RADIOACTIVE IRON STUDIES IN ROUTINE HAEMATOLOGICAL PRACTICE

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In recent years considerable use has been made of radioactive iron in the study of normal and abnormal iron metabolism, and its value as a research tool is well recognized (Finch *et al.*, 1949 ; Huff *et al.*, 1950, 1951, 1953 ; Ledlie and Baxter, 1954 ; Badenoch and Callender, 1954 ; Moore and Dubach, 1955 ; Cartwright and Wintrobe, 1955).

The object of this paper is to discuss the value of intravenous radioactive iron studies in routine haematological practice and to assess whether these investigations can contribute anything to the diagnosis and treatment of individual patients which cannot be obtained by simpler techniques.

Methods and Materials

Preparation of Radioactive Iron for Injection

There are two radioactive isotopes of iron which may be used for biological investigations. ^{55}Fe emits low-energy *x*-radiation, but the difficulties of measurement of this isotope preclude its use in routine clinical practice. ^{59}Fe emits penetrating gamma radiation (1.1 and 1.3 meV) as well as low-energy beta particles, and is much more suitable for routine work in which measurements of absorbed iron are made external to the body surface. This isotope has been used in the present work.

^{59}Fe is obtained as ferric chloride in 0.3/N HCl from Oak Ridge, U.S.A., through the Isotope Division, Atomic Energy Research Establishment, Harwell. The activity of the solution supplied is usually 30 microcuries (μC) in a total volume of 2 ml. The specific activity of the solution supplied for this study has varied between 350 and 1,500 μC per mg., so that the samples obtained have contained between 20 and 90 μg. of elemental iron.

In the present series of patients two techniques have been used to prepare the iron for injection :

Method 1.—The iron is combined with β-globulin in a buffered solution as described by Ledlie and Baxter (1954).

Method 2.—The iron is added to 0.1% sodium citrate in isotonic saline and allowed to stand for 15 minutes at room temperature. It has been shown by Loeffler *et al.* (1955) that iron injected in tracer doses in citrate buffer is bound to the metal-binding protein of the recipient's plasma and that plasma clearances are comparable to those obtained with method 1.

With either technique it is convenient to prepare 13 ml. of a solution having a total activity of approximately 13 μC. Exactly 10 ml. of this solution is injected intravenously, and the remainder is retained for counting and calculation of the total injected dose.

Technique for Following the Disposal of Injected Iron

1. *Plasma Clearance.*—The prepared iron solution is injected intravenously and samples of venous blood are withdrawn at known times during the first hour. Samples are usually taken at 15, 25, 40, and 60 minutes after injection and counted in the well counter. The times are varied if abnormal clearance times are expected. The results show an exponential clearance of iron from the plasma (Fig. 1).

2. *Surface Counting.*—With the directional scintillation counter, changes in activity are recorded over the prae-

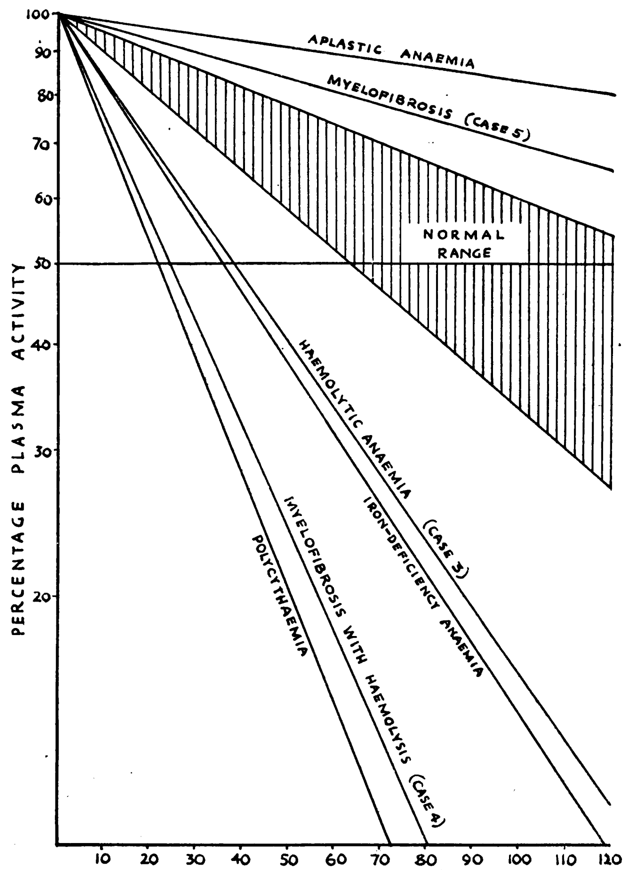

FIG. 1.—Plasma clearance rates in normal and abnormal subjects. Plotted on semi-logarithmic paper.

