NATURE OF ANAEMIA IN RHEUMATOID ARTHRITIS

VI. METABOLISM OF ENDOGENOUS IRON

BY

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Hypoferraemia is a common feature of rheumatoid arthritis (Bruzzone and Massimello, 1940; Nilsson, 1948; Alexander and Duthie, 1962; Roberts, Hagedorn, Slocumb, and Owen, 1963). The cause of the low serum levels of iron remains obscure (Raymond, Bowie, and Dugan, 1965).

Using small amounts of transferrin bound ^{59}Fe , Freireich, Ross, Bayles, Emerson, and Finch (1957b), Ebaugh (1958), Weinstein (1959), Roberts and others (1963), and Raymond and others (1965) showed that the utilization of exogenous iron for erythrocyte production was unimpaired, that the total daily production of erythrocyte haemoglobin was normal, and that injected iron was rapidly cleared from the plasma in patients with rheumatoid arthritis. A similar rapid clearance of large doses of intravenous iron was reported by Nilsson (1948) and Roy, Alexander, and Duthie (1955).

The results of studies using endogenous iron, derived from senescent red cells labelled with ^{59}Fe , were reported by Freireich, Miller, Emerson, and Ross (1957a) in dogs with turpentine abscesses, and by Weinstein (1959) in one patient with rheumatoid arthritis. Both investigations suggested that a possible factor leading to hypoferraemia might be an abnormal handling of iron derived from senescent

red cells with failure of the red cells to release iron at a normal rate to the plasma.

It was decided to investigate the metabolism of endogenous iron in a group of patients with rheumatoid arthritis in an attempt to test this hypothesis. Loading with endogenous iron was achieved by intravenous nicotinic acid as described by Laurell (1953) and Gydell (1958a and b, 1959, 1960a), and by infusions of aged autologous blood (Gydell, 1960b). The effect of corticosteroid therapy upon the endogenous iron metabolism was studied in one patient with polymyalgia rheumatica and in one patient with rheumatoid arthritis.

Material and Methods

Clinical Material

Eight patients, five males and three females, admitted to the Rheumatic Diseases Unit, Northern General Hospital, Edinburgh, were studied. Seven were suffering from definite or classical rheumatoid arthritis in an active phase; one male patient had polymyalgia rheumatica. Only patients in whom there was no cause for anaemia other than the presenting disease were included. All patients were receiving the same basic regimen of rest in bed, salicylates, splintage, and physiotherapy. Two patients (Table I) had received parenteral iron immediately before admission to hospital. Three patients were

TABLE I
CHARACTERISTICS OF CONTROLS AND PATIENTS

Subjects	Sex	Age (yrs)	Hb (g./100 ml.)	ESR (mm./hr)	Plasma Iron ($\mu\text{g.}/100\text{ ml.}$)	Total Iron-Binding Capacity	Duration of Disease (yrs)
Controls	1	M	28	15.3	5	96	
	2	M	32	15.3	3	140	
	3	M	32	16.9	4	86	
	4*	M	30	14.6	2	68	
	5	F	23	13.6	4	220	
Patients	1	M	48	12.5	47	34	10
	2†	M	55	13.7	81	120	20
	3	M	63	11.6	96	44	10
	4	M	60	12.4	79	46	7 mths
	5‡	M	59	12.4	38	60	5 mths
	6	F	58	11.6	31	48	20
	7†	F	62	9.2	31	86	18 mths
	8	F	48	10.9	70	24	5

*Studied while taking high doses of salicylates.

†Both had received parenteral iron therapy immediately before admission to hospital.

‡Suffering from polymyalgia rheumatica.

investigated after injection both of nicotinic acid and of aged autologous blood. The effect of injecting nicotinic acid intravenously before and after prednisolone therapy was studied in one patient, and before and after corticotrophin therapy in one patient.

The effect of injecting nicotinic acid intravenously in five normal subjects (four males and one female) was studied. One male was given high doses of salicylates for 3 days before and on the day of the study. Two of the male subjects were also given aged autologous blood.

Methods

Nicotinic Acid.—This was administered intravenously as described by Gydell (1958, 1960b). 50 mg. sodium nicotinate, diluted in 20 ml. isotonic saline were injected. The injection was given slowly over a mean time of 10 minutes. Mild tingling and facial flushing sometimes occurred. No serious side-effects were observed.

Aged Autologous Blood.—100 ml. blood were collected under sterile conditions into 20 ml. 3·8 per cent. sodium citrate and stored for 14-16 days at 4° C. Sterility was confirmed 48 hours before infusion. 2 hours before infusion the blood was taken from the refrigerator and gently mixed before injection.

In all experiments blood was collected from the recipient immediately before injection and at 30 minutes, 60 minutes, and hourly thereafter. Plasma iron and bilirubin were measured in each sample. Care was taken to avoid contamination of specimens with iron during collection and subsequent testing. The plasma iron levels were not done fasting, but the specimens were taken, both in the normal subjects and in the patients, at the same times throughout the day and the meals given were comparable. Plasma haemoglobin (measured by the method of Dacie and Lewis, 1963) was estimated in three normal subjects, in three patients after injection of nicotinate, and in all five subjects injected with aged blood. The haemoglobin was measured against a cyanhaemoglobin standard (100 per cent. = 14·6 g./100 ml.) in an "EEL" haemoglobinometer. The erythrocyte sedimentation rate (ESR) was measured by the Westergren method modified by Dawson (1960). A stained

smear of peripheral blood was examined. Plasma iron concentration and total iron-binding capacity were measured by the methods of Ramsay (1957a and b respectively). The plasma bilirubin was measured by the method of Haslewood and King (1937).

Results

The haemoglobin, ESR, plasma iron, total iron-binding capacity, and the duration of the disease in the patients are recorded in Table I. With the exception of Cases 2 and 7, who had recently received parenteral iron, the plasma iron levels of the patients were low. Seven patients had iron-binding capacities similar to those found in the normal controls, and in one female patient this measurement was low (Case 8). In Case 7, who was the most anaemic, sternal marrow biopsy was performed and serum B₁₂ and urinary formaminoglutamic acid were estimated. These tests were normal and a small amount of stainable iron was present in the marrow.

Effects of Injection of Nicotinic Acid

The effect on the plasma iron in the individual cases is recorded in Table II, and the mean values and ranges of plasma iron are shown in Fig. 1 (opposite).

There was a smaller and more gradual rise in plasma iron in patients compared with controls. The two patients who had just completed courses of parenteral iron and had high initial plasma irons were excluded from this analysis. Both of these patients, despite high initial plasma iron, had qualitatively the same type of response as the other patients with rheumatoid arthritis. In Case 3, the rise in plasma iron was as great as in some of the controls, but the subsequent fall was more rapid. The pattern of response in the control who was taking salicylates was similar to that in the other controls.

TABLE II
EFFECT OF NICOTINIC ACID ON PLASMA IRON CONCENTRATION

Hours after Injection			0	½	1	2	3	4	5	6	7
Plasma Iron Concentration (μg./100 ml.)	Controls	1	96	152	186	190	204	—	—	—	—
		2	140	152	170	—	174	—	140	—	—
		3	86	106	126	—	128	—	96	—	—
		4*	68	108	150	186	190	—	120	156	76
		5	220	228	256	276	304	340	172	108	—
	Patients	1	34	50	56	60	36	—	—	—	—
		2†	120	146	150	48	—	44	32	40	—
		3	44	68	92	128	98	84	74	—	—
		4	46	50	58	58	66	—	36	—	—
		5‡	60	60	80	80	72	56	56	—	—
		6	48	66	68	40	52	—	28	26	—
		7†	86	102	114	86	94	—	68	—	—
		8	20	—	32	36	24	20	—	—	—

*Studied while taking high doses of salicylates.

†Both had received parenteral iron therapy immediately before admission to hospital.

‡Suffering from polymyalgia rheumatica.

Fig. 1.—Changes in plasma iron after intravenous injection of nicotinic acid—mean and range.

The results for plasma bilirubin in the two groups are shown in Fig. 2. The rate and magnitude of the rise in the plasma bilirubin was less in patients than in controls. In both patients and controls the peak concentration of bilirubin was reached at the first hour.

No increase of plasma haemoglobin was observed following injection of nicotinic acid in three controls and three patients.

Effect of Aged Autologous Blood

The results are shown in Fig. 3 (overleaf), and for comparison the changes after injection of nicotinic acid in the same subjects are shown in Fig. 4 (overleaf).

In the two controls and the three patients investigated after infusion of aged blood, the rate, magnitude, and duration of the rise in plasma iron were greater and the fall was more prolonged than after nicotinic acid.

The rise and fall in plasma bilirubin after the infusion of aged autologous blood were similar to those found after nicotinic acid.

Fig. 2.—Changes in plasma bilirubin after intravenous injection of nicotinic acid—mean and range.

The plasma haemoglobin was measured at the same times as plasma iron and bilirubin in all five subjects. No rise occurred. No haemolysis was demonstrated in any of the five specimens of aged blood before transfusion.

Effects of Prednisolone and Corticotrophin on the Response to Nicotinic Acid

Case 5, a male with polymyalgia rheumatica, was studied before and on the tenth day of treatment with prednisolone. Case 8, a female with classical rheumatoid arthritis, was similarly studied before and on the tenth day of treatment with corticotrophin. The effect on plasma iron levels is shown in Fig. 5 (overleaf).

In Case 5 the plasma iron was low before prednisolone was given, and the pattern of response to intravenous nicotinic acid was similar to that found in rheumatoid arthritis. After 10 days of prednisolone (15 mg. per day), the plasma iron level had risen considerably and the duration and magnitude of the rise in plasma iron after nicotinic acid injection was within the limits found in normal subjects. In Case 8, although there was no alteration in the plasma iron level after ACTH (20 I.U. given intramuscularly for 10 days), a greater increase in plasma iron followed nicotinic acid and the level had not returned to the initial value at 6 hours.

Fig. 3.—Changes in plasma iron after transfusion of aged autologous blood.

Fig. 4.—Changes in plasma iron after intravenous injection of nicotinic acid.

Discussion

The effects of intravenous injection of nicotinic acid on the plasma iron and plasma bilirubin demonstrated in this study were similar to those reported by Laurell (1953) and Gydell (1960b). The changes which followed transfusion of aged autologous blood were similar to those observed by

Fig. 5.—Changes in plasma iron after nicotinic acid (a) before and after prednisolone in Case 5, (b) before and after corticotrophin in Case 8.

Gydell (1960b). Changes in plasma iron and bilirubin induced by nicotinic acid, although quantitatively different from, were qualitatively comparable to those which followed the transfusion of aged blood. These results supported the conclusions of Laurell (1953) and Gydell (1960b) that nicotinic acid acts primarily upon the erythrocytes which then undergo extravascular haemolysis. A possible source of error in the use of nicotinic acid was that different amounts of endogenous iron might be released by the action of nicotinic acid at various levels of haemoglobin. Gydell (1960a) compared the haemoglobin destruction induced by nicotinic acid in normal controls and patients with polycythaemia and others with haemolytic disease. He concluded that the effect of nicotinic acid varied more with the basal rate of haemoglobin catabolism than with the total amount of circulating haemoglobin. The consistent pattern of alterations in plasma iron observed in normal subjects and patients in this series, despite a considerable variation in the individual haemoglobin levels, was in keeping with this conclusion. It was shown that the initial haemoglobin levels did not influence the plasma iron response after the infusion of 50 ml. aged autologous blood. Thus the technically simpler and safer procedure of intravenous injection of nicotinic acid would appear to be a reliable method of inducing a satisfactory load of endogenous iron.

A smaller rise in plasma iron followed intravenous nicotinic acid in patients with rheumatoid arthritis than in controls. This may have been due to a

diminished susceptibility of the red cells in rheumatoid arthritis to the action of nicotinic acid. However, infusion of aged blood demonstrated similar differences between controls and patients. It was unlikely, therefore, that there was any difference in sensitivity of erythrocytes to nicotinic acid.

The differences of response found in patients with rheumatoid arthritis may be explained either by a decreased release of endogenous iron from haemoglobin into the plasma or by an increased clearance of endogenous iron from the plasma. In both patients and controls more iron was released from aged blood than after injection of nicotinic acid, but in both situations there was a smaller rise in plasma iron in patients than in controls. The shapes of the curves of plasma iron in the two groups, however, and the time taken for the plasma iron levels to return to the initial values were similar. If increased plasma clearance had been a major factor in the patients, their curves should have shown a more rapid fall. A more likely explanation of the findings would be that less iron reached the plasma. In support of this, hyperplasia of the lymphoid tissue and spleen is common in rheumatoid arthritis, and the reticulo-endothelial system is also the site of extravascular haemolysis. Gardner and Roy (1961) reported a *post mortem* study of patients who died while suffering from rheumatoid arthritis, a proportion of whom had not received medicinal iron in any form. In this group they demonstrated an increased content of haemosiderin in liver and spleen but normal values in other tissues. This suggested a relative excess of iron retained in the reticulo-endothelial system even in the presence of a low plasma iron. The excess of iron could be due to an abnormal retention of endogenous iron from effete red cells.

The differences in plasma bilirubin levels between patients and controls were very similar to those found in respect of plasma iron. So closely did the bilirubin curves parallel the iron curves that it is probable that the factors causing the abnormality in plasma iron response to intravenous nicotinic acid and aged blood were also responsible for the difference in plasma bilirubin levels. The results, therefore, suggested that pigments formed from the degradation of haemoglobin were retained in the reticulo-endothelial system as well as iron.

The return to a more normal pattern of response induced by the administration of prednisolone and corