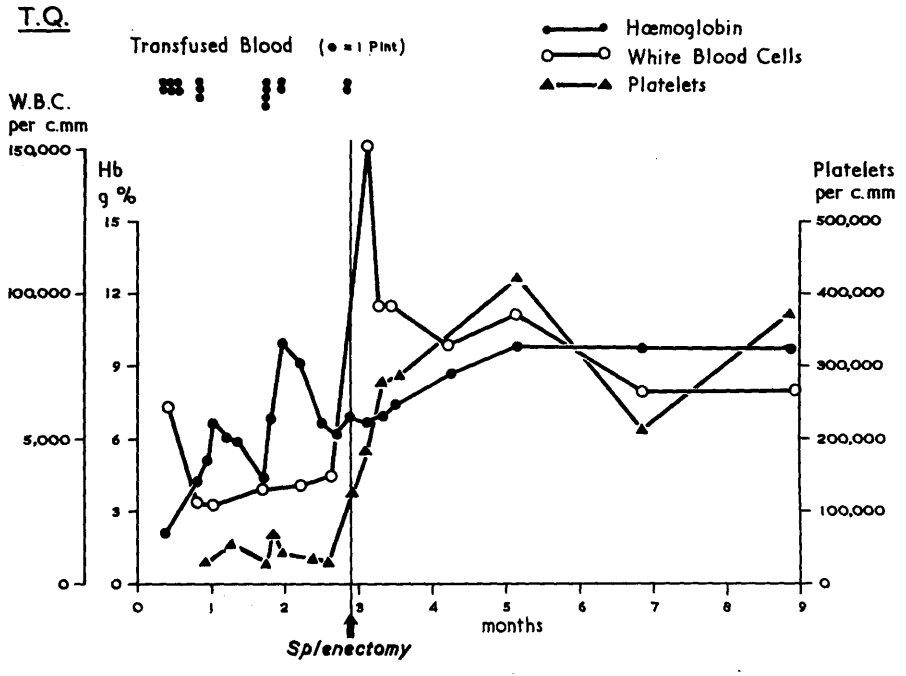


Fig. 97 Effect of splenectomy on patient T.Q. with myelofibrosis.
(reproduced by permission from 'Clinical Radiology')

/and only a small degree of splenic erythropoiesis. The radiochromium studies showed $T\frac{1}{2}$ to be reduced to 14 days and the spleen:liver ratio to be 3:1. Thus the spleen had only a minor productive function but a major destructive function. On this basis splenectomy was carried out. The effect on the blood findings is seen in fig.97. The haemoglobin rose and the need for transfusion ceased. The platelet count rose to normal or high levels and the white count, after an initial post operative peak, remained near normal figures. The patient's general condition was greatly improved and one year later she was still at her work and has remained well without further treatment.

Patient M.C. had gross splenomegaly and over a period of 2 - 3 years her condition deteriorated steadily. Radioiron studies showed no significant splenic activity and the radiochromium studies showed $T\frac{1}{2}$ to be reduced to 14 days and the spleen:liver ratio to be 2.65:1. This again demonstrated that

/the spleen was predominantly an organ of destruction and that splenectomy was indicated. The spleen was removed but unfortunately the patient died in an unexpected post operative collapse after a technically satisfactory operation.

'Intermediate' Case

Patient M.B. with a condition intermediate between chronic myeloid leukaemia and myelofibrosis had been treated previously on several occasions by splenic irradiation. She developed a marked haemolytic anaemia and required frequent blood transfusion. This was not affected by a further course of splenic irradiation and removal of the spleen seemed the only hope for alleviation of her condition. The radiochromium $T\frac{1}{2}$ had earlier been 12 days, at which time she was not in need of transfusion, and the rate of haemolysis obviously had increased since then. Radioiron studies had demonstrated splenic erythropoiesis and no marrow activity but in view of the severity of the haemolysis it was felt

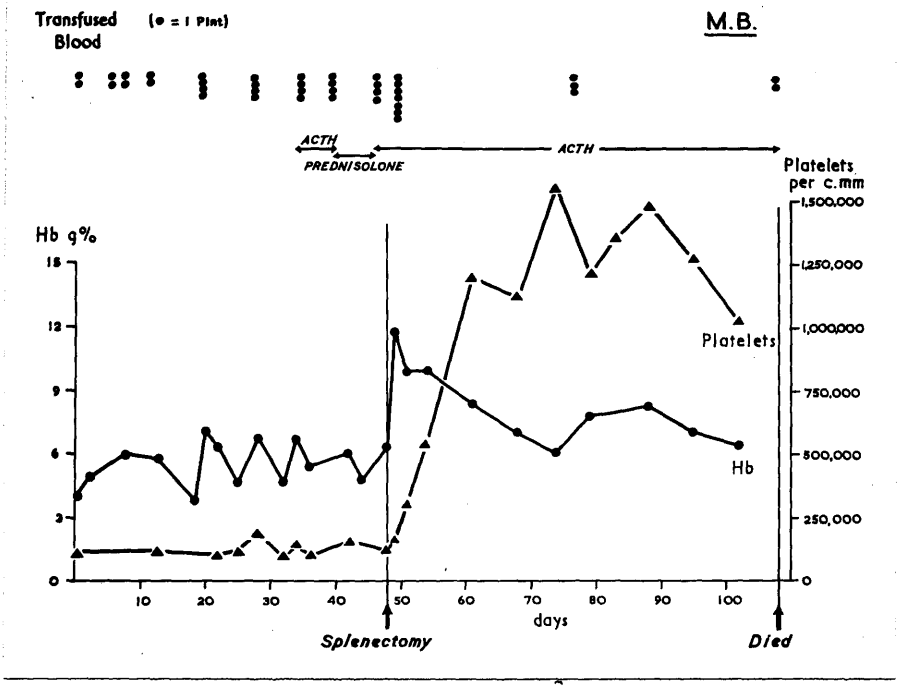


Fig. 98 Effect of splenectomy on patient M.B. with 'intermediate' disease.

/justifiable to proceed with splenectomy in the hope that other sites of erythropoiesis might compensate for the loss of the splenic source of red cells.

Following splenectomy the need for transfusion was greatly reduced and the level of haemoglobin was more easily maintained (fig.98). The platelets however, rose to over one million per c.mm. The patient's general condition improved considerably for a time but she developed severe bacterial infections and died about 2 months later. During this period her liver increased in size and soon extended to the right iliac fossa. This occurrence has previously been noted (Nelson, 1954; Richmond and Duncan, 1956) and presumably is due to an attempt by the liver to compensate for the removal of a major source of red cells.

'Transition' Myeloproliferative disease

Patient J.McD had a 'transition' disease which was considered to lie between

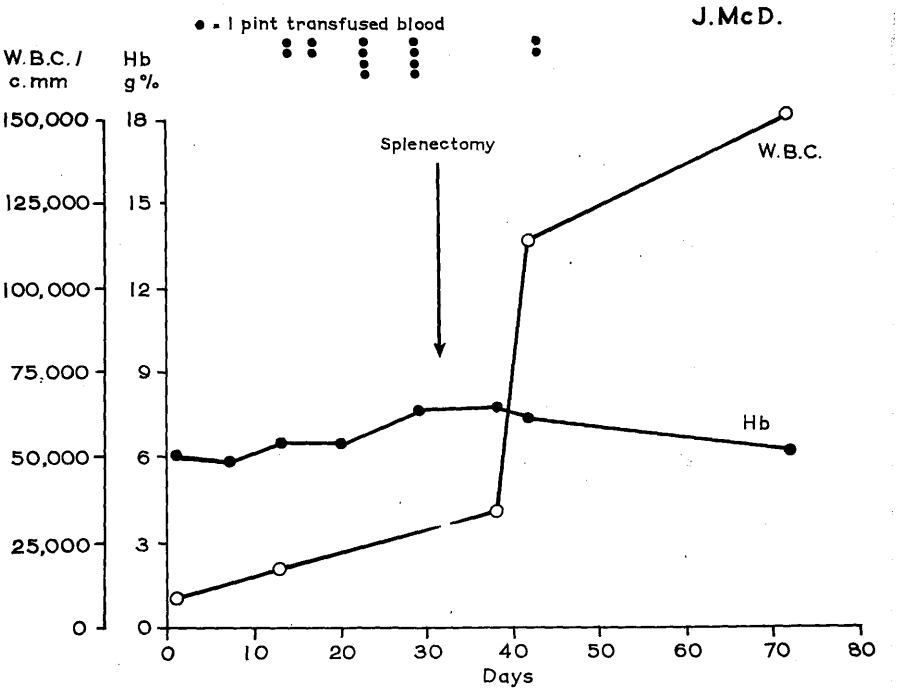


Fig. 99 Effect of splenectomy on patient J.McD. with 'transition' disease.

/polycythaemia vera and chronic myeloid leukaemia. She had a large abdominal mass in which erythropoietic tissue was demonstrated by radioiron studies; this mass was, therefore, thought to be an atypical spleen. The marrow, however, still retained some erythropoietic function. Radiochromium studies showed slightly increased haemolysis with $T\frac{1}{2}$ of 20 days and the spleen:liver ratio was 2.7:1. The patient required frequent blood transfusions. These radioisotopic results suggested that if the abdominal mass was spleen then it seemed mainly to sequester and destroy red cells but had a little erythropoietic function. On these grounds and on the demonstration of persistent marrow erythropoiesis, laparotomy was performed. At operation the mass proved to be spleen on a long pedicle and was therefore removed. Following splenectomy the requirements for transfusion diminished and the haemoglobin level was better maintained (fig.99). The white cell count, however, rose to leukaemic levels with 67 per cent neutrophils and

/21 per cent myeloblasts.

Histological examination of the spleen showed that the normal architecture had been replaced by sheets of myeloid cells many of which were primitive.

Thus, the impression that this case was in a 'transition' phase between polycythaemia vera and leukaemia appears confirmed and splenectomy seems to have precipitated the change to leukaemia. This patient was still well six months after operation but had required busulphan for her leukaemia.

Discussion

The place of splenectomy in myeloproliferative disease is limited but in selected cases may prove beneficial. Four of the twenty seven patients with myeloproliferative disease were considered suitable for splenectomy which was then carried out. In one patient post operative collapse ensued and the patient died. In the other three patients the operation met with varying degrees of success. One has remained extremely well for over one year;

/the second improved for a period but then succumbed to severe infections which were almost certainly related to hypogammaglobulinaemia; the third patient improved considerably but developed leukaemia.

Thus, the operation has proved to be a success when used to control a severe haemolytic anaemia with associated splenic sequestration of red cells. Unfortunately, these patients had complicating features which modified their outlook. The radioisotopic studies have, therefore, proved useful in determining the haematological suitability of such patients for splenectomy, and in one case have proved to be of diagnostic value. It is suggested that all patients with myeloproliferative disease who are being considered for splenectomy should have radioiron and radiochromium studies carried out as these investigations provide an accurate and reliable indication of the role of the spleen in the production and destruction of the blood. It is essential that the patient's general condition also is

/taken into account and that splenectomy is not performed solely on the results of these tests. For example, patient J.G. had $T\frac{1}{2}$ of 17 days and the spleen:liver ratio was 2.5:1. Splenectomy was not carried out as the patient's general condition remained satisfactory and his erythrocyte production was able to compensate for the sequestration and increased rate of haemolysis of the red cells.

When radioisotopic studies are carried out to assess a patient's suitability for splenectomy, less is left to chance and the operation is placed on a more logical and sound basis.

Summary

Four cases of myeloproliferative disease were subjected to splenectomy mainly on the basis of radioiron and radiochromium studies. The presence of a haemolytic anaemia and splenic sequestration of red cells is considered an indication for splenectomy provided the patient's condition requires treatment.

In three cases the operation met with success although this was modified by other complications in two of these patients.

The radioisotopic studies have proved helpful in assessing a patient's suitability for splenectomy. The development of frank leukaemia after operation in one 'transition' case is considered further evidence in favour of relationship of the myeloproliferative disorders.

Chapter 19

IRRADIATION

OF THE SPLEEN

For over fifty years radiotherapy has been the treatment of choice in chronic myeloid leukaemia. Recently other forms of treatment have come into use but as yet there is no convincing proof of any superiority over radiotherapy. In the case of myelofibrosis, however, radiotherapy is by no means accepted generally as a suitable form of therapy. Some authorities consider splenic irradiation to be totally ineffective or even harmful (Jackson et al, 1940; Block and Jacobson, 1950; Cook et al, 1953) and fatal results have been recorded (Jackson et al, 1940; Cook et al, 1953; Cartwright, 1955). Others (Korst et al, 1956) note a fall in the leucocyte count but find no apparent change in the course of the disease. A limited use of local irradiation to relieve splenic pain is advocated by some writers (Marson and Meynell, 1952; Richmond and Duncan, 1956) but Hickling (1953) draws attention to the good general results which can be obtained in some patients with myelofibrosis.

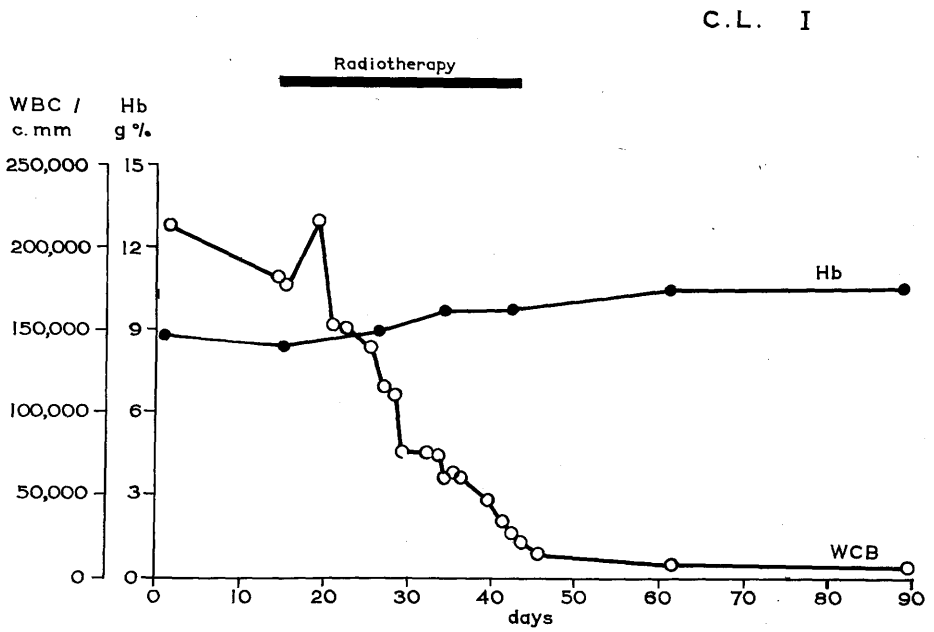


Fig. 100 Effect of splenic irradiation on patient C.L. with chronic myeloid leukaemia.

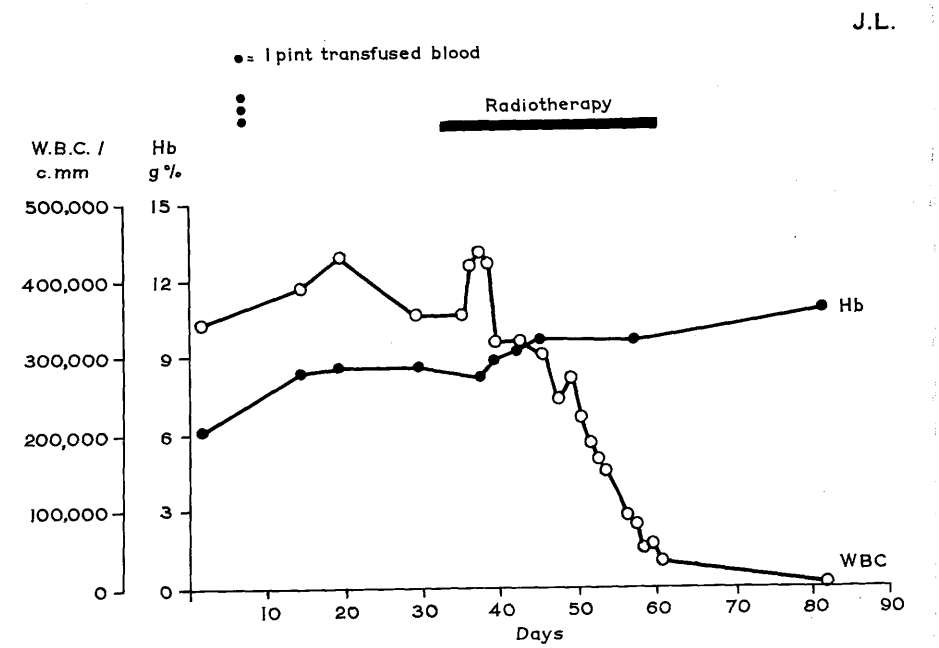


Fig. 101 Effect of splenic irradiation on patient J.L. with chronic myeloid leukaemia.

/ There is, therefore, considerable controversy as regards the place of splenic irradiation in the treatment of myelofibrosis and a comparison is made of the effect of this form of therapy in chronic myeloid leukaemia and in myelofibrosis.

Chronic Myeloid Leukaemia

Of the five patients with chronic myeloid leukaemia, four were treated by splenic irradiation in doses of 506 r to 911 r given over 10 to 23 treatments. The conditions accepted as indications for therapy were 1) a falling haemoglobin; 2) a high and rising white cell count; and 3) clinical deterioration including splenic discomfort. In all cases a good response was obtained; the patient's general condition improved, the white cell count fell to normal figures and the haemoglobin level usually rose. A typical response as obtained in patient C.L. is illustrated in fig.100. It is of interest that in this patient the haemoglobin level rose during the period of irradiation.

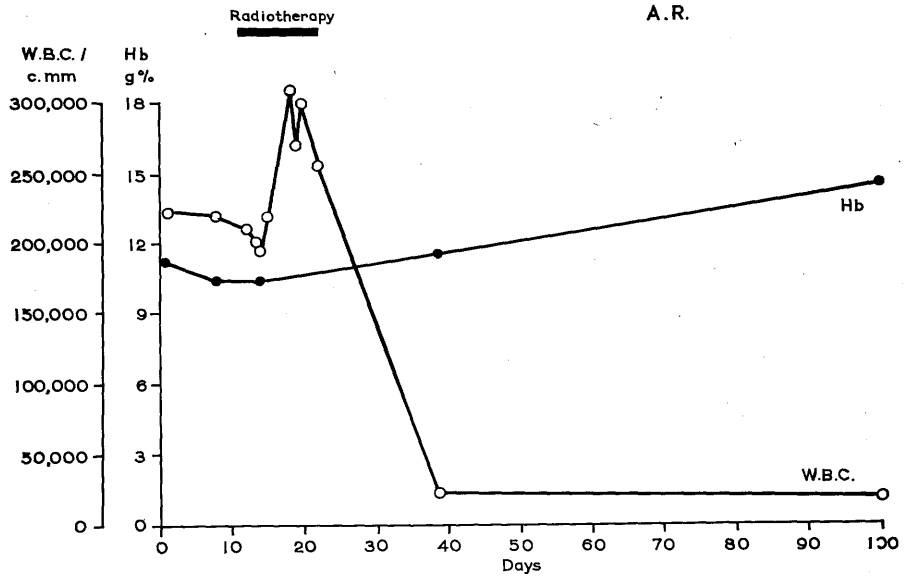
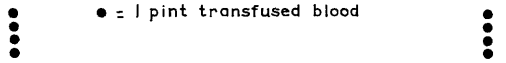


Fig. 102 Effect of splenic irradiation on patient A.R. with chronic myeloid leukaemia.



E.W.

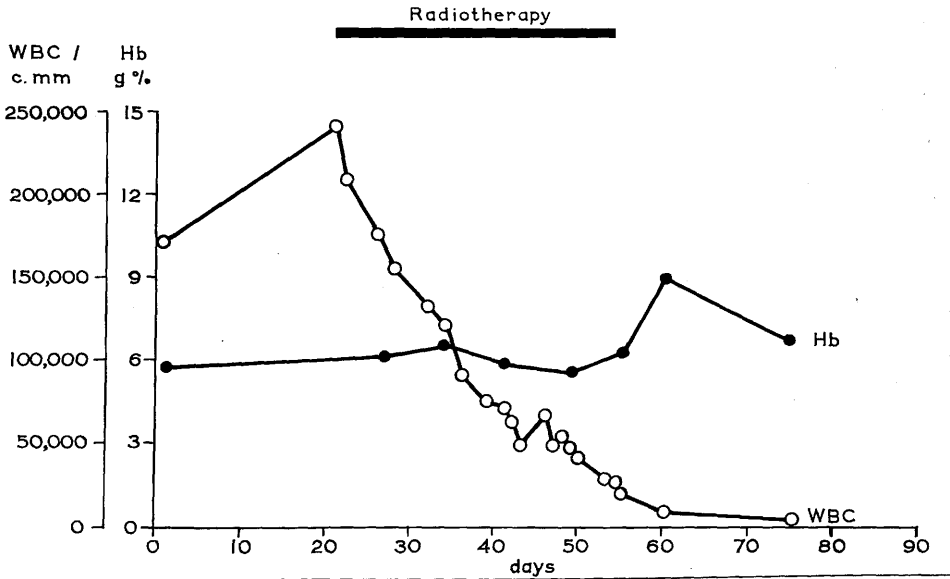


Fig. 103 Effect of splenic irradiation on patient E.W. with chronic myeloid leukaemia.

/Another patient, J.L., also had a rise of haemoglobin during splenic irradiation (fig.101). Such a definite response in the level of haemoglobin did not always occur and in patient A.R. there was a delayed rise which occurred towards the end of treatment or immediately after (fig.102). In patient E.W., however, the haemoglobin level did not rise significantly during therapy and even after a blood transfusion the higher level was not maintained (fig.103). The most satisfactory response appears to be a rise during treatment as one patient with this survived 2 years and the other is still alive and well 2½ years later. Patient W.R., who had a delayed response died 2 years later and patient E.W. with no significant response died within a year.

These results, though small in number, suggest that the response of the haemoglobin to splenic irradiation may give an indication of the stage of the disease.

It is worthy of note that three of the

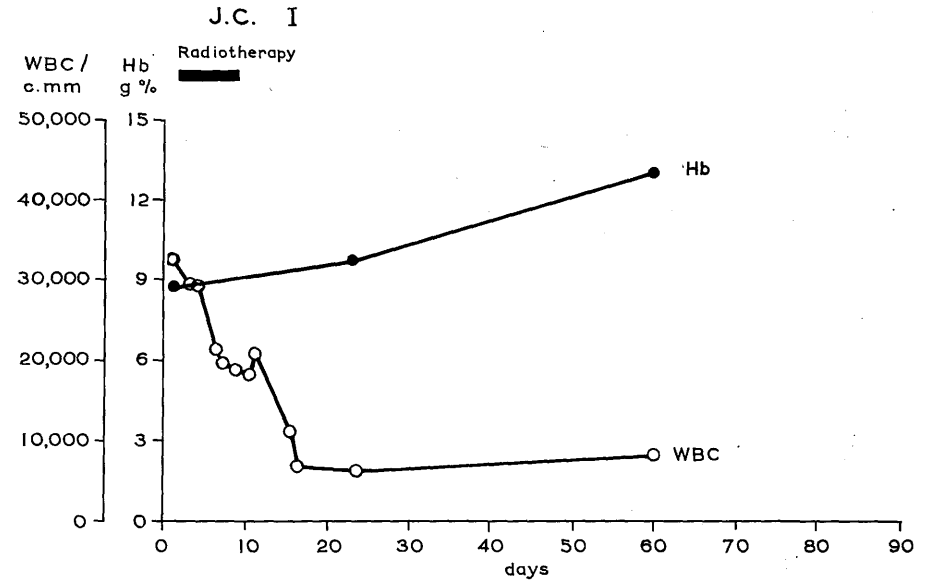


Fig. 104 Effect of 1st course of splenic irradiation on patient J.C. with myelofibrosis. (reproduced by permission from 'Clinical Radiology').

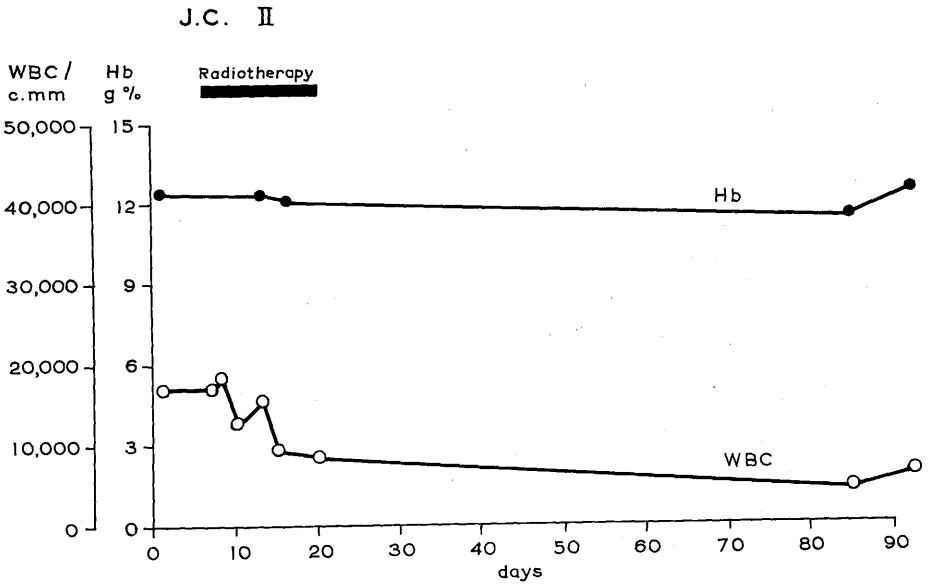


Fig. 105 Effect of 2nd course of splenic irradiation on patient J.C. with myelofibrosis.

/four patients treated by splenic irradiation had been shown by radioiron studies to have splenic erythropoiesis. This appears not to have influenced the results of treatment and the patient who died within a year had no evidence of splenic erythropoiesis on either radioiron studies or splenic puncture.

Myelofibrosis

Two patients with myelofibrosis were treated by splenic irradiation and one of these was treated on three separate occasions. The doses of splenic irradiation in this patient were, successively, 290 r delivered in 6 treatments, 248 r in 10 treatments and 364 r in 8 treatments. In the other patient 324 r was delivered over 12 treatments. All four treatments produced a satisfactory response in that the patient's general condition improved, and the white cell counts and haemoglobin levels usually were restored to more normal figures.

Splenic irradiation was considered to be indicated in patient J.C. on each occasion

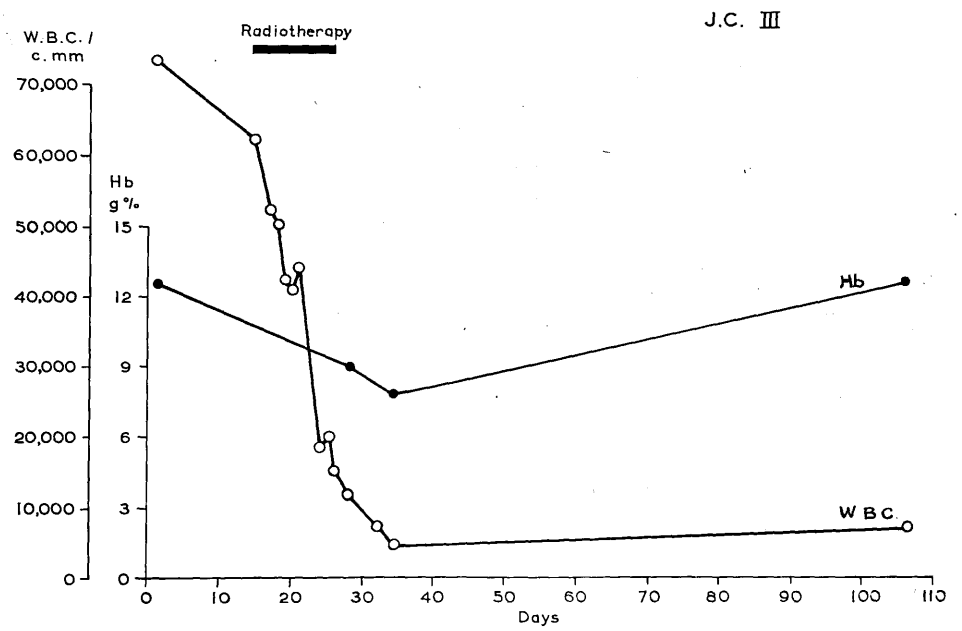


Fig. 106 Effect of 3rd course of splenic irradiation on patient J.C. with myelofibrosis.

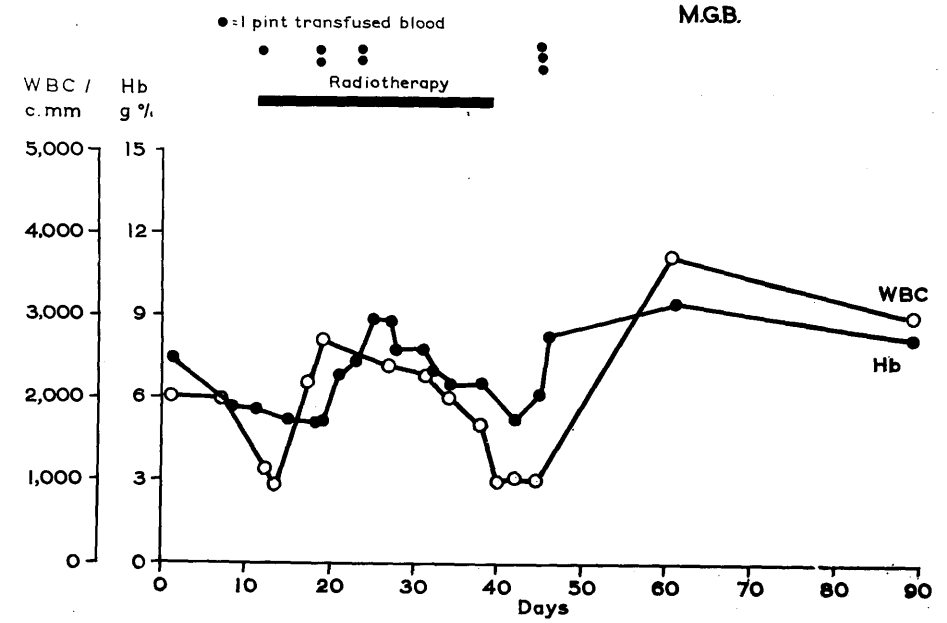


Fig. 107 Effect of splenic irradiation on patient M.G.B. with myelofibrosis.

/mainly because of the disability produced by the grossly enlarged spleen. At the time of the first treatment the white cell count was 32,400 cells per c.mm. and the haemoglobin 8.7 g per cent. The white cell count was restored to normal and the haemoglobin to 13 g per cent (fig.104). On the second occasion there was little haematological upset but the white cell count fell to normal and the satisfactory haemoglobin level was maintained (fig.105). The third treatment brought about a fall of the white cell count from 73,200 cells per c.mm. to normal and although the haemoglobin level fell during treatment it soon recovered to over 12 g per cent (fig.106). On each occasion there was a satisfactory clinical response. Splenectomy had been considered inadvisable because of the presence of splenic erythropoiesis and the lack of significant splenic sequestration.

Patient M.G.B. had increased haemolysis, $T\frac{1}{2}$ 17 days, and the spleen/liver ratio was 2.4/1

/which is considered borderline evidence of sufficient red cell sequestration to justify splenectomy. This patient was also shown to have splenic erythropoiesis and the examination of the blood revealed a leucopenia (2,000 cells per c.mm.). She had recently had a myocardial infarction and was in cardiac failure and so splenectomy could not be considered. She was treated by splenic irradiation in a dose of 324 r in 12 treatments over 28 days. The haemoglobin level improved and the need for transfusion ceased and the white cell count rose to over 3,000 per c.mm. shortly after treatment was completed. (fig.107). This patient required no further treatment for her myelofibrosis for the next 6 months at which time unfortunately, she had a further myocardial infarction and died.

Thus, in each of these patients, splenic irradiation proved a satisfactory and effective form of therapy despite the presence of erythropoietic tissue in the spleen.

If the response of the haemoglobin to

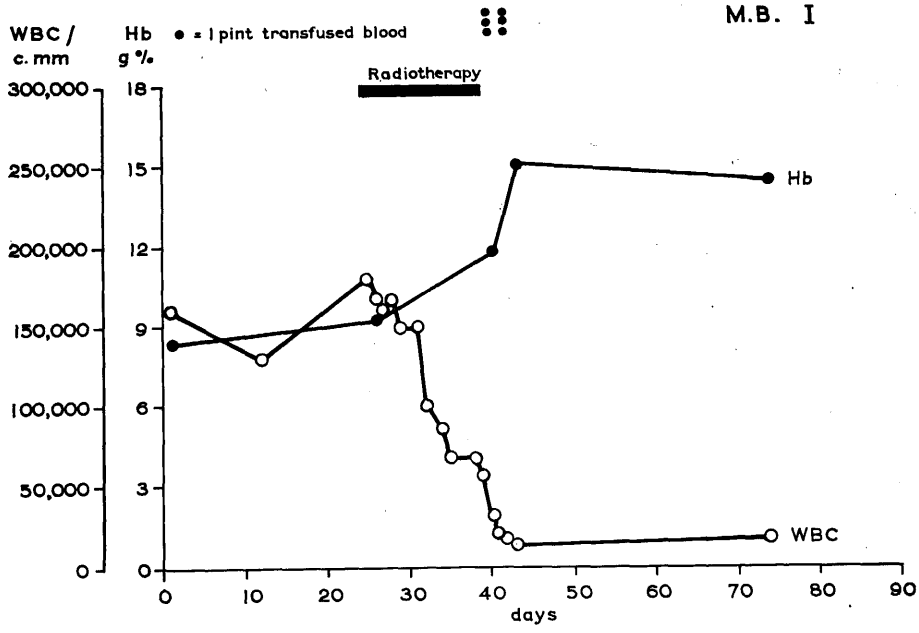


Fig. 108 Effect of 1st course of splenic irradiation on patient M.B. with 'intermediate' disease.

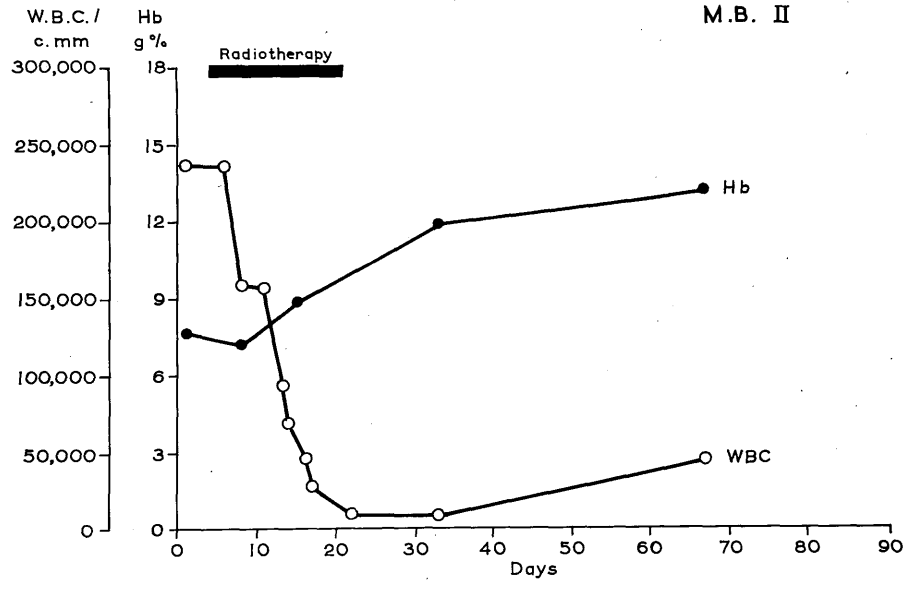


Fig. 109 Effect of 2nd course of splenic irradiation on patient M.B. with 'intermediate' disease.

