Role of the reticuloendothelial system in the anaemia of rheumatoid arthritis

A study using the ^{59}Fe -labelled dextran model

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Anaemia associated with a low serum iron is one of the commonest systemic accompaniments of rheumatoid arthritis. It often reflects the severity of the inflammatory process, and although it bears some resemblance to iron deficiency anaemia (hypochromia, low serum iron, high serum copper, and increased erythrocyte protoporphyrin content) it is clearly a separate entity, as shown by its poor response to iron therapy, normal iron-binding capacity, and increased iron stores.

There are often multiple factors involved in the causation of the anaemia (Mowat, 1971); however, an invariable feature is an abnormality of iron metabolism, and this is usually of major importance. It has been suggested that, in rheumatoid arthritis, as in other chronic inflammatory diseases, there is a failure of iron re-utilization after senescent red cells have been taken up by the reticuloendothelial system (Cartwright, 1966). In previous studies to elucidate this mechanism, ^{59}Fe -labelled erythrocytes from homologous donors have been administered (Noyes, Bothwell, and Finch, 1960; Haurani, Young, and Tocantins, 1963). This procedure is now considered potentially hazardous because of the possible transmission of serum hepatitis. Accordingly, an alternative method of studying the release of iron from reticuloendothelial cells employing ^{59}Fe -labelled iron dextran has been used.

Studies of the metabolic fate of iron dextran in animals and man have shown that, after intravenous administration, it is first cleared into the reticuloendothelial system and later liberated into the circulation as transferrin-bound iron, which is then utilized by developing erythrocytes (Wood, Milner, and Pathak, 1968; Grimes and Hutt, 1957; Goldberg, 1958; Garby and Sjölin, 1957). The breakdown of iron dextran in the reticuloendothelial cells can be considered analogous to that of haemoglobin (Henderson and Hillman, 1969), and, if the iron load is kept between 0.15 and 0.25 mg./kg. body weight, there is a constant retention of iron in the reticuloendothelial system in normal individuals (Noyes and

others, 1960). The present study uses this model to follow the uptake, release, and utilization of reticuloendothelial sequestered iron in patients with rheumatoid arthritis.

Material and methods

SUBJECTS

Five patients with definite seropositive rheumatoid arthritis (Ropes, Bennett, Cobb, Jacox, and Jessar, 1959) of more than 5 years' duration were studied. They all had active synovitis in a minimum of three major joints. In addition, one haematologically normal patient (hospitalized for medical treatment of a duodenal ulcer) and one patient with a severe iron deficiency anaemia (secondary to menorrhagia) were similarly investigated.

None of the patients had taken any iron preparation for at least 3 months before the study.

All patients, with the exception of the haematologically normal patient, had a full haematological investigation, including bone marrow aspiration.

ISOTOPE ADMINISTRATION

^{59}Fe iron dextran (prepared by Dr. G. Moss of Fisons Pharmaceuticals Ltd.) with an activity of 1 to 2 μCi . was diluted in 10 ml. of patient's plasma and injected intravenously over the course of 1 min. The total iron load was 0.2 to 0.3 mg./kg. body weight. The syringe was weighed before and after injection and the amount injected accurately determined. A 1-ml. aliquot of the injected mixture was diluted 1:100, and 1 ml. of this solution kept as a standard.

RADIOACTIVITY MEASUREMENTS

Blood samples (2 ml.) were collected into heparin every hour for the first 6 hrs and then at intervals over the subsequent 14 days. After centrifugation the radioactivity was determined for the plasma and red cells (haemolysed by saponin) in an auto-gamma counter.

Surface counting was performed at similar intervals over the sacrum, liver, and spleen by means of a collimated scintillation counter. Sufficient counts were obtained to give a standard deviation of less than 2 per cent. All counts were adjusted for background and decay.

In two patients with rheumatoid arthritis and bilaterally inflamed knees (E, F), counting was performed over the

knees and thigh, in order to determine intra-articular iron accumulation as previously described (Bennett, Williams, Lewis, and Holt, 1973).

One of the rheumatoid patients ('D') had undergone a routine ferrokinetic study with ^{59}Fe transferrin (Dacie and Lewis, 1968) 1 year previously as part of a study on iron accumulation in rheumatoid knees (Bennett and others, 1973); she was not iron deficient at that time.