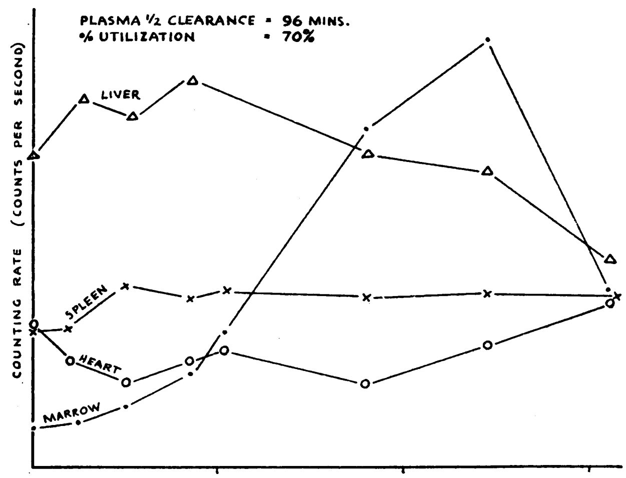

FIG. 2.—External counting rates. Normal adult. Plotted with activity on a linear ordinate scale against time on a log abscissa scale.

cordium (a measure of whole-blood activity), the spleen, the liver, and the sacrum (a measure of marrow activity) as described by Ledlie and Baxter (1954). Changes in activity over these four sites are measured during the 10-day period following injection (Fig. 2). For convenience the results are plotted with activity on a linear ordinate scale against time on a log abscissa scale.

3. *Red-cell Utilization of Injected Iron.*—The percentage of the injected dose utilized for haemoglobin synthesis increases from about 12 hours after injection and reaches a maximum between 7 and 10 days. It can be calculated from the following data : (*a*) the total R.B.C. volume estimated from the patient's weight and haematocrit, correcting for body haematocrit according to the formulae of Mollison (1951); (*b*) the total injected dose in counts/sec.; and (*c*) the activity in counts/sec./ml. of red cells.

$$\text{Percentage utilization} = \frac{\text{R.B.C. vol. in ml.} \times \text{activity in counts/sec./ml. of red cells} \times 100}{\text{total activity injected in counts/sec.}}$$

Apparatus and Technique of Measurement

1. *Measurement of Blood Samples.*—In this work a well-type scintillation counter has been used for the measurement of blood samples. In order to take advantage of recently developed plastic phosphors, this well counter was made in the hospital physics workshop, but suitable, if not so convenient, counters are available commercially. The scintillator is a cylinder, 3 in. (7.5 cm.) in height and 2 in. (5 cm.) in diameter, of polyvinyl toluene containing a mixture of terphenyl butadiene and tetraphenyl butadiene. Owing to the relatively large size of the cylinder, it is possible to drill an adequate hole in the material for insertion of the sample, which is therefore almost completely surrounded by the scintillation material. The scintillator is mounted above the cathode of a photo-multiplier supplied by E.M.I. (type 6097), and the whole assembly is mounted in a light-tight metal container enclosed in a hollow thick lead cylinder. The blood samples contained in thin flat-bottomed glass tubes are placed inside the "well," and the pulses, after suitable amplification, are counted by an EKCO scaler (type N529). The photo-multiplier is operated at 1,550 volts between anode and cathode and a discriminator bias of 40 volts. Under these conditions the absolute efficiency (counts per disintegration) of the well counter is approximately 11%. This compares favourably with instruments described by other authors (Belcher, 1953 ; Haigh, 1954). The equipment has been found trouble-free and is extremely simple to use. All measurements are corrected for radiation background. Variations in counter sensitivity are controlled by measuring the activity of a standard solution of ^{59}Fe before the measurement of each set of blood samples.

2. *Surface Measurement.*—The technique and apparatus used for surface counting is similar to that described by Ledlie and Baxter (1954). The wide-angle directional scintillation counter (type BN101), supplied by Messrs. Burndept Limited, is used with its lead collimator removed, and the pulses are amplified and measured by an EKCO rate-meter (type N522). Corrections for variations in counter sensitivity are determined before each clinical measurement by measuring the activity of a standard solution of ^{59}Fe contained in a "perspex" jig, which is applied in conditions of fixed geometry to the scintillation counter.

Radiation Hazards in Routine Use of ^{59}Fe

When contemplating the use of radioactive isotopes in the hospital on a routine basis it is essential to consider very carefully whether the radiation hazards to the patient and staff are likely to be important. From data published in the "Recommendations of the International Commission on Radiological Protection" (*British Journal of Radiology*, 1955) the maximum permissible weekly dose of 0.3 rem is delivered to the whole body of a "standard man" by a continuous body burden of 13 μC of ^{59}Fe. Thus an injected dose of 10 μC results in a maximum dose-rate of 0.23 rem/week to the whole body, which decreases subsequently with an effective half-life of 27 days. Since the maximum permissible dose has been laid down for occupational workers who are at continuous risk, we believe that the radiation hazard to the patient is negligible. The risk to staff is even less, provided simple precautions are observed.

Results

Interpretation

The results obtained by the use of the techniques which have been described are apt to be misinterpreted if all the factors which may modify them are not taken into consideration.

The immediate effect of injection of a tracer dose of iron is to label the plasma iron pool. The subsequent clearance of active iron from the plasma and its utilization in haemoglobin synthesis or storage will depend chiefly on two factors : the level of iron stores in the body, and the rate and volume of red-cell production by the marrow.

For example, a patient with high iron stores and a normal red-cell production, as in idiopathic haemochromatosis, will appear to have a relatively slow plasma clearance and a

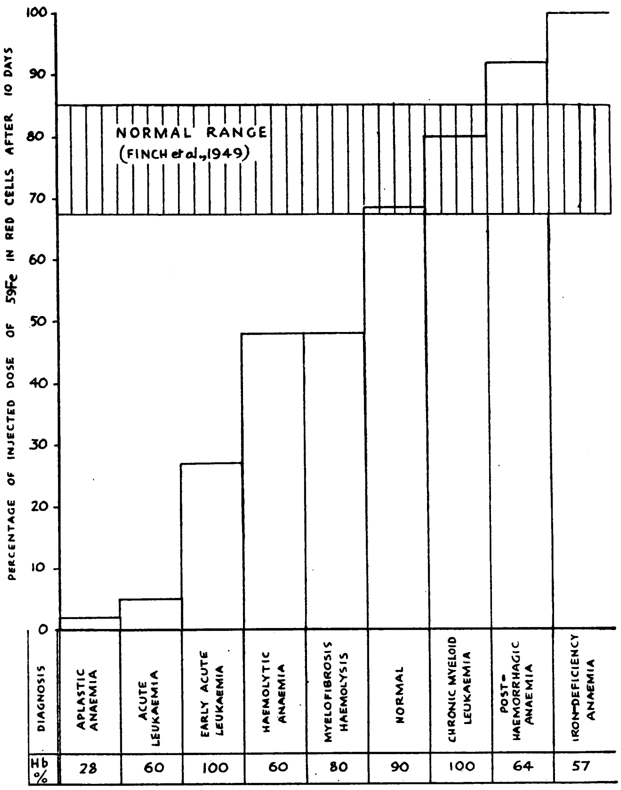

FIG. 3.—Utilization of injected iron for haemoglobin synthesis.

reduced utilization of iron merely because the iron pool which has been labelled is much larger than normal. Conversely, when the labelled pool is smaller than normal, as in iron-deficiency states, the plasma clearance will appear to be more rapid and the iron utilization greater than normal (Figs. 1 and 3). Under these circumstances such results do not mean that red-cell production is greater than normal.

When the surface-counting technique is used it is also necessary to recognize that the observed activity over any site is determined not only by the uptake of iron by the organ but also by the varying activity of the blood flowing through the organ.