/splenic irradiation is now considered it is seen that patient J.C. had a satisfactory rise of haemoglobin during the first treatment. At the second treatment the haemoglobin remained steady at an already satisfactory level and at the third treatment it fell during irradiation but later rose again to a satisfactory figure. This appears to be consistent with the observations made on chronic myeloid leukaemia and confirms the suggestion that the response of the haemoglobin may be an indication of the stage of the disease.

'Intermediate' cases

The 'intermediate' cases were each treated by splenic irradiation. Patient M.B. was treated this way on five occasions, of which the first four produced a satisfactory clinical response and a fall in the white cell count. The first treatment brought about a rise of haemoglobin the exact time of which is obscured by blood transfusion at the conclusion of treatment (fig.108). The second and third treatments (figs. 109 & 110), caused a rise

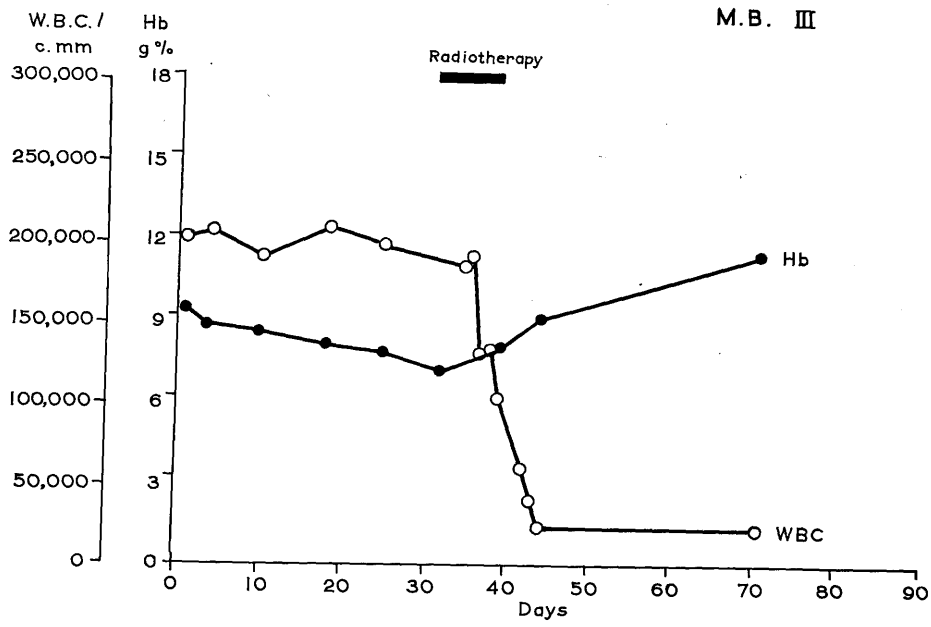


Fig. 110 Effect of 3rd course of splenic irradiation on patient M.B. with 'intermediate' disease.

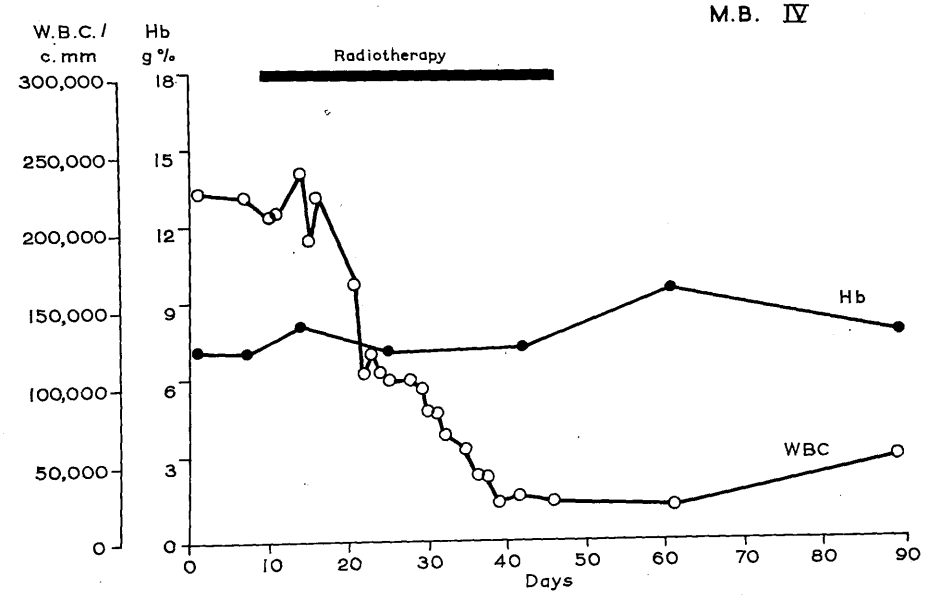


Fig. 111 Effect of 4th course of splenic irradiation on patient M.B. with 'intermediate' disease.

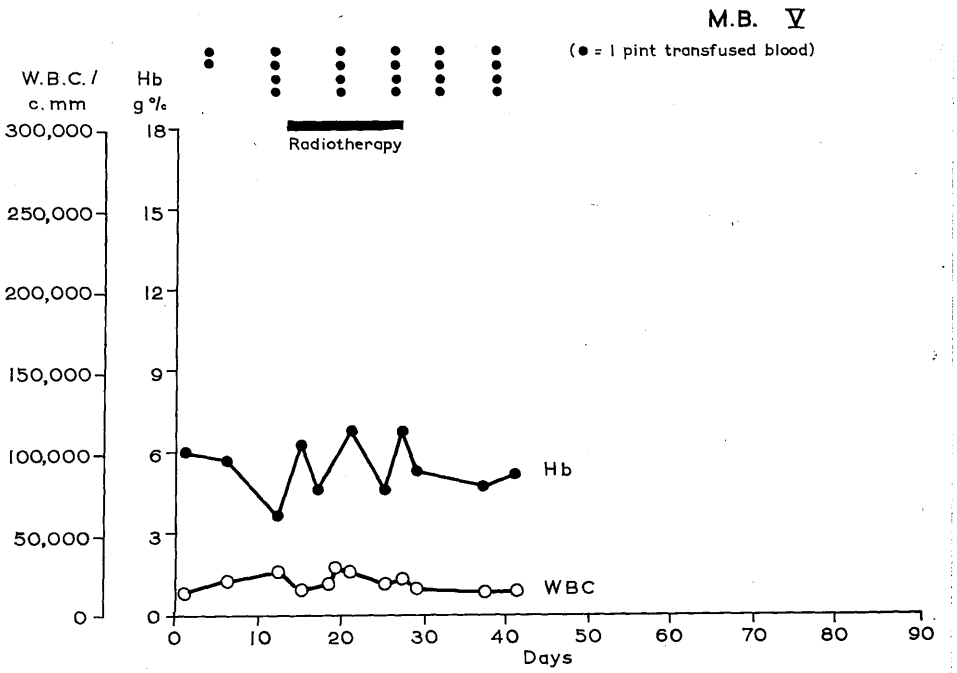


Fig. 112 Effect of 5th course of splenic irradiation in patient M.B. with 'intermediate' disease.

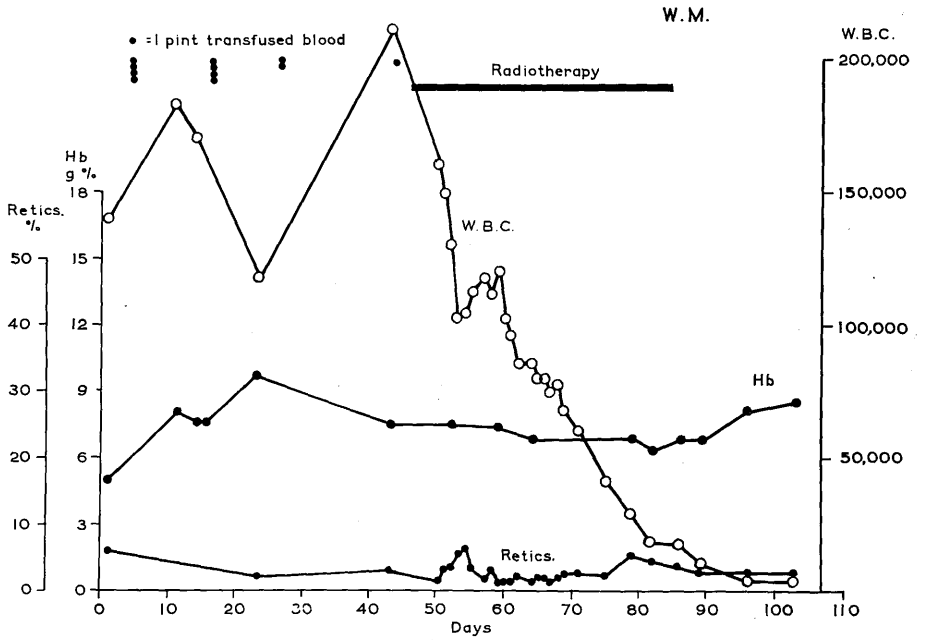


Fig. 113 Effect of splenic irradiation on patient W.M. with 'intermediate' disease.

/of haemoglobin during treatment and the fourth treatment (fig.111) brought about a less marked rise to a less well maintained level on completion of the course of radiotherapy. By the time of the fifth treatment the patient had gross haemolysis and required frequent transfusion of blood. Splenic irradiation then had no apparent effect on the haemoglobin level and the requirements for transfusion were not altered (fig.112).

Patient W.M. had one course of splenic irradiation. The white cell count fell from over 200,000 per c.mm. to normal figures. The need for transfusion ceased and the haemoglobin rose slowly after therapy had been completed (fig.113). There was marked clinical improvement. This patient survived for about 15 months after therapy and the late response of the haemoglobin is in keeping with a moderately advanced stage of the disease.

Extramedullary erythropoiesis was present in the spleen, in each of these patients and marrow uptake of radioiron was depressed.

/There were no ill effects from irradiation of the spleens which contained erythropoietic tissue.

Discussion

From the results of splenic irradiation in the above cases of chronic myeloid leukaemia, myelofibrosis and 'intermediate' disease, it seems that this form of therapy can be equally effective in each condition. Whereas splenic irradiation is probably the treatment of choice in chronic myeloid leukaemia, it requires careful consideration before being used in the treatment of myelofibrosis, and even then must be well controlled and possibly given in lower dosage over a long period. With these precautions satisfactory results can be obtained (Goldberg and Seaton, 1960).

Hickling (1953) found that in myelofibrosis the patients with high white cell counts were the ones who responded to splenic irradiation. Patient M.G.B. however, who had a leucopenia, had a good response to splenic irradiation; this demonstrates that a high white cell count

/is not necessary for radiotherapy to prove effective, and that factors such as haemolysis and 'hypersplenism' must be taken into account in assessing the patient's suitability for splenic irradiation.

It is suggested by the results of these 14 treatments with splenic irradiation that the stage of the disease may be assessed from the response of the haemoglobin to therapy. If the disease is in a fairly early stage the haemoglobin will rise during treatment, if later, there may be a delayed response, and later still the haemoglobin may be unaffected or may even fall during therapy.

The similarity of response in chronic myeloid leukaemia and myelofibrosis to splenic irradiation, regardless of the presence of absence of splenic erythropoiesis in either disease is considered to favour a relationship between these diseases. Further, this response is also found in the 'intermediate' cases and suggests that even in the transition from one disorder to another, the disease is fundamentally

/unchanged.

Summary

Splenic irradiation is shown to be a satisfactory form of therapy, not only in chronic myeloid leukaemia but also in some cases of myelofibrosis and 'intermediate' cases.

In myelofibrosis careful consideration should be given to the need for radiotherapy before this is recommended. If cases are selected carefully and if the course is planned and controlled, the results may be entirely satisfactory.

Splenic erythropoiesis is not necessarily a contraindication to splenic irradiation and good results may be obtained even in the presence of splenic erythropoiesis. The response of the haemoglobin to radiotherapy may give an indication of the stage of development of the disease.

Chapter 20

THE PROGRESSION

OF

MYELOPROLIFERATIVE DISEASE

It is well known that the average length of survival from the onset of symptoms in chronic myeloid leukaemia is about three years (Scott, 1957) and in myelofibrosis rather longer (Whitby and Britton, 1957). In chronic myeloid leukaemia the condition of the patient may be improved with treatment, which is required usually on several occasions during the course of the disease, but it is doubtful if the length of survival is prolonged (Scott, 1957). In myelofibrosis, however, treatment is probably required less frequently and when it is required it appears on occasions to be life saving.

Thus, two diseases so similar in many ways seem to run a different course. Nine patients with chronic myeloid leukaemia, myelofibrosis or 'intermediate' disease have been under observation for around two years or more and the progress of these patients is reported.

Chronic myeloid leukaemia

Patient E.W. was observed for 27 months during which time she was treated initially

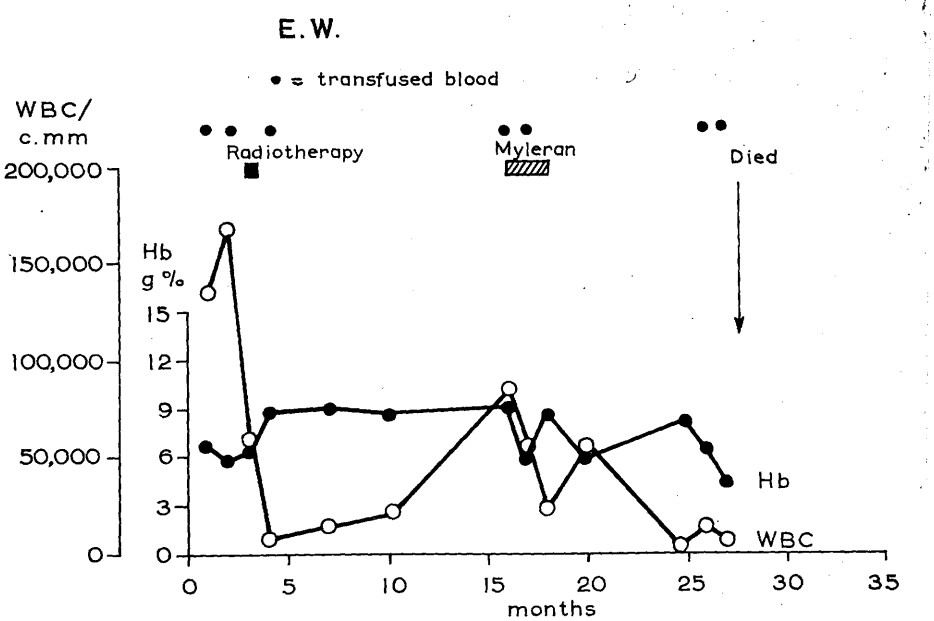


Fig. 114 The course of chronic myeloid leukaemia in patient E.W.

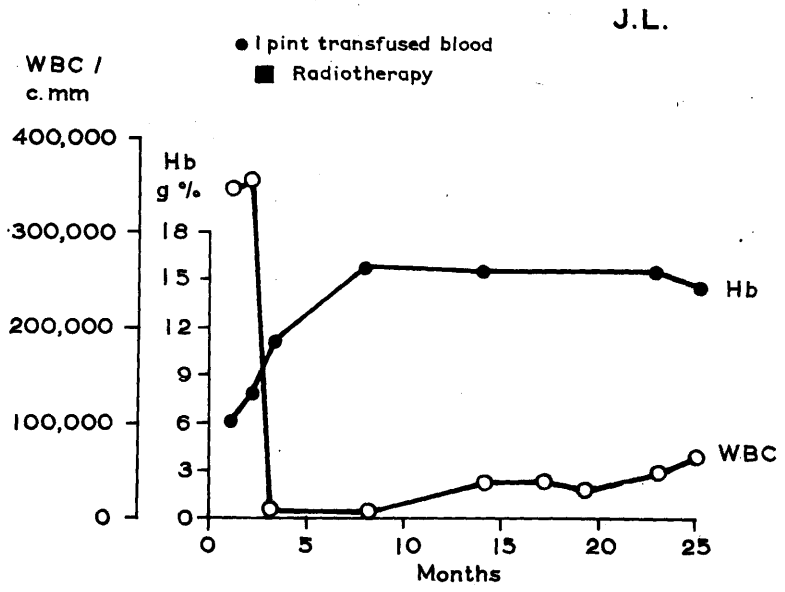


Fig. 115 The course of chronic myeloid leukaemia in patient J.L.

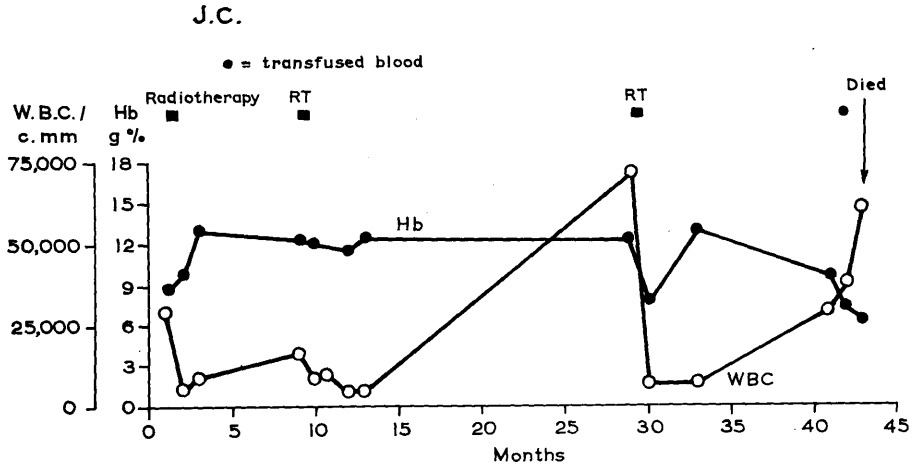


Fig. 116 The course of myelofibrosis in patient J.C.

/Myelofibrosis

Patient J.C. was observed for three and a half years and was treated with radiotherapy on three occasions. The course of the disease as it affected the haemoglobin and white cell count is seen in fig.116. The haemoglobin level is on the whole less variable than in chronic myeloid leukaemia but in the terminal stages it falls steadily. The white cell count is much lower in comparison to chronic myeloid leukaemia though the fluctuation is exaggerated on the graph by the different scale. There is, however, some similarity though this pattern is much less undulant.

Patient M.C. was under observation for over ten years. She was diagnosed originally as 'refractory macrocytic anaemia' and treated with 'Anahaemin' with apparently some response (fig.117). Later vitamin B_{12} was substituted without benefit and iron therapy produced temporary improvement. Over her last 18 months, the haemoglobin level fell steadily until she died. Again, the course is seen to be less

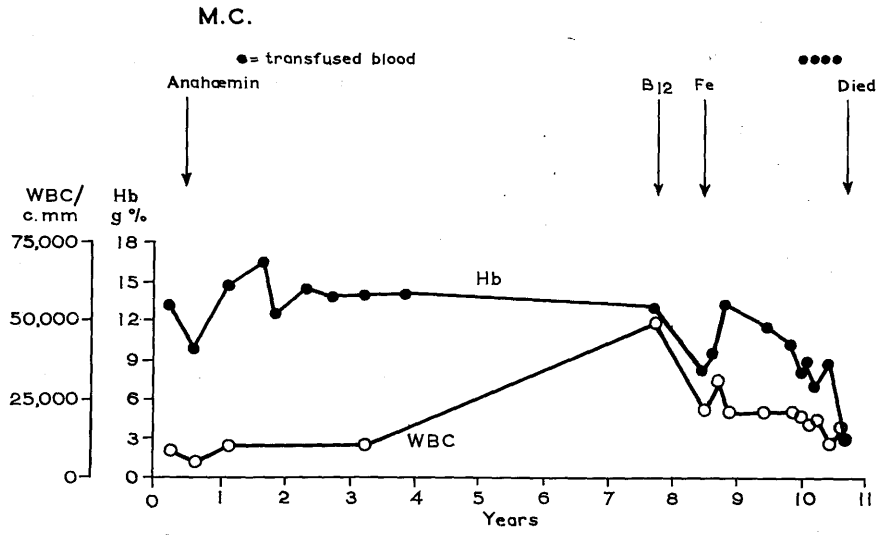


Fig. 117 The course of myelofibrosis in patient M.C.

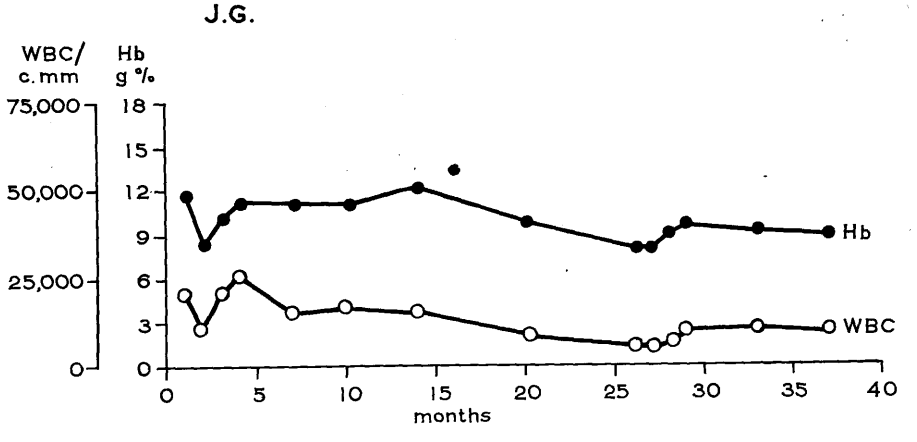


Fig. 118 The course of myelofibrosis in patient J.G.

/fluctuant than that of chronic myeloid leukaemia.

Patient J.G. was observed for over three years with little significant change in the haemoglobin or white cell count over this period (fig.118).

Patient S.H. also was observed for over three years and again there is no significant change in the blood levels during this time (fig.119).

Thus, whereas the course of chronic myeloid leukaemia is punctuated by periods of marked alteration in the haemoglobin and white cell levels which usually necessitates therapy, the course of myelofibrosis appears to follow a less undulant pattern. Only one case of myelofibrosis had a pattern like that of chronic myeloid leukaemia and even in this case the haematological variations were less well marked.

'Intermediate' cases

Patient M.B. had a course similar to chronic myeloid leukaemia; there was a periodic fall in the haemoglobin and a rise in the

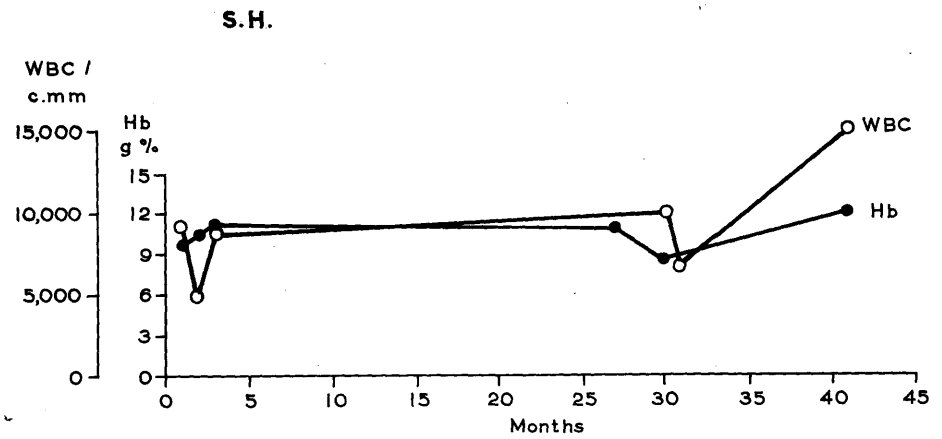


Fig. 119 The course of myelofibrosis in patient S.H.

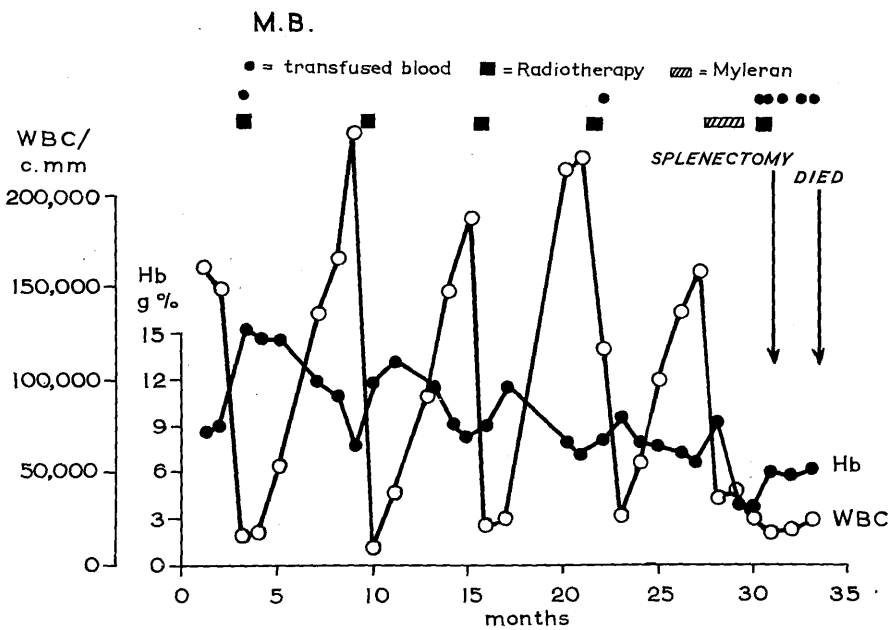


Fig. 120 The course of 'intermediate' disease in patient M.B.

/white cell count which necessitated treatment (fig.120). Over the three year period, however, there was a progressive deterioration in the haemoglobin level. Though splenectomy produced a temporary improvement, this was not sustained even with repeated blood transfusion.

Patient W.M. had a truly 'intermediate' course (fig.121). The initial high white cell count responded to splenic irradiation as did the haemoglobin. Although regular transfusion of blood was required there was never any great change in the haemoglobin level and this was in fact improving before death. Latterly, however, the white cell count started to rise again.

Thus, of the two 'intermediate' cases, one had a course which closely resembled chronic myeloid leukaemia and the other a course similar to chronic myeloid leukaemia in some ways and to myelofibrosis in others.

Discussion

From these results it would appear that myelofibrosis runs a course which produces

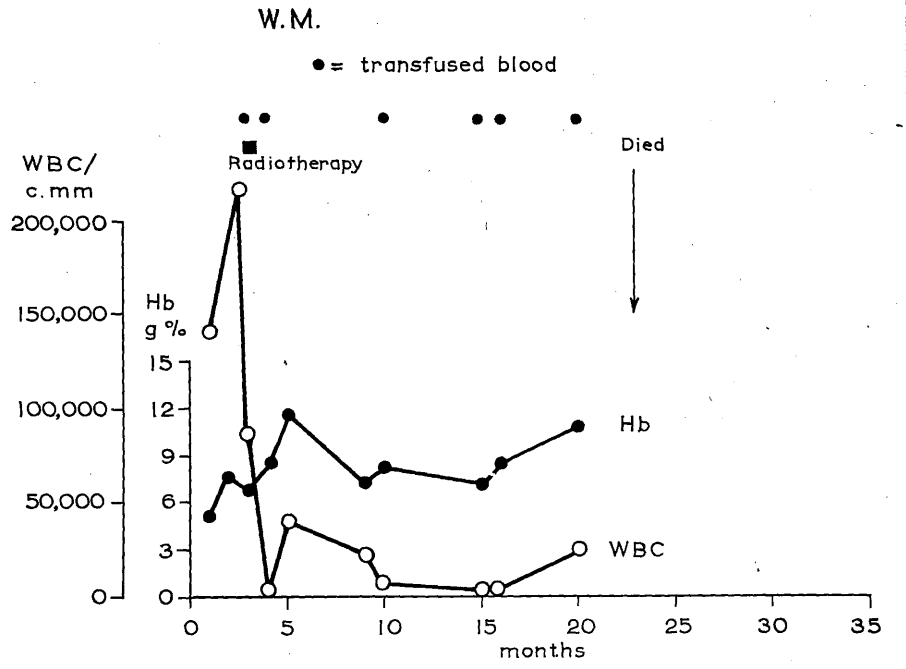


Fig. 121 The course of 'intermediate' disease in patient W.M.

/less disturbance of the haemoglobin and white cell count than does chronic myeloid leukaemia. Furthermore, it takes longer to reach the terminal phase which usually is ushered in by a progressive fall in the haemoglobin. Treatment, other than simple remedies, is seldom required in the early stages and it is not uncommon to see cases such as J.G. and S.H. progress uneventfully for several years. Patient M.C. is, of course, remarkable and survived almost eleven years. In the terminal phase the haemoglobin fell steadily but it is noteworthy that this took place over a period of one and a half years. In patient J.C. the haemoglobin fell for about ten months before death occurred.

In chronic myeloid leukaemia the course is much more fluctuant with repeated alterations in the haemoglobin level and white cell count related to deterioration and therapy. As a result of this pattern it is obvious that the haemoglobin and white cell counts

Patient	Diagnosis	Interval (yrs.)	Test	^{59}Fe uptake Marrow	^{59}Fe uptake Spleen	^{59}Fe Utilisation (%)	^{51}Cr $T\frac{1}{2}$ (days)	Transfusion requirements
C.L.	C.M.L.	1	1	0	+++	73	20	0
			2	0	+++	54	-	0
R.C.	Mf.	1	1	0	+	53	15	0
			2	0	+	132	16.5	0
M.C.	Mf.	2	1	0	0	100	13	0
			2	0	0	16	14	++
J.C.	Mf.	$2\frac{1}{2}$	1	0	++	55	22	0
			2	0	+	34	25	+
J.G.	Mf.	2	1	0	0	33	17	0
			2	-	-	-	11	0
W.McA	Int.	$1\frac{1}{2}$	1	0	++	16	15	+
			2	0	+++	65.3	20	+
M.B.	Int.	1	1	0	++	97	12	+
			2	0	0	-	-	+++

Table 43 The change in the results of radioisotope investigation after an interval of time in seven patients with myeloproliferative disease.
(C.M.L. = chronic myeloid leukaemia; Mf.= myelofibrosis; Int.= 'intermediate' disease).

/are not reliable in determining when a terminal stage is reached. In the two patients who died the fall in haemoglobin took place over a short two month period in contrast to the longer periods seen in the cases of myelofibrosis.

Thus, myelofibrosis appears to be a much less disturbing disease more likely to run a longer course and less liable to require any major form of therapy than chronic myeloid leukaemia. When haematological deterioration occurs in myelofibrosis the disease is likely to be entering a terminal phase but this is not necessarily so in chronic myeloid leukaemia.

RADIOISOTOPE STUDIES

Radioisotope studies were repeated in four patients with myelofibrosis, one with chronic myeloid leukaemia and two with 'intermediate' disease. The results are seen in table 43.

Chronic myeloid leukaemia

In the one patient on whom the studies

/were repeated there appears to be no obvious change in the pattern of production and destruction of red cells.

Myelofibrosis

Patient R.C. shows no significant change except for the rise of iron utilisation to the obviously fallacious figure of 132 per cent. The patient was much more anaemic at this time and the great increase in iron utilisation is probably partly related to marrow turnover and to iron deficiency.

In patient M.C. the iron utilisation had fallen to the very low figure of 16 per cent which indicates severely deficient erythropoiesis. There was no significant change in haemolysis at this time and so the increased transfusion requirement is probably the result of hypoplasia. This pattern is seen also in patient J.C. The only test to be repeated on patient J.G. was the red cell survival and this showed an increased rate of haemolysis. This patient, however, was well and no treatment was required thus suggesting that erythropoiesis was able

/to keep pace with the increased rate of haemolysis.

'Intermediate' disease

Patient W.McA's transfusion requirements remained the same over one and a half years. His red cell production capacity, as judged by iron uptake and iron utilisation, appeared to have improved and there was less haemolysis. His death shortly after these studies were carried out was due to cerebral haemorrhage and was not caused by any change in his haematological state, as is so common in these patients.

Patient M.B. had the iron uptake only repeated and this showed loss of splenic erythropoiesis. This however must have played only a small part in the production of anaemia as her requirements for transfusion were so great that excessive haemolysis must have been present.

Comment

While one patient showed actual improvement in the haematological status

/and two showed no change there was evidence of deterioration in four patients. Of these four patients three had myelofibrosis and one had 'intermediate' disease. There are obviously two main causes for the development of severe anaemia; firstly, the rate of haemolysis may increase and the limited red cell production be unable to compensate; secondly, erythropoiesis may decrease until there is insufficient erythropoietic tissue to maintain a steady haemoglobin at even a lower level. It appears that each of these may play a part in any patient and that either may be the dominant factor.

Summary

The progression of the disease was studied in nine patients with chronic myeloid leukaemia, myelofibrosis or intermediate disease.

Myelofibrosis appears to cause less disturbance of the haematological state than chronic myeloid leukaemia.

When severe haematological deterioration occurs in myelofibrosis it is likely to be terminal but in some patients years may pass before this occurs. In chronic myeloid leukaemia frequent treatment is usually required for haematological deterioration.

The development of anaemia may be due to hypoplasia or excessive haemolysis and either may be the dominant factor.

SUMMARY OF

SECTION V

The general aspects of treatment of myeloproliferative disease are reviewed briefly. Some patients require no therapy for long periods and others respond satisfactorily to simple measures. Only in polycythaemia vera, however, does treatment appear to affect the survival time.

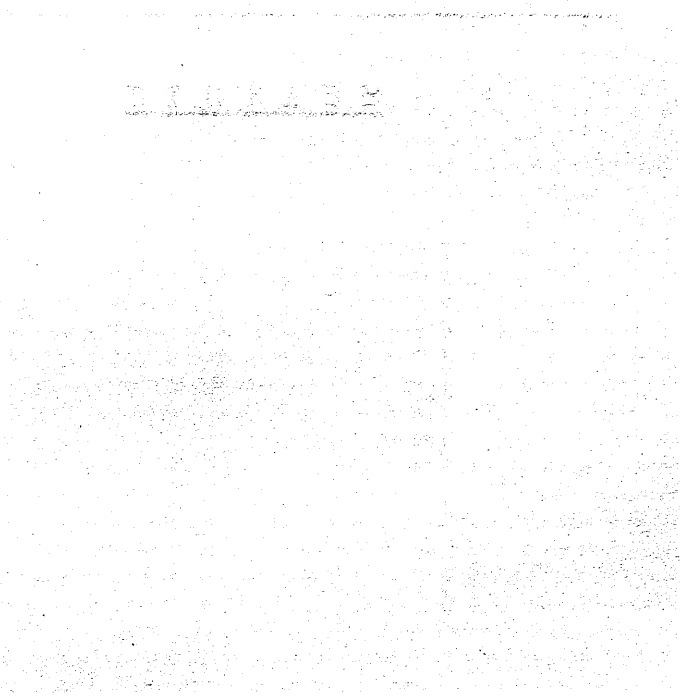
Splenectomy is shown to be an effective form of therapy in some cases of myelofibrosis and the radioisotope studies are of help in determining the patients' suitability for this procedure.

Irradiation of the spleen is found similarly to affect chronic myeloid leukaemia and myelofibrosis. The presence of splenic erythropoiesis is not necessarily a contraindication provided that the dosage is then adjusted suitably. The nature of the response to radiotherapy may provide a guide to the stage of progress of the disease.

The radioisotope studies have helped to define the mechanism of the anaemia in these disorders and thus to place treatment on

/a more logical basis.

The progression of myelofibrosis and of chronic myeloid leukaemia has been studied. Chronic myeloid leukaemia appears to run a more undulant course which is of shorter duration than that of myelofibrosis. When haematological deterioration occurs in myelofibrosis this usually is an indication that the disease is approaching its terminal phase.



SECTION VI

THE PATHOGENESIS OF MYELOPROLIFERATIVE DISEASE

Chapter 21

A CONCEPT

OF

MYELOPROLIFERATIVE DISEASE

The myeloproliferative disorders are regarded by some workers as a group of diseases with a superficial resemblance but without any aetiological relationship; other workers, however, regard them as a group of closely related diseases.