CALCULATIONS

(i) Plasma volume

Plasma counts over the first 6 hrs were plotted against time on logarithmic-linear graph paper—the initial plasma activity was obtained by extrapolation to zero. Thus

$$\text{Plasma volume} = \frac{\text{Activity injected}}{\text{Initial activity in plasma}} = \frac{\text{C.p.m. in 1 ml. standard} \times \text{Dilution of standard} \times \text{Volume injected (ml.)}}{\text{C.p.m. in 1 ml. of plasma}}$$

(ii) $T_{1/2}$ dextran clearance = The time in hours for the plasma radioactivity to fall to half its initial value

$$\text{(iii) Blood volume} = \frac{\text{Plasma volume} \times 100}{100 - (\text{PCV} \times 0.9)}$$

(iv) Red cell volume = Blood volume – Plasma volume

(v) Red cell utilization of ^{59}Fe dextran

$$\text{Red cell utilization} = \frac{\text{Red cell volume} \times \text{C.p.m. due to 1 ml. RBCs}}{\text{C.p.m. in 1 ml. of standard} \times \text{Volume injected (ml.)}}$$

$$\text{Where counts due to 1 ml. RBCs} = \frac{\text{Whole blood C.p.m.} - \text{Plasma C.p.m.} \times \frac{100 - \text{PCV}}{100}}{\text{PCV}}$$

(vi) Reticuloendothelial mobilization

All surface counts were expressed as a percentage of the counts at zero time (taken as 100 per cent.) after correction for background and decay. The percentage count at 14 days, was subtracted from the greatest percentage count in the first few days. The sum of the individual differences for the spleen, liver, and sacrum was taken to represent the total reticuloendothelial mobilization of ^{59}Fe at 14 days after injection.

Results

HAEMATOLOGICAL STATUS (Table I, opposite)

Patient 'A' was considered haematologically normal with a haemoglobin of 13.5 (g./dl.), normal peripheral blood film, and normal values for serum iron and iron-binding capacity. A bone marrow biopsy was not therefore requested.

Patient 'B' had a classical picture of an iron deficiency anaemia with a low haemoglobin associated with a hypochromic microcytic peripheral blood smear, low serum iron with an elevated total iron-binding capacity. Her bone marrow was negative for iron stores by Perl's stain. The cause of the iron deficiency was a combination of menorrhagia and

poor nutrition; she had a prompt reticulocytosis when given oral iron supplements.

Patients 'C', 'D', 'E', and 'F' had seropositive rheumatoid arthritis and were all anaemic. In all cases serum iron was low with a normal total iron-binding capacity, and bone marrow biopsy revealed plentiful iron stores in the macrophages but poor incorporation of iron into developing red cells. The serum vitamin B_{12} and folic acid levels were normal in all four patients.

Patient 'G' was a 63-year-old man who had suffered from seropositive rheumatoid arthritis for 30 years. He was iron deficient on the criteria of a microcytic

hypochromic peripheral blood smear, serum iron, and iron-binding capacity values, and absent stainable iron on a bone marrow biopsy. On oral iron supplements he had a reticulocyte response which was only 3.5 per cent. at 8 days. No source of bleeding was found and the anaemia was ascribed to poor nutrition.

^{59}Fe IRON DEXTRAN STUDIES (Table II)

(i) $T_{1/2}$ iron dextran clearance

This was most rapid in the patient with classical iron deficiency without any underlying chronic inflammatory disorder ('B'). The longest clearance was seen in the normal subject ('A'). The rheumatoid patients were distributed between these two extremes.

(ii) Iron dextran utilization

Both iron-deficient patients ('B' and 'G') showed the greatest utilization at 14 days, with 100 and 99 per cent. respectively. The normal subject ('A') had 81 per cent. incorporation at 14 days. All the patients with rheumatoid arthritis, with the notable exception of 'G' (with concomitant iron deficiency), had impaired utilization ranging from 22 to 54 per cent. (mean 38).

Table I *Haematological profile of patients studied*

Patient	Condition	Haemoglobin (g./dl)	Serum iron (µg./dl)	Total iron-binding capacity (µg./dl)	Plasma volume (ml.)	Red cell volume (ml.)	Marrow iron stores
A	Normal	13.5	110	340	3664	1724	Not done
B	Iron deficiency	6.9	9	550	2802	884	Absent
C	RA	9.1	34	231	2405	935	Plentiful
D	RA	12.5	35	250	3111	1645	Plentiful
E	RA	9.9	34	280	2631	1085	Plentiful
F	RA	9.0	17	243	2018	824	Plentiful
G	RA + Iron deficiency	12.7	56	440	2824	1554	Absent