If any quantitative estimation of iron metabolism is required it is, of course, necessary to estimate the significance of these factors and to make a quantitative correction of the observed results (Huff *et al.*, 1951 ; A. J. Grimes, 1955, personal communication). This requires considerable elaboration of the techniques, but in routine haematological practice such refinement is unnecessary. In those patients in whom tracer iron studies can contribute to the diagnosis and management of the disease adequate information can usually be derived from the uncorrected results, provided the possible effects of variation in iron stores and organ blood-flow are recognized. All surface-counting data shown in this paper are uncorrected for organ blood-activity.

Normal Findings

In the normal subject the injected iron is cleared from the plasma exponentially, with a half-clearance of 70–120 minutes (Ledlie and Baxter, 1954) (Fig. 1). This clearance is usually evident in the surface counts over the heart, which fall rapidly over the first two to three hours (Fig. 2). During this time surface counts over the sacrum begin to rise, reaching a peak at about 20–30 hours. Red cells containing active haemoglobin begin to appear in the peripheral blood after about 10 hours, and there is a progressive increase in red-cell activity up to 10 days. This increase, which is accompanied by a rising heart count, is associated with a gradual fall in the marrow surface-count. The liver shows a slight initial rise followed by a gradual fall, while the spleen shows no change or parallels the changes over the heart. In the normal individual, approximately 70–80% of the injected dose eventually appears in red cells, and this maximum level is reached at 7–14 days (Finch *et al.*, 1949) (Fig. 3).

Abnormal Patterns

A total of 70 patients was investigated, using one or more of the techniques described. The preliminary diagnoses in these patients are shown in the Table. Some of the patients

Preliminary Diagnoses in 70 *Cases*

Normal	3	Refractory anaemia	7
Leukaemias	17	Idiopathic haemolytic anaemia	3
Iron-deficiency anaemias	5	Myelofibrosis	6
Megaloblastic anaemias	1	Carcinomatosis	1
Polycythaemia	3	Rheumatoid arthritis	1
Uraemia	11	Post-haemorrhagic anaemia	2
Fibrosis of the liver	1	Chronic infection	1
Aplastic anaemia	3	Malignant lymphoma	5

were selected for investigation because the mechanism of their anaemia was obscure. The remainder were investigated in order to establish, for reference purposes, the various radioactive patterns associated with recognized types of anaemia. Three main patterns were observed in these patients.

1. Patients in whom there was a normal plasma clearance, a normal or only slightly reduced maximal percentage utilization, and normal surface counts. This pattern was found in several patients suffering from leukaemia, uraemia, and malignant lymphoma who showed only slight anaemia.

2. Patients in whom there was a rapid plasma clearance and a high percentage utilization of ^{59}Fe for haemoglobin synthesis. In these patients the surface counts usually showed no diagnostic alteration of the marrow pattern. This pattern was found in iron-deficiency anaemias, post-haemorrhagic anaemias, polycythaemia, and some "refractory anaemias" (Figs. 1 and 3).

3. Patients in whom there was a slow plasma clearance and a low percentage utilization. The majority of these patients showed a flat marrow curve associated with a gradually rising liver uptake (Fig. 5). The liver curve was considerably modified in patients who had received multiple transfusions and who presumably had increased iron stores. These results, which suggest depressed erythropoiesis, were commonly observed in patients with aplastic anaemia, in some "refractory anaemias," in some leukaemias with low haemoglobin, and in some cases of myelofibrosis (Figs. 1 and 3).

Apart from these groups a number of unusual patterns were found. In some, useful information, particularly in the demonstration of haemolysis and extramedullary erythropoiesis, was obtained (Cases 3, 4, and 5). In others, although the interpretation remains uncertain, the findings suggest that this technique will be of value in other studies which aim at clarifying the mechanism of anaemia in leukaemia and renal failure.

On the basis of iron tracer studies the patients in this series could be divided into two main groups. In the first and larger group the mechanism of their anaemia had been clearly established by routine haematological techniques, and in these patients, although the iron studies invariably confirmed the diagnosis, they made no additional contribution to the practical management of the patient. In the second and smaller group of patients a presumptive diagnosis had usually been established by the routine haematological techniques. The iron studies were of particular value in such patients because they established a definite diagnosis and gave a fairly clear dynamic picture of erythropoietic activity. They provided, therefore, a rational basis for treatment. The following cases illustrate some of these points.

Case 1 : Hypoplastic Anaemia

A woman aged 58 was admitted for investigation of anaemia of eight months' duration. She had been treated elsewhere with iron, liver, folic acid, and vitamin B_{12} without response. Apart from the anaemia there were no abnormal physical signs.

Routine Findings.—Hb, 6.2 g./100 ml. ; R.B.C., 1,500,000 per c.mm. ; P.C.V., 18% ; M.C.H.C., 34% ; M.C.V., 120 cubic microns ; W.B.C, 4,100 per c.mm. (polymorph neutrophils 28%, band cells 14%, lymphocytes 52%, monocytes 6%) ; platelets, 152,000 per c.mm. ; reticulocytes less than 1% of R.B.C. ; direct Coombs test negative. Sternal marrow —a rather dilute specimen showing normoblastic erythropoiesis.

^{59}Fe Study (Fig. 4).—There was reduced marrow uptake, reduced percentage utilization, and increased deviation of iron to the liver—findings which confirmed the diagnosis

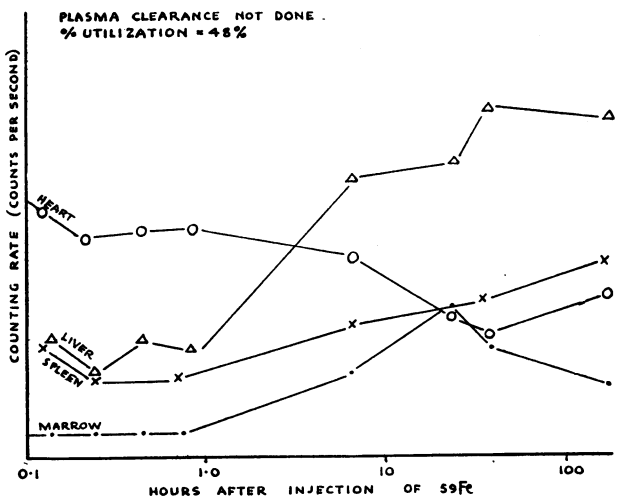

FIG. 4.—External counting rates. Hypoplastic anaemia (Case 1).

of hypoplastic anaemia. In addition, it was established that there was still appreciable erythropoietic activity, and this suggested that the prognosis was probably better than in Case 2. The patient has been satisfactorily maintained with relatively infrequent transfusions.

Case 2 : Aplastic Anaemia

A man aged 76 was admitted to hospital with symptoms of anaemia for two months. Apart from severe anaemia and slight icterus, he showed no abnormal physical signs. He had been treated with large doses of vitamin B_{12} on the basis of a clinical picture and a peripheral blood count which were highly suggestive of pernicious anaemia.

Investigations.—Hb, 3.85 g./100 ml.; R.B.C., 740,000 c.mm. ; P.C.V., 11% ; M.C.V., 146 cubic microns ; M.C.H.C., 33% ; reticulocytes, 1% ; film—macrocytosis and anisocytosis. An occasional normoblast was seen. Marrow (×2) : a dilute specimen containing relatively few marrow cells ; a few cells of the erythropoietic series had the appearance of transitional megaloblasts.

^{59}Fe Study (Fig. 5).—This patient showed a negligible marrow uptake, slow plasma clearance, a grossly reduced utilization (2%), and increased deviation of iron to the liver. These findings showed almost complete functional failure of erythropoiesis, and indicated that the altered appearance of the red cells and their precursors was due to an aplastic anaemia rather than a nutritional anaemia.

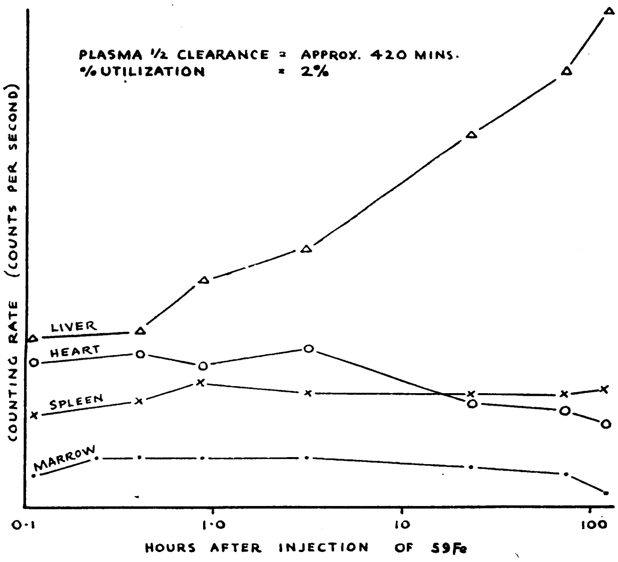

FIG. 5.—External counting rates. Aplastic anaemia (Case 2).

Case 3 : Haemolytic Anaemia

A woman aged 68 was admitted for investigation of anaemia and splenomegaly. The spleen was palpable below the umbilicus.

Investigations.—Hb, 8.8 g./100 ml.; R.B.C., 3,000,000 per c.mm.; M.C.V., 93 cubic microns; M.C.H.C., 31%; W.B.C., 2,800 per c.mm. (polymorph neutrophils 47%, lymphocytes 53%); platelets, 80,000 per c.mm.; direct Race–Coombs test, negative; reticulocytes, 4%; sternal marrow, normal erythropoiesis.

^{59}Fe Study (Fig. 6).—This patient showed a rapid plasma clearance, a normal marrow uptake, and a reduced percentage utilization. These findings are extremely suggestive of a haemolytic process with increased marrow turnover and shortened red-cell survival. This was confirmed by cell-survival studies, and there was an extremely satisfactory response to splenectomy. The low percentage utilization, as found in this patient, is a characteristic feature of haemolytic anaemia, and there is at present no satisfactory explanation of this. It seems probable that there is a larger, slowly turned-over iron pool in the reticulo-endothelial system and that iron enters this pool either direct from the plasma or following its liberation as a result of early random destruction of red cells.

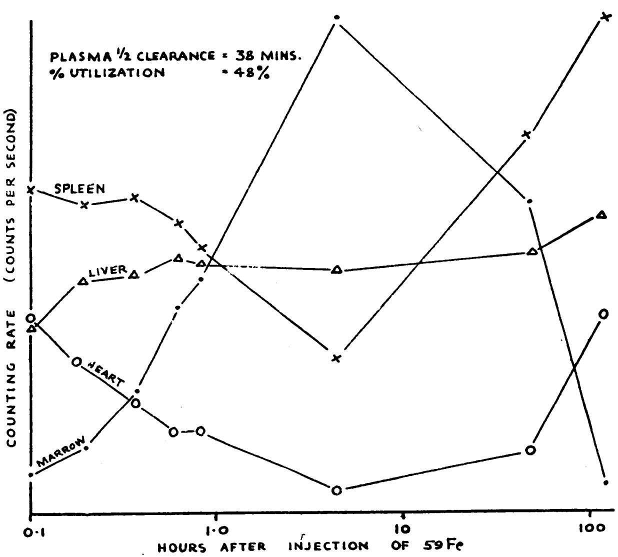

FIG. 6.—External counting rates. Haemolytic anaemia (Case 3).

Cases 4 and 5 : Myelofibrosis

In both these patients, who had enormous spleens, the diagnosis of myelofibrosis had been established by marrow biopsy, and the question of splenectomy was considered. It was therefore of some interest to assess the relative erythropoietic activity of the spleen and the bone marrow.

Case 4

A man of 58 with symptoms of splenomegaly was admitted only for a decision on splenectomy.

Routine Findings.—Hb, 11.9 g./100 ml.; M.C.H.C., 31%; M.C.V., 118 cubic microns; W.B.C., 4,500 per c.mm. (polymorph neutrophils 70%, lymphocytes 20%, monocytes 6%, metamyelocytes 3%, neutrophil myelocytes 1%); reticulocytes, 3%; platelets, 368,000 per c.mm.; direct Race–Coombs test, negative.

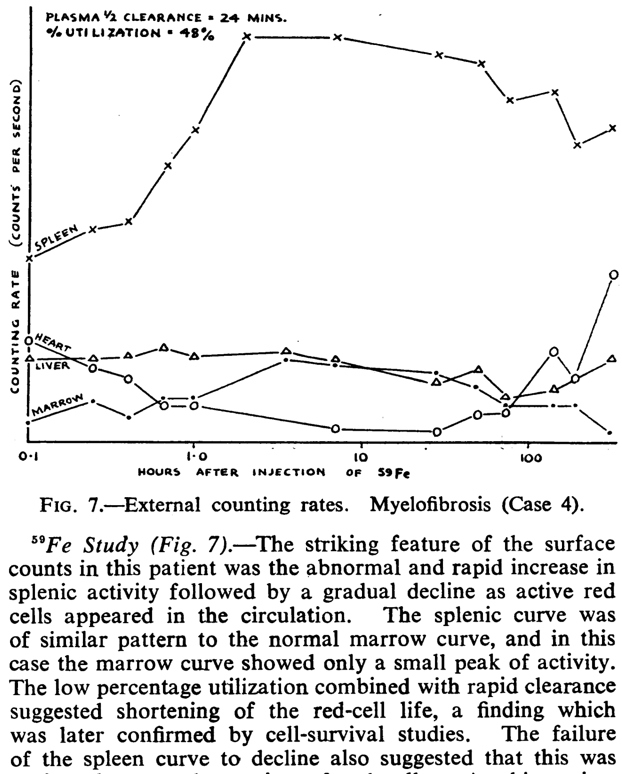

FIG. 7.—External counting rates. Myelofibrosis (Case 4).

^{59}Fe Study (Fig. 7).—The striking feature of the surface counts in this patient was the abnormal and rapid increase in splenic activity followed by a gradual decline as active red cells appeared in the circulation. The splenic curve was of similar pattern to the normal marrow curve, and in this case the marrow curve showed only a small peak of activity. The low percentage utilization combined with rapid clearance suggested shortening of the red-cell life, a finding which was later confirmed by cell-survival studies. The failure of the spleen curve to decline also suggested that this was a site of excess destruction of red cells. As this patient had a constant haemoglobin of 80% it is reasonable to suggest that his decreased red-cell survival was almost compensated by an increased production of cells from his marrow, spleen, and possibly liver. The exact contribution of each is difficult to assess owing to variation in surface-counting conditions for the different organs, but, as there was evidence of a splenic contribution to erythropoiesis in a patient with compensated haemolysis, splenectomy was considered inadvisable at present.

Case 5

A man of 59, with symptoms of anaemia and splenomegaly since 1946, was initially diagnosed and treated as a case of chronic myeloid leukaemia, but in 1954 marrow biopsy established a diagnosis of myelofibrosis. In the last two years his transfusion requirements have increased, a shortened red-cell survival had been demonstrated, and he now needs 3 pints (1.7 litres) of blood every 10 days to maintain his haemoglobin.

Routine Findings (at Time of ^{59}Fe Study).—Hb, 9.77 g./100 ml.; W.B.C., 28,000 per c.mm.; reticulocytes less than 1%; platelets, 200,000 per c.mm.

^{59}Fe Study (Fig. 8).—The findings in this patient contrast with those in Case 4. The rise in splenic activity is more delayed, there is a parallel and abnormal increase in liver

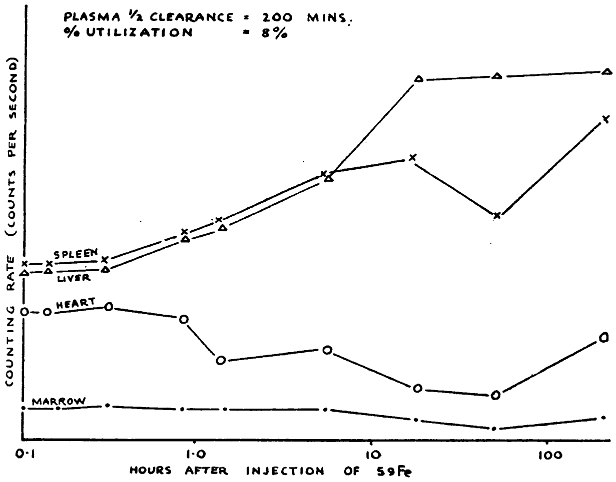

FIG. 8.—External counting rates. Myelofibrosis (Case 5).

activity, the marrow curve is flatter than normal, and he has a slow plasma clearance and decreased utilization. The general picture is that of reduced marrow erythropoiesis with iron deviation to liver and spleen as in aplastic anaemia. There is possibly some splenic erythropoiesis, but the shape of the splenic curve, particularly in the later phase, suggests that the spleen is also involved in a haemolytic process.

In this patient the tracer findings and red-cell survival studies establish that the anaemia is due to a combination of haemolysis and failure of red-cell production. In contrast to Case 4, they also demonstrate that the role of the spleen is haemolytic rather than erythropoietic. Splenectomy is therefore the logical treatment, but unfortunately his cardiac and respiratory condition precludes the operation.

Discussion

The experiences recorded here make it possible to assess the value of iron tracer studies in routine haematological practice. From the practical point of view these investigations present little difficulty, provided the advice and assistance of an experienced physicist are available for the initial installation, calibration, and subsequent maintenance of the apparatus. The techniques are within the scope of any routine hospital laboratory which has haematological responsibilities. No special isotope facilities are necessary, and the work may be safely done in the general ward and the general laboratory.

It is necessary to decide, however, whether the information obtained is of sufficient practical value to justify the outlay in time and material. From our experience it is quite obvious that this technique is not an indispensable aid to diagnosis and treatment of the majority of patients with blood disorders.

It is, however, clear that in a number of patients iron tracer studies demonstrate objectively an erythropoietic abnormality which can only be inferred from even the most comprehensive routine investigations. Furthermore, estimation of red-cell survival and radioactive iron investigations are at present the only readily available laboratory techniques by which the dynamics of erythropoiesis can be studied.

For these reasons we feel that the techniques described in this paper have a definite place in the routine investigation of haematological disorders. They not only contribute to an increased understanding of normal and abnormal erythropoiesis but provide a logical basis for treatment of the individual patient.

Summary

Seventy patients have been studied following the intravenous injection of tracer doses of radioactive iron (^{59}Fe).

The behaviour of the tracer was followed by estimating plasma clearance and the percentage of the injected dose appearing in red cells over a 10- to 14-day period.

Measurements of distribution within the body were made by external surface-counting techniques.

The methods and hazards are discussed, and an attempt is made to assess the value of these techniques in the diagnosis and treatment of individual patients.

We wish to thank the consultant physicians of St. Thomas's Hospital for permission to study patients under their care, Dr. A. J. Grimes for his advice and criticism, Mr. J. Coles for his work in preparing the ^{59}Fe for injection, and Miss P. Skoyles for technical assistance. We would also like to express our thanks to Dr. E. M. Ledlie and Dr. E. H. Belcher, of the Royal Marsden Hospital, for their very generous help and encouragement during the initial phases of this work. The expenses of this investigation were partly borne by the Endowment Fund, St. Thomas's Hospital.

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Many members of the British Medical Association who attended the Annual Meeting in Toronto last year visited the Mountain Sanatorium at Hamilton, Ontario, where the Hamilton Academy of Medicine and the Hamilton Health Association acted as hosts at an alfresco lunch. The Hamilton Health Association has now been in existence for over 50 years, and it was in 1906 that the Mountain Sanatorium was opened with two tents. To-day it is a modern institution with accommodation for about 800 patients for the treatment of all forms of tuberculosis. This expansion has been made possible by strong public support. In the latest annual report of the sanatorium, Dr. H. T. Ewart, the medical superintendent, reports that in Ontario there are more than 5,000 patients in hospital for the treatment of tuberculosis. He goes on to say that tuberculosis still kills more Canadians than all other infectious diseases combined. Efforts are continuing to search out new cases of the disease, and the Mountain Sanatorium has been selected to be the centre of treatment of Eskimo patients from the eastern Arctic region. Among the 900 patients admitted during 1955 about 100 were English, Scottish, Irish, or Welsh by birth. Dr. Joseph H. Lee, head of the department of medicine, writes in the report about the decreasing use of pneumothorax in treatment. 1942 was the peak year for pneumothorax refills, when the total reached 13,990. A slow decline then set in until by 1955 the number of refills given totalled only 195, divided equally between pneumothorax and pneumoperitoneum. During 1955 only one pneumothorax was induced.