The suggestion of relationship of some of these disorders was put forward by Vaughan and Harrison (1939) and has been supported by Heller et al (1947) and Rosenthal (1950). Dameshek (1951) publicised this view and extended the range of speculation to include the whole group of myeloproliferative disorders and has since been supported by Robson (1953), Hutt et al (1953) and Goldberg and Seaton (1959).

Other workers, however, oppose this view and regard the diseases as unrelated thus favouring the suggestion of Donhauser (1908) that a primary interference in the bone marrow leads to secondary and compensatory extramedullary erythropoiesis in the spleen and other organs. Wyatt and Sommers (1950) compared myelofibrosis and hepatic cirrhosis; they felt that there

/were many causes for these conditions and concluded that both disorders were morphological entities without aetiological unity. Wintrobe (1951) felt that there was no sound basis for assuming myelosclerosis to be a variant of chronic leukaemia. More recently Leonard et al (1957) gave their opinion that the group of disorders were not simply facets of a single disease and felt that if 'transition' forms occurred at all commonly then the constant patterns which they found would not have been expected.

In the present study, clinical and haematological assessments have been made of each of the diseases constituting the myeloproliferative group and the mechanism of blood production and destruction has been studied by radioactive isotopes. Further points have been investigated and the indications for, and effect of, treatment have been studied.

The onset and the signs and symptoms of chronic myeloid leukaemia and myelofibrosis

/have been shown to be similar and while some points of resemblance are found on examination of the blood and bone marrow it is on histological grounds that the two disorders are conclusively differentiated. The mechanisms of blood production and destruction have been studied and the same patterns were found in each disease. These points are considered to favour a relationship between the two diseases and the occurrence of two 'intermediate' cases provides further evidence for this view. Polycythaemia vera, megakaryocytic thrombocythaemia and Di Guglielmo's disease have all been studied in the same way and the results discussed. Again the evidence appears to favour a close aetiological relationship of the whole group of myeloproliferative disorders and the four 'transition' cases provide strong support for this.

The treatment of myeloproliferative disease has been reviewed briefly and the main problems of the roles of splenic

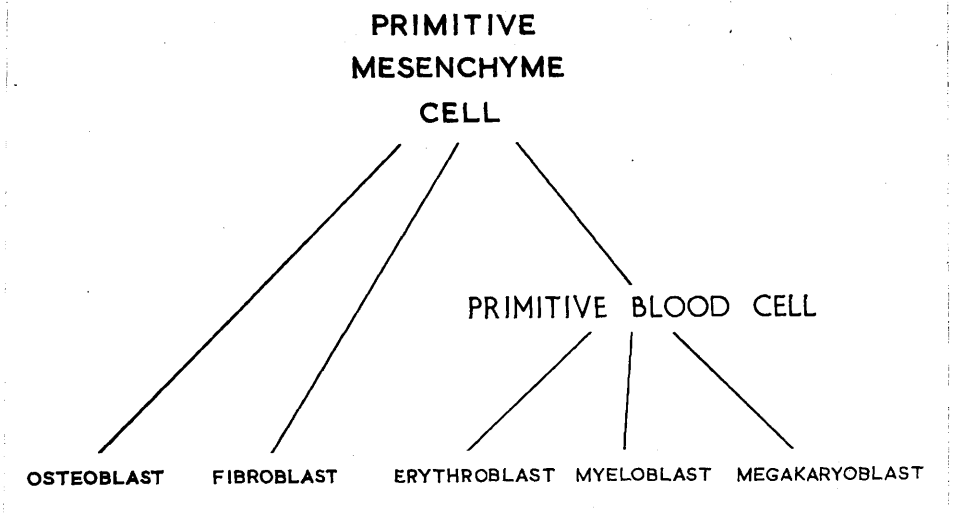


Fig. 122 The primitive mesenchymal cell.

/irradiation and splenectomy in the treatment of myelofibrosis and other myeloproliferative states have been investigated. The use of radioactive iron and radioactive chromium in assessing the mechanisms and sites of blood production and destruction seems to place these forms of therapy on a more logical basis and it has been shown that with these methods satisfactory results may be obtained. Further, the effects of treatment have been observed and the results appear to strengthen the case for relationship between these diseases.

Thus the evidence gained by this study suggests that although each component disease is a separate morphological entity, the myeloproliferative group of disorders are closely related and the not uncommon occurrence of 'transition' forms appears to provide the final evidence for this aetiological relationship.

The primitive mesenchymal cell is considered to be multipotential and thus

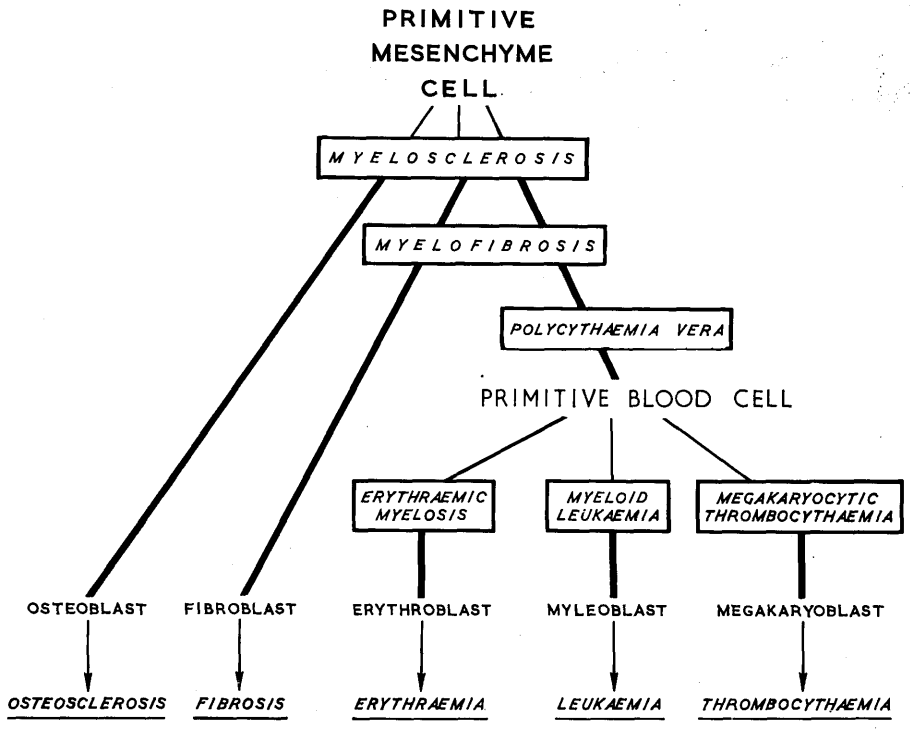


Fig. 123 A concept of myeloproliferative disease.

/is capable of differentiating into osteoblast, fibroblast or any of the precursors of the cells of the blood (fig.122). Thus, if the myeloproliferative disorders are produced by an abnormality in development this may lead to involvement of any of the succeeding cell types or a combination of these (fig.123). Thus, chronic myeloid leukaemia, Di Guglielmo's disease and megakaryocytic thrombocythaemia may be circumscribed types of abnormality occurring at a late stage in development and consequently affecting one major cell type only. Polycythaemia vera may be caused by abnormality at a rather earlier stage of differentiation and thus may involve all three cellular components of the blood. Myelofibrosis and myelosclerosis could be the results of developmental abnormality at a very early phase of differentiation and could involve all the succeeding phases resulting in osteosclerosis, fibrosis and leucopoietic,erythropoietic or megakaryocytic proliferation in the bone marrow. Transition

/forms are then easily explained by a variation in the predominant cell type involved or by the progression of abnormality to an earlier phase of differentiation thus involving other cell types.

The results of the work composing this thesis substantiate this concept that the myeloproliferative disorders are variable expressions of abnormal development of the primitive mesenchymal cell.

APPENDIX

CASE SUMMARIES

The case summaries are presented in order to provide an overall picture of each case without the detail as described in various chapters.

Chronic myeloid leukaemia

Patient E.W., a female, aged 69 years, was first seen in June 1957 because of intermittent symptoms of anaemia during the previous few years. She also complained of abdominal discomfort and loss of weight. The main findings on examination were of pallor, hepatomegaly, splenomegaly and a few palpable nodes in the axillae. The diagnosis of chronic myeloid leukaemia was made on investigation of the blood and marrow.

Blood transfusion was required on several occasions and she was then treated with splenic irradiation. She had a good response to this and remained well for a little over a year before she required further blood transfusion and radiotherapy. The response was less satisfactory than on the first occasion and within

/a few months busulphan had to be given. Despite this, however, deterioration continued and she developed bronchopneumonia and then congestive cardiac failure. Further treatment was of no avail and she died in September 1959.

Patient J.L., a male, aged 33 years, attended hospital first in April 1958 because of tiredness, dyspnoea, sweating and marked weight loss despite a good appetite. His first attendance was at an Endocrine Clinic to which he was referred because of the above history, but the suspected diagnosis was not confirmed. Abdominal examination revealed hepatomegaly and splenomegaly and a few nodes were found in the axillae. The diagnosis of chronic myeloid leukaemia was made on blood and bone marrow examination. He was very anaemic but responded well to splenic irradiation and blood transfusion. His one complication was of priapism which was treated with anticoagulant drugs.

Following irradiation his spleen shrank to the costal margin, the blood picture returned to normal and the patient's general condition

/was greatly improved. This satisfactory state of affairs continued for almost two years though latterly the white cell count was beginning to rise again. He has as yet required no further treatment and remains symptom free.

Patient C.L., a female, aged 47 years, first attended the urological unit of the Western Infirmary in June 1957 because of increased frequency of micturition and dysuria. The cause of this was a urethral caruncle but the patient also complained of weight loss of four stone in four months. Abdominal examination revealed splenomegaly and marked bruising was noted on the legs. The diagnosis of chronic myeloid leukaemia was made on blood and bone marrow examination.

The patient was treated by splenic irradiation with considerable benefit. The improvement however, lasted only for a few months and she required treatment on two more occasions before she died in July 1959.

Patient A.R., a female, aged 59 years, first attended hospital in January 1956 because of abdominal pain, dyspnoea on exertion and spontaneous bruising. Examination revealed multiple ecchymoses and gross splenomegaly was present.

She was treated by splenic irradiation with marked symptomatic improvement but there was no alteration in the level of the white cell count until the completion of the course and the spleen did not change in size.

She remained relatively well for a further one and a half years before being readmitted with the same symptoms as before. No abnormality was found on investigation of the clotting mechanism but following iliac crest trephine biopsy there was considerable haemorrhage. A few days later in October 1957, she died following a cerebral vascular accident.

Patient M.M., a female, aged 51 years, had had symptoms of abdominal discomfort and dyspnoea on exertion for only a few weeks when she first attended in February 1959. Examination revealed hepatomegaly, splenomegaly,

/widespread large palpable nodes and a right sided pleural effusion. The diagnosis of chronic myeloid leukaemia was made on blood and bone marrow examination. X-ray of chest showed a right sided pleural effusion and marked leukaemic infiltration of the pulmonary parenchyma. The patient developed bronchopneumonia but despite antibiotic therapy she died.

Myelofibrosis

Patient J.C., a female, aged 42 years, presented first in March 1956 with swelling of the abdomen of one year's duration. She also complained of weight loss and spontaneous bruising. Examination revealed gross splenomegaly and the diagnosis of myelofibrosis was established by iliac crest trephine biopsy.

She was treated by splenic irradiation and had a satisfactory response which lasted, however, for a few months only before a further course was required. A few months later she developed gout in her right foot.

Thereafter followed gradual deterioration

/and another course of splenic irradiation in 1958 had little effect except to lower the white cell count.

In 1959 she developed congestive cardiac failure and died in September of that year.

Patient T.Q., a female, aged 56 years, was first seen in December 1958. Her complaints were of the symptoms of anaemia, nose bleeds and right sided abdominal discomfort. Examination revealed congestive cardiac failure, pallor, hepatomegaly and gross splenomegaly. She was very anaemic due to an increased rate of haemolysis and required frequent blood transfusion. Radioisotope studies demonstrated that the spleen was the site of sequestration and destruction of red cells; although there was some splenic erythropoiesis there was also some continuing marrow activity.

On these grounds splenectomy was performed after the cardiac failure was cleared; there was a very satisfactory result. The patient has remained well since, has required no further therapy and is still at work.

Patient M.C., a female aged 62 years, first presented at hospital in June 1949 because of abdominal swelling which proved to be due to gross splenomegaly. She was investigated and found to have a mild macrocytic anaemia which was treated with thyroid extract. She became more anaemic in the next few months and was then changed to 'Anahaemin' to which she had a 12 per cent reticulocyte response. She remained fairly well for the next three years and although marrow puncture was attempted on several occasions the result was always a 'dry tap'.

In 1952 a bone marrow trephine biopsy was carried out and the diagnosis established as myelofibrosis. She was not seen again until 1956 when she attended because of tiredness. Again there was very mild anaemia and the 'Anahaemin' was stopped and 'Cytamen' substituted. She became more anaemic over the next few months and this was found to be due to iron deficiency which responded well to parenteral iron therapy.

In 1959 she again became anaemic and

/developed a urinary tract infection and septicaemia. With antibiotic therapy she recovered but the anaemia was found to be due to splenic sequestration of red cells and increased haemolysis. Splenectomy was therefore carried out but the patient unfortunately died in a sudden post operative collapse after a technically satisfactory operation.

Patient S.H., a female, aged 64 years, presented first in March 1957 with intermittent claudication due to true peripheral vascular disease. She was found to have splenomegaly and the diagnosis of myelofibrosis was established by iliac crest trephine. This diagnosis, however, was incidental as she has required no treatment for myelofibrosis, though her vascular condition has necessitated her being in hospital for most of the past four years. It is of interest that she did not trouble about the symptoms of mild anaemia and weight loss which had been present for around three years and

/that her first attendance was only because her intermittent claudication severely limited her activity. This underlines the insidious onset of myelofibrosis.

Patient A.S., a female, aged 49 years, first attended in May 1959 because of weight loss and abdominal discomfort of about six months duration. A few small lymph nodes were palpable in the right axilla and there was hepatomegaly and marked splenomegaly. The diagnosis of myelofibrosis was established by iliac crest trephine.

The only treatment required was for iron deficiency anaemia and this produced a satisfactory response. The patient has remained well since.

Patient J.G., a male, aged 62 years, was first seen in May 1957 when he complained of the symptoms of mild anaemia for the previous few weeks. Examination revealed hepatomegaly and marked splenomegaly.

The diagnosis of myelofibrosis was established by iliac crest trephine.

/ The patient required no specific therapy and remained well for a further three years. Oral iron therapy was then administered but in view of his satisfactory general state no major therapy was undertaken although radiochromium studies indicated that the spleen was sequestering and destroying red cells.

Patient R.C., a female, aged 58 years, was seen first in April 1959 because of weight loss and angina of effort of three years duration. Examination revealed hypertension, anaemia and gross splenomegaly. Investigations showed that no treatment was necessary other than for iron deficiency. Following this treatment she remained well for a further fifteen months when she required a blood transfusion and further iron therapy. She has remained well since.

Patient M.G.B., a female, aged 63 years, first complained of right sided abdominal discomfort and weight loss of 1 year's duration in June 1959. She then was pale, had an easily palpable liver and spleen

/and was in cardiac failure secondary to myocardial infarction. Megaloblasts were found in the peripheral blood but there was no real response to vitamin B_{12} except a) to correct the serum B_{12} level and b) to eliminate megaloblasts from the blood. The diagnosis of myelofibrosis was established by iliac crest trephine.

She required blood transfusion and despite leucopenia was treated with splenic irradiation. There was a satisfactory response to this treatment and she remained well for a further six months before succumbing to her second myocardial infarction. This patient had radiological defects in the skull possibly secondary to myelofibrosis but unfortunately no autopsy was obtained.

Patient W.P., a male, aged 56 years, had a three week history of left sided upper abdominal pain when he first attended in January 1960. Examination revealed a few small nodes in the axillae and groins. The liver and spleen were both markedly enlarged.

/The diagnosis of myelofibrosis was established by iliac crest trephine.

This patient was found to have a haemolytic anaemia with splenic sequestration; the marrow and spleen both showed erythropoietic activity and so the haemolysis was treated by steroid therapy. With this treatment he improved greatly and has remained well since.

'Intermediate' disease

Patient W.M., a male, aged 57 years, was first seen in January 1957 because of enlargement of the abdomen and symptoms of anaemia. He had mild cardiac failure, was pale and anaemic and had greatly enlarged liver and spleen. Investigation revealed some features of chronic myeloid leukaemia and some of myelofibrosis and he was classified as 'intermediate'.

He was treated with splenic irradiation and had a satisfactory response. About six months later however his symptoms recurred but blood transfusion was sufficient on this occasion to provide improvement. Thereafter

/he remained relatively well for about a year when further transfusion was required. He died at home a few months later as a result of a cerebrovascular accident.

Patient M.B., a female, aged 34 years, presented first in March 1956 with abdominal swelling and the symptoms of anaemia during the previous few months. She was found to have hepatomegaly and marked splenomegaly.

Investigation revealed some features of chronic myeloid leukaemia and some of myelofibrosis. She was, therefore, classified as 'intermediate' disease.

Treatment with splenic irradiation was required on five occasions during the next two and a half years. Eventually she developed severe haemolysis which necessitated frequent blood transfusion. Splenectomy was carried out with an apparently successful result in that the blood picture improved remarkably and blood transfusion requirements were greatly reduced. Wound infection and abscess formation occurred, probably partly

/due to hypogammaglobulinaemia, and the patient died about two months after operation.

Polycythaemia Vera

Patient A.McA., a male, aged 70 years, first attended for examination in April 1958. He had a 15 year history of dyspepsia due to proved duodenal ulcer and had had melaena and haematemesis on two previous occasions. Furthermore he had a history of jaundice in 1914. He was admitted to the wards because of recent melaena and symptoms of anaemia. Liver and spleen were both palpable as had been noted on the occasion of his last melaena in 1954. He also had gouty arthritis of his hands.

He was found to be rather anaemic but with elevated platelet and white cell counts.

Investigation confirmed the presence of a duodenal ulcer, there was no upset of liver function and the diagnosis of polycythaemia vera was established on later examination of the bone marrow.

Treatment with radiophosphorus was delayed until his bleeding had settled and his

/blood values had risen to above normal. He had a good response to this therapy and has remained well since.

Patient M.S., a female, aged 56 years, first attended hospital in 1951 when she was found to have a mild hydronephrosis. At that time plethora was noted. In 1953 she again attended because of abdominal pain. She was then found to have a dusky red complexion, splenomegaly was noted and the haemoglobin was recorded as 140 per cent. Treatment was by radiotherapy and thereafter repeated venesections until July 1958. She defaulted for a year and was then found to be in a mental hospital. By this time she was again polycythaemic and the diagnosis was confirmed by marrow histology and was treated by venesection and then radiophosphorus with a satisfactory response. She has remained well since and has also improved mentally.

Patient F.N., a female, aged 55 years, was first seen in August 1959 because of

/chest pain, breathlessness, cough and spit. She was seen to be plethoric but cyanotic and was found to have hypertension but no splenomegaly. The diagnosis of polycythaemia vera was confirmed on blood and marrow examination.

She was treated with radiophosphorus and improved haematologically and symptomatically for three months. Thereafter although she remained symptom free her polycythaemia returned.

Patient J.G., a male, aged 61 years, was diagnosed as having polycythaemia vera in 1936 and was treated by phenylhydrazine and blood-letting. In 1958 he developed severe gouty arthritis of the hands and feet. His haemoglobin thereafter remained around normal but the white cell count was constantly elevated to about 40,000 per c.mm. Marrow examination was typical of polycythaemia vera.

In 1959 his haemoglobin rose again to high levels and he was then treated with

/radiophosphorus with some haematological improvement.

Patient J.C., a male, aged 50 years, first attended an Orthopaedic department because of bone pain. He was noted to be plethoric and had a palpable spleen. Blood and marrow examination confirmed the diagnosis of polycythaemia vera.

Treatment was with radiophosphorus and he responded satisfactorily.

Di Guglielmo's disease

Patient G.McF., a male, aged 77 years, was admitted for investigation of a normochromic anaemia in September, 1958. His complaints were of the symptoms of anaemia of about four months duration. There were no physical findings of significance.

Further examination of the blood revealed the presence of occasional nucleated red cells and marrow examination showed irregular areas of hyperplasia. Erythropoiesis was predominantly megaloblastic and many multinucleated well haemoglobinised red cells

/were seen. The diagnosis of Di Guglielmo's disease was thus made.

Vitamin B_{12} and folic acid studies revealed nothing to account for megaloblastic erythropoiesis and the administration of these substances produced no benefit. Further treatment with blood transfusion and Prednisolone was of no avail and the patient's condition steadily deteriorated until he died in November 1958.

Megakaryocytic thrombocythaemia

Patient J.D., a female, aged 67 years, was admitted first to another hospital in August 1957 because of spontaneous bruising. Physical examination revealed also hepatomegaly and splenomegaly. She was found to have elevated white cell and platelet counts and investigation of the clotting system gave evidence of haemorrhagic megakaryocytic thrombocythaemia.

During the next two years despite several haematemeses and episodes of melaena, she gradually became polycythaemic. The treatment of choice appeared to be

/radiophosphorus; this was given and the response was very satisfactory. The patient has remained well since and has had no further haemorrhagic episodes.

'Transition' myeloproliferative disease

Patient J.N., a male, aged 54 years, was first seen in November 1959 when he complained of joint pains. He was found to have splenomegaly and a few palpable axillary nodes.

Investigations of the blood and marrow suggested a diagnosis of polycythaemia vera but further study using radioisotopes suggested that the patient was in fact in a state of transition from polycythaemia vera to myelofibrosis.

No treatment was required for his haematological state and he has since remained well.

Patient J.McG., a male, aged 74 years, first attended hospital in March 1959 because of the symptoms of anaemia and a bleeding tendency. Physical examination revealed

/marked pallor and hepatomegaly but no splenomegaly.

White cell count was usually within normal limits but platelet counts were persistently over half a million per c.mm. Marrow examination revealed marked megakaryocytic proliferation and gross myeloid hyperplasia.

Further investigation suggested that the disease was in a state of transition between megakaryocytic thrombocythaemia and chronic myeloid leukaemia.

The patient was treated with repeated blood transfusion and was given radiophosphorus on two occasions. While there was considerable haematological response the patients' general condition deteriorated steadily over the next few months and he died in October 1959.

Patient J.McD., a female, aged 64 years, first attended another hospital in 1956 because of weakness and lassitude. She was found to have hepatomegaly and splenomegaly and blood examination revealed no diagnostic features. Marrow histology, however, showed

/30 - 50 per cent of the cells to be myeloblasts. In 1959 she was very anaemic and had hepatomegaly. There was also an abdominal mass which appeared to come from the pelvis and the spleen was palpable just below the costal margin i.e. much less large than in 1956.

Investigation showed that the abdominal mass was in fact an ectopic spleen. In view of the evidence of hypersplenism at that time splenectomy was performed. Following operation the white cell count rose to leukaemic levels and had to be controlled with 'Busulphan'. Otherwise the patient was much improved and has since required no further therapy.

The investigations suggest that this case was in a state of transition probably from polycythaemia vera to myeloid leukaemia.

Patient A.McG., a female, aged 74 years, first presented in 1946 with swelling of the legs, thrombophlebitis and bleeding after dental extraction. No definite diagnosis

/was made. In 1950 she was found to have polycythaemia vera with splenomegaly and was treated by repeated venesection. In 1956 she developed gouty arthritis and by 1959 was anaemic and had thrombocythaemia. The marrow appearances were those of polycythaemia vera with exaggerated proliferation of the megakaryocytes.

Further investigation suggested that the patient was in a state of transition between spent polycythaemia vera and megakaryocytic thrombocythaemia.

Her gout was controlled by probenecid and the only other treatment required was the administration of iron. With this treatment she has remained well.

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