Table II *⁵⁹Fe-labelled iron dextran studies*

Patient	T _{1/2} dextran (hrs)	Per cent. dextran utilization at 14 days	Per cent. maximal hepatic uptake*	Per cent. hepatic mobilization at 14 days	Per cent. maximal splenic uptake*	Per cent. splenic utilization 14 days	Per cent. maximal sacral uptake*	Per cent. splenic utilization at 14 days	Per cent. total reticuloendothelial mobilization
A	33	81	173	51	117	18	136	26	31
B	13	100	129	42	136	30	136	45	39
C	25	44	134	11	112	9	111	22	14
D	18	25	198	10	127	10	115	17	12.3
E	24	54	273	25	132	10	101	20	18.3
F	20	22	151	29	133	7	120	28	21.3
G	24	99	200	65	122	19	133	26	36

* Percentage of original counts (at time of first measurement on Day 0).

(iii) *Comparison of ⁵⁹Fe transferrin and ⁵⁹Fe dextran*
Patient 'D' had undergone a routine ferrokinetic study 1 year previously and a marked difference in the handling of the two iron presentations is seen in Fig. 1. With ⁵⁹Fe transferrin the greatest accumulation occurred over the sacrum within 24 hrs, its subsequent mobilization being associated with a 100 per cent. erythrocyte utilization at 7 days. With ⁵⁹Fe iron dextran, the greatest accumulation occurred over the liver, 198 per cent. of the original activity at Day 7, only 115 per cent. of original activity appearing in the sacrum. The total mobilization at 14 days was 12.3 per cent. and this was reflected in a poor erythrocyte utilization which was only 25 per cent. at 14 days.

(iv) *Reticuloendothelial distribution and mobilization*
In all patients surface counting showed a rapid initial accumulation of iron dextran in the liver (Table I and Figs 2 and 3). Accumulation in the spleen showed a

similar time course but was of much smaller magnitude. This iron was rapidly mobilized in both the normal iron-deficient and the rheumatoid iron-deficient patients. By contrast the four rheumatoid patients with normal iron stores did not show much mobilization. Accumulation over the sacrum (representing the bone marrow) was greatest in two iron-deficient patients 'B' (Fig. 2) and 'G' (Fig. 3), with values of 136 and 133 per cent. of original activity respectively. The normal patient ('A') had a similar accumulation of 136 per cent. But the four other rheumatoid patients had lower values ranging from 101 to 120 per cent. accumulation with a mean of 112 per cent. Mobilization of this accumulated iron dextran was greatest in the two iron deficient patients and the haematologically normal patient, but the patients with rheumatoid arthritis tended to retain their reticuloendothelial deposits of iron. When the total mobilization from the liver, spleen, and sacrum

FIG. 1 Comparison of ⁵⁹Fe dextran and ⁵⁹Fe transferrin kinetics in one patient (D). ⁵⁹Fe dextran Δ , ⁵⁹Fe transferrin $\bullet$

FIG. 2 Comparison of ⁵⁹Fe dextran uptake, mobilization, and utilization in a rheumatoid patient (E) $\blacktriangle$, an iron-deficient patient (B) $\circ$, and a haematologically normal patient (A) $\bullet$

FIG. 3 ⁵⁹Fe Dextran study in an iron-deficient rheumatoid patient (G)

was plotted against the utilization at 14 days, a positive correlation was observed (Fig. 4). The regression equation was $y = 6.8x + 0.29$, with $r = 0.90$; even for this limited number of subjects this was significant at a level of $P < 0.05$.

FIG. 4 Correlation of reticuloendothelial iron mobilization with subsequent incorporation into circulating erythrocytes

(v) Knee v. thigh counts

No accumulation of ⁵⁹Fe iron dextran was observed in the four affected knees of the two rheumatoid patients 'E' and 'F' over the course of 14 days.

Using ^{59}Fe transferrin, Freireich, Ross, Bayles, Emerson, and Finch (1957), Weinstein (1959), and Raymond, Bowie, and Dugan (1965), showed that exogenous iron is rapidly cleared from the plasma and efficiently incorporated into the circulating erythrocytes in patients with rheumatoid arthritis. However, endogenous iron, derived from senescent red cells labelled with ^{59}Fe , resulted in a poor iron incorporation into developing erythrocytes in dogs with turpentine abscesses (Freireich, Miller, Emerson, and Ross, 1957).

This led to the hypothesis that, in chronic inflammatory states, there was a defective re-utilization of iron from senescent red cells. Proof of this in human subjects came from the work of Haurani, Burke, and Martinez (1965) and Haurani and others (1963), using radio-iron-labelled red cells and haemoglobin in patients with malignant conditions and patients with chronic inflammatory diseases.

More recently, Beamish, Davies, Eakins, Jacob, and Trevett (1971) have demonstrated defective reticuloendothelial iron release in patients with chronic inflammatory states, using as an index the ratio of the percentage utilization of ^{55}Fe transferrin to ^{59}Fe iron dextran.

Our study which, for the first time, correlates the mobilization of sequestered iron from individual reticuloendothelial sites with its subsequent utilization in developing erythrocytes, extends further the concept of a defective reticuloendothelial iron release in rheumatoid arthritis.

It is interesting to note that, in the one patient with both rheumatoid arthritis and iron deficiency anaemia, the defect in reticuloendothelial iron release was overcome and there was a subsequent ^{59}Fe utilization into circulating erythrocytes of 99 per cent. (Fig. 3).

As more than 90 per cent. of the daily bone marrow iron requirements comes from the re-utilization of iron from senescent red blood cells, the fate of the iron released from haemoglobin within the reticuloendothelial cells is of major importance in stable erythropoiesis. Normally, this iron is released into a labile intracellular pool some of which passes into the plasma, and, transported on transferrin, is available for use by developing red cells. The iron remaining in the reticuloendothelial cells is normally easily mobilized by venesection (Noyes and others, 1960) and is preferentially re-utilized even in the presence of increased reserve iron stores (Dern and Hart, 1961).

What prevents the normal re-utilization of red cell iron in chronic inflammatory diseases is not clear. Starting with the single observation of an increased amount of haemosiderin and decreased levels of ferritin in the liver and spleen in chronic inflammatory

disease (Heilmeyer, 1958), one can postulate a preferential aggregation of ferritin molecules to form the less readily mobilized haemosiderin aggregates. Such a hypothesis is supported by *in vitro* experiments showing the conversion of ferritin to haemosiderin by oxidizing agents (Matioli and Baker, 1963) and the increased oxidative metabolism found in inflammation (Mazur, Carleton, and Carlsen, 1961). In such a scheme, intracellular ferritin is envisaged as being in dynamic equilibrium with the intravascular transferrin-bound iron on one hand and with stable intracellular deposits of haemosiderin on the other. At the present time the factors influencing this equilibrium are incompletely understood.

It has been suggested that the synovial membrane acts as part of the reticuloendothelial system in rheumatoid arthritis (Mowat, 1971), thus accounting for the observed increase in iron deposits at this site (Mowat and Hothersall, 1968; Senator and Muirden, 1968).

Our findings in this study do not support this hypothesis, as there was no accumulation of iron in four inflamed knees of two patients with rheumatoid arthritis. In addition, our previous work has shown that inflamed rheumatoid knees do not take up iron directly from transferrin but that the accumulation of iron at this site is temporarily related to its incorporation into circulating erythrocytes (Bennett and others, 1973). In conjunction with the direct demonstration of bleeding into a rheumatoid knee (Bennett, Hughes, Bywaters, and Holt, 1972), we support the hypothesis that the observed synovial iron deposits are a result of intermittent intra-articular bleeding with subsequent phagocytosis of the erythrocytes by the Type A cells of the synovial membrane (Muirden, 1970).

Another hypothesis to account for the altered iron kinetics in rheumatoid arthritis involves a role for 'lactoferrin' (Mowat, Disney, and Vaughan, 1971); an iron-binding protein that has been demonstrated in neutrophil leucocytes (Masson, Heremans, and Schonne, 1968) and more recently in synovial fluid (Bennett, Eddie-Quartey, and Holt, 1973). However, the small amounts of lactoferrin found in these situations and the kinetics of iron accumulation in the joints and reticuloendothelial system suggest that its role in affecting iron metabolism in rheumatoid arthritis could, at the most, be only of minor significance.

Summary

The reticuloendothelial handling of iron in rheumatoid arthritis has been studied using ^{59}Fe -labelled dextran. Rheumatoid patients with the anaemia of chronic inflammation showed excessive uptake of iron into the liver with little subsequent mobilization.

One rheumatoid patient, who was also iron deficient, was able to mobilize his hepatic iron stores normally. The ability to incorporate into circulating erythrocytes iron which has been stored in the reticulo-

endothelial system, was closely correlated with its subsequent mobilization from the liver.

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