

## Role of ineffective erythropoiesis in the anaemia of rheumatoid arthritis

DIANA SAMSON, D. HALLIDAY, AND J. M. GUMPEL

*From the Department of Haematology, Division of Clinical Investigation and Rheumatic Diseases Study Group, Northwick Park Hospital and Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ*

**SUMMARY** The importance of inadequate haemoglobin synthesis and ineffective erythropoiesis in the anaemia of rheumatoid arthritis was studied by measuring the incorporation of  $^{15}\text{N}$  glycine into haemoglobin haem and early labelled bilirubin in a patient with severe anaemia before and after response to gold therapy. Initially, total erythroid haem turnover was decreased but haem turnover due to ineffective erythropoiesis was markedly increased, accounting for 29% of total erythroid haem turnover. Gold therapy resulted in marked clinical improvement, accompanied by a rise in haemoglobin to normal. Total erythroid haem turnover increased and the percentage ineffective erythropoiesis fell to normal. Ineffective erythropoiesis may thus be an important reversible factor in the production of the anaemia of rheumatoid arthritis.

Anaemia is frequently present in patients with active rheumatoid arthritis (Jeffrey, 1953; Freireich *et al.*, 1957; Roberts *et al.*, 1963; Mowat, 1971). In common with the anaemia of other chronic disorders it is characterized by a low serum iron and a low total iron-binding capacity with normal or increased reticuloendothelial iron stores. It is usually normocytic and normochromic but microcytosis or hypochromia may also be seen, either singly or in association (Cartwright, 1966). The mean cell life is usually slightly shortened; there is rapid clearance of iron from the plasma but incorporation of exogenous  $^{59}\text{Fe}$  transferrin into red cells is normal or only slightly reduced (Freireich *et al.*, 1957; Weinstein, 1959; Roberts *et al.*, 1963). It is therefore thought that the anaemia results from failure of red cell production rate to increase to compensate for the slight increase in the rate of destruction. Although  $^{59}\text{Fe}$  bound to transferrin is incorporated reasonably well into red cells, endogenous iron, derived from red cells labelled with  $^{59}\text{Fe}$  or from  $^{59}\text{Fe}$  dextran, is poorly utilized. Impaired release of iron from the reticuloendothelial system is therefore thought to be the major cause of the inadequate haemoglobin synthesis (Weinstein, 1959; Haurani *et al.*, 1965; Lawson *et al.*, 1967; Beamish *et al.*, 1971; Bennett *et al.*, 1974). Failure of erythropoietin production may be an additional factor (Cartwright, 1966).

Ineffective erythropoiesis has not been considered a feature of the anaemia of chronic disorders, although morphological evidence of dyserythropoiesis is commonly seen in the bone marrow. We therefore undertook a quantitative study of ineffective and effective haem synthesis in a patient with classical anaemia of rheumatoid arthritis by measuring total bilirubin turnover rate and the incorporation of  $^{15}\text{N}$  glycine and  $^{15}\text{N}$   $\delta$ -aminolaevulinic acid (ALA) into haemoglobin and early labelled bilirubin. After administration of  $^{15}\text{N}$  glycine and its incorporation into haem, labelled red cells appear in the circulation reaching a plateau at about 10 days. Their subsequent destruction gives rise to a peak of labelled bile pigment excretion at the end of the mean cell life span. A peak of labelled bile pigment production also occurs during the first 10 days after  $^{15}\text{N}$  glycine administration, which in normal subjects accounts for 10–20% of total labelled pigment production. This early labelled peak arises partly from intramedullary cell death, i.e. ineffective erythropoiesis, but some early labelled bile pigment also arises from the breakdown of hepatic haem proteins so that the contribution of the hepatic component of the early peak must be assessed in order to quantify ineffective erythropoiesis. In contrast to glycine, exogenous ALA which is the successor of glycine in the haem synthetic pathway, is incorporated almost exclusively into hepatic haem (Berlin *et al.*, 1969). The early labelled bilirubin peak resulting from the administration of  $^{15}\text{N}$  ALA can therefore be used to

calculate hepatic haem turnover and hence the hepatic contribution to the early labelled peak resulting from  $^{15}\text{N}$  glycine administration. Using the clearance of  $^{14}\text{C}$  bilirubin to measure total haem turnover rate, the rates of haem turnover due to effective erythropoiesis and ineffective erythropoiesis can then be calculated, as previously described (Samson *et al.*, 1976a).

### Case report

A woman, born in 1909, has had mild psoriasis for many years, and in 1964 developed a widespread symmetrical polyarthritis, diagnosed as rheumatoid arthritis on the basis of a repeatedly positive sheep cell agglutination test (titre of 1:256). In January 1971 there was a flare-up of the disease and in July 1971 the haemoglobin was 10.5 g/dl and the erythrocyte sedimentation rate (ESR) 56 mm/h; subsequent levels are shown in Table 1. Radiographs showed extensive symmetrical erosive disease. She was first seen at Northwick Park Hospital in August 1971, at which time she was taking phenylbutazone 100 mg tds, dihydrocodeine 60 mg, and Distalgesic. She had also been taking oral iron for a year, which was then stopped. In December 1971 Hoffman's operation was performed on both feet and she had a further 3 months' course of iron, after which no more iron therapy was given. In February 1972 5 mCi yttrium 90 was injected into each knee, and in June

1972 an ulnar head resection and synovectomy of the right wrist was performed, and benorylate was substituted for phenylbutazone.

In September 1972 there had been no response to the yttrium injections; in view of this, the widespread erosive joint disease, the high ESR, and the anaemia, she was judged to have sufficient generalized active rheumatoid arthritis to merit chrysotherapy.  $^{15}\text{N}$  glycine studies were carried out at this time, and subsequently gold therapy was started. After 1210 mg myocrisin there was marked clinical improvement; all manifestations of active rheumatoid arthritis had disappeared and the gold was continued at 50 mg fortnightly as the only medication. In February 1974 the interval between injections was increased to 4 weeks and in December 1975 to 6 weeks. At this time a total dose of 2710 mg had been given with no side effects. At no time during the course of her disease was there any abnormality in liver function tests.

Before starting gold therapy she had a persistent normochromic microcytic anaemia which had not responded to oral iron (see Table 1). No evidence of blood loss from the gut was found and there was no postmenopausal bleeding. The anaemia was associated with a markedly raised ESR, a low serum iron, and a low normal total iron binding capacity. Bone marrow aspiration showed adequate reticulo-endothelial iron stores and normoblastic erythropoiesis with morphological evidence of dyserythro-

Table 1 *Haematology parameters in relation to therapy in patient*

Date	Hb (g/dl)	PCV (%)	MCV (fl)	Fe ( $\mu\text{mol/l}$ )	TIBC ( $\mu\text{mol/l}$ )	ESR (mm/h)	Total gold dose (mg)	Drugs, operations etc.
Normal range	11.5-16.5	35-47	80-90	12-27	40-75	0-10	—	
1971								
July	10.5					56		
Aug	9.6	28.9	75			128		Phenylbutazone 100 mg tds, iron dihydrocodeine, Distalgesic
Oct	8.6	25.4	75	4.0	49.5	135		
Dec	9.7	29.4	80			122		Admitted for investigation Hoffman's operation
1972								
Jan	8.9	26.2	76	4.5	45	134		
Feb	9.4			7.0	44.7	96		
Mar	9.1	26.6	75			140		
June	8.2	24.8	73	2.9	55.8	142		Benorylate 10 ml bd, wrist surgery, 1 pint blood
Sept	8.7	29.5	75			123		
Oct	9.3	29.1	76	3.0	47.7			
Nov 1973	8.7	26.5	74	4.3	43.2	140	Started	Benorylate, Distalgesic
Jan	10.3	29.9	72			75	610	
July	10.7	32.1	76	3.4	55.2	70	1210	
1974								
Feb	12.6	37.2	82	11.7	57.0	22	1710	None
Nov	13.0	38.4	86			12	2110	
1975								
Dec	13.4	34.0	82			15	2710	

PCV = packed cell volume; MCV = mean corpuscular volume; TIBC = total iron binding capacity.

poiesis, i.e. ragged cytoplasm, indistinct nuclear outline, and karyorrhexis. Ferrokinetic studies showed a rapid plasma iron clearance ( $T_{\frac{1}{2}}^{59\text{Fe}}$  55 min, normal range 60–140) and reduced incorporation into circulating red cells (43% utilization on day 14, normal range 70–80). 6 months after starting gold therapy there was some improvement of the haemoglobin and ESR, and after 18 months these had returned to normal. Serum iron rose spontaneously to normal and there was also a slight increase in total iron binding capacity (Table 1).

## Methods

These have been described in full elsewhere (Samson *et al.*, 1976a). The patient was admitted to a metabolic ward for a 20-day study period immediately before gold therapy. On day 0, 20 mg  $^{15}\text{N}$  ALA was given by rapid intravenous injection, and on day 10, when the early peak resulting from ALA administration was over, 50 mg  $^{15}\text{N}$  glycine was given by rapid intravenous injection. Stercobilin was isolated from individual stool samples by the method of Watson (1934) as modified by Gray and Scott (1959). Blood samples were taken three times weekly and haemin isolated by the method of Labbe and Nishida (1957). The atom percent excess  $^{15}\text{N}$  in the stercobilin and haemin samples was determined in an MS20 mass spectrometer. The total daily bilirubin production rate was determined by the clearance of  $^{14}\text{C}$  bilirubin, and from this and the atom percent excess  $^{15}\text{N}$  of the stercobilin the percentage incorporation of both precursors into bilirubin over the 10-day period was calculated (see Table 2). Similarly, from the  $^{15}\text{N}$  concentration of the haemin and the total red cell mass, measured with  $^{51}\text{Cr}$ , the percentage incorporation of glycine into haemoglobin haem was calculated (Table 2). In October 1974, after 2 years of gold therapy, the total bilirubin turnover rate and the incorporation of  $^{15}\text{N}$  glycine into haemoglobin haem were again measured. The patient gave informed consent to the studies, which had been approved by Northwick Park Hospital Ethical Committee and the MRC Isotope Advisory Panel.

## CALCULATIONS

### Initial study

Total bilirubin turnover rate (BRT), bilirubin production from hepatic haem turnover ( $\text{BR}_{\text{HEP}}$ ), from ineffective erythropoiesis ( $\text{BR}_{\text{IE}}$ ), and from the destruction of circulating red cells ( $\text{BR}_{\text{RBC}}$ ), and the percentage ineffective erythropoiesis were calculated from the total haem turnover rate (bilirubin production rate) and the isotope incorporation as previously described (Samson *et al.*, 1976a). The potential haemoglobin, i.e. that which would have been achieved at the same total erythroid haem turnover rate if there had been no increase in ineffective erythropoiesis, was also calculated (Samson *et al.*, 1976b). Haem turnover rates are obtained by converting bilirubin production rates to molar units.

### Repeat study

Let  $\text{BRT}_2$ ,  $\text{BR}_{\text{IE}_2}$ , and  $\text{BR}_{\text{RBC}_2}$  be the new values for  $\text{BRT}_1$ ,  $\text{BR}_{\text{IE}}$ , and  $\text{BR}_{\text{RBC}}$  and  $\text{GRBC}_2$  be the incorporation of  $^{15}\text{N}$  glycine into haemoglobin haem on this occasion, where  $\text{GRBC}_1$  was the incorporation into haemoglobin haem in the initial study. Assuming that the incorporation of  $^{15}\text{N}$  glycine into haemoglobin haem is proportional to the rate of effective haem synthesis,

$$\frac{\text{GRBC}_2}{\text{BR}_{\text{RBC}_2}} = \frac{\text{GRBC}_1}{\text{BR}_{\text{RBC}_1}}$$

$$\text{BR}_{\text{RBC}_2} = \frac{\text{BR}_{\text{RBC}_1} \times \text{GRBC}_2}{\text{GRBC}_1}$$

Further, assuming hepatic haem turnover to have remained constant,

$$\text{BR}_{\text{IE}_2} = \text{BRT}_2 - \text{BR}_{\text{RBC}_2} - \text{BR}_{\text{HEP}}$$

and % ineffective erythropoiesis =  $\frac{\text{BR}_{\text{IE}_2}}{\text{BR}_{\text{IE}_2} + \text{BR}_{\text{RBC}_2}}$ .

## Results

Initially the incorporation of  $^{15}\text{N}$  glycine into haemoglobin haem was markedly reduced, but the incorporation into early labelled bilirubin was

Table 2 Incorporation of  $^{15}\text{N}$  ALA and  $^{15}\text{N}$  glycine into haem and early labelled bilirubin (% administered dose)

Precursor	Product	Normal range*	Patient before gold therapy	Patient after gold therapy
Glycine	Hb haem	1.24 ± 0.24	0.56	0.95
Glycine	Early labelled bilirubin	0.23 ± 0.06	0.37	—
ALA	Early labelled bilirubin	18.40 ± 7.77	22.20	—

\*Samson *et al.* (1976a).

increased (Table 2). The incorporation of  $^{15}\text{N}$  ALA into early labelled bilirubin was normal, indicating that hepatic haem turnover was within the normal range ( $1.56 \mu\text{mol/kg}$  per day, mean  $\pm$  SD  $1.30 \pm 0.55$ ). The increased incorporation of  $^{15}\text{N}$  glycine into the early labelled peak was therefore due to increased ineffective erythropoiesis,

Total haem turnover, measured by the clearance of  $^{14}\text{C}$  bilirubin, was reduced ( $5.96 \mu\text{mol/kg}$  per day, mean  $\pm$  SD  $6.75 \pm 0.46$ ) (see Fig.) and since hepatic haem turnover was normal, this was due to a decreased total erythroid haem turnover ( $4.40 \mu\text{mol/kg}$  per day, mean  $\pm$  SD  $5.46 \pm 0.50$ ). Because of the increased percentage ineffective erythropoiesis (29%, mean  $\pm$  SD  $8 \pm 3$ ) the rate of effective haem synthesis was considerably reduced ( $3.13 \mu\text{mol/kg}$  per day, mean  $\pm$  SD  $5.03 \pm 0.51$ ) and the haem turnover rate due to ineffective erythropoiesis was actually increased ( $1.27 \mu\text{mol/kg}$  per day, mean  $\pm$  SD  $0.45 \pm 0.17$ ). The potential haemoglobin, i.e. that which would have been achieved at the same level of total erythroid haem turnover if the percentage ineffective erythropoiesis had been normal, was  $11.3 \text{ g/dl}$ .

When studied after gold therapy, total haem turnover had risen to normal ( $7.35 \mu\text{mol/kg}$  per day). Assuming hepatic haem turnover to have remained constant, this indicated a rise in total erythroid haem turnover to  $5.78 \mu\text{mol/kg}$  per day. According

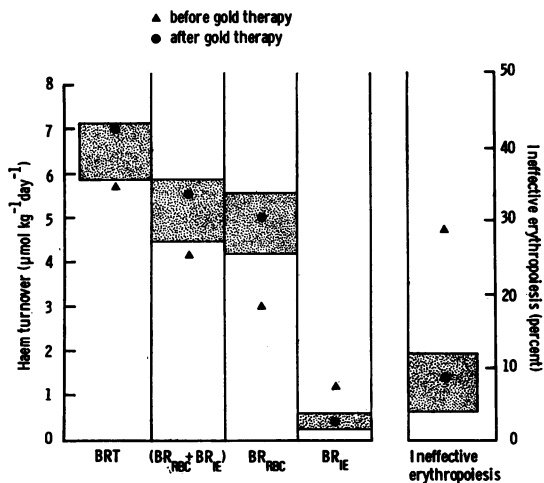


Fig. Haem turnover and ineffective erythropoiesis before and after gold therapy in the patient studied. BRT is total haem turnover rate (total bilirubin turnover rate).  $BR_{RBC}$  and  $BR_{IE}$  are haem turnover rates due respectively to effective and ineffective erythropoiesis, and  $(BR_{RBC} + BR_{IE})$  is total erythroid haem turnover rate. The shaded area shows the range of values in 4 control subjects (Samson et al., 1976a).

to the above calculations, effective haem turnover was now  $5.29 \mu\text{mol/kg}$  per day, so that ineffective haem turnover was  $0.49 \mu\text{mol/kg}$  per day, i.e. 10% of total erythroid haem turnover. Although there may be a small error in this estimate owing to the assumption of a constant value for hepatic haem turnover, the difference from the pretreatment value is too great to be accounted for by a variation in hepatic haem turnover rate. In view of the consistently normal liver function tests, there was no evidence that liver function had altered between the two periods of study.

## Discussion

Before gold therapy the patient had a moderately severe anaemia associated with active generalized rheumatoid arthritis. The slight microcytosis, low serum iron and iron binding capacity with adequate marrow iron stores were all characteristic of the anaemia of chronic disorders. At this time total erythroid haem turnover was marginally reduced (see Fig.), consistent with failure of iron utilization and/or insufficient erythropoietin production. Ineffective erythropoiesis accounted for 29% of total erythroid haem turnover, so that effective haem turnover was considerably reduced. In the absence of an increase in ineffective erythropoiesis the haemoglobin would have been  $11.3 \text{ g/dl}$ , which is approximately halfway between the actual level ( $8.7 \text{ g/dl}$ ) and the expected normal level. Failure of haemoglobin synthesis and ineffective erythropoiesis were therefore of equal importance in the production of the anaemia in the present case. The low incorporation of  $^{59}\text{Fe}$  into red cells is consistent with this interpretation. Since  $^{59}\text{Fe}$  incorporation is usually normal or only slightly reduced in the anaemia of rheumatoid arthritis the increase of ineffective erythropoiesis in the present case may be unusually high. Nevertheless it is likely that ineffective erythropoiesis is a factor in the production of this type of anaemia.

After successful treatment of the arthritis with gold, all the parameters returned to normal (see Tables 1, 2, Fig.). Both the failure of haemoglobin synthesis and the ineffective erythropoiesis were therefore reversed by gold therapy.

## References

- Beamish, M. R., Davies, A. G., Eakins, J. D., Jacobs, A., and Trevett, D. (1971). The measurement of reticuloendothelial iron release using iron-dextran. *British Journal of Haematology*, **21**, 617-622.
- Bennett, R. M., Holt, P. J. L., and Lewis, S. M. (1974). Role of the reticuloendothelial system in the anaemia of rheumatoid arthritis. *Annals of the Rheumatic Diseases*, **33**, 147-152.

- Berlin, N. I., Berk, P. D., and Howe, R. B. (1969). Disorders of bilirubin metabolism. *Duncan's Diseases of Metabolism*, 6th ed. Ed. by P. K. Bondy. Saunders, London.
- Cartwright, G. E. (1966). The anemia of chronic disorders. *Seminars in Hematology*, **3**, 351-375.
- Freireich, E. J., Ross, J. F., Bayles, T. B., Emerson, C. P., and Finch, S. C. (1957). Radioactive iron metabolism and erythrocyte survival studies of the mechanism of the anemia associated with rheumatoid arthritis. *Journal of Clinical Investigation*, **36**, 1043-1058.
- Gray, C. H., and Scott, J. J. (1959). The effect of hemorrhage on the incorporation of a  $-^{14}\text{C}$  glycine into stercobilin. *Biochemical Journal*, **71**, 38-42.
- Haurani, F. I., Burke, W., and Martinez, E. J. (1965). Defective re-utilization of iron in the anemia of inflammation. *Journal of Laboratory and Clinical Medicine*, **65**, 560-570.
- Jeffrey, M. R. (1953). Some observations on anemia in rheumatoid arthritis. *Blood*, **8**, 502-518.
- Labbe, R. F., and Nishida, G. (1957). A new method of haemin isolation. *Biochimica et Biophysica Acta*, **26**, 437.
- Lawson, A. A. H., Owen, E. T., and Mowat, A. G. (1967). Nature of anaemia in rheumatoid arthritis. VII. Storage of iron in rheumatoid disease. *Annals of the Rheumatic Diseases*, **26**, 552-559.
- Mowat, A. G. (1971). Hematologic abnormalities in rheumatoid arthritis. *Seminars in Arthritis and Rheumatism*, **1**, 195-219.
- Roberts, F. D., Hagedorn, A. B., Slocumb, C. H., and Owen, C. A. (1963). Evaluation of the anemia of rheumatoid arthritis. *Blood*, **21**, 470-476.
- Samson, D., Halliday, D., Nicholson, D. C., and Chanarin, I. (1976a). Quantitation of ineffective erythropoiesis from the incorporation of ( $^{15}\text{N}$ )  $\delta$ -amino-laevulinic acid and ( $^{15}\text{N}$ ) glycine into early labelled bilirubin. I. Normal subjects. *British Journal of Haematology*, **34**, 33-34.
- Samson, D., Halliday, D., Nicholson, D. C., and Chanarin, I. (1976b). Quantitation of ineffective erythropoiesis from the incorporation of ( $^{15}\text{N}$ )  $\delta$ -amino-laevulinic acid and ( $^{15}\text{N}$ ) glucine into early labelled bilirubin. II. Anaemic patients. *British Journal of Haematology*, **34**, 45-53.
- Watson, C. J. (1934). An improved method for the isolation of crystalline stercobilin. *Journal of Biological Chemistry*, **105**, 469-472.
- Weinstein, I. M. (1959). A correlative study of the erythrokinetics and disturbances in iron metabolism associated with the anemia of rheumatoid arthritis. *Blood*, **14**, 950-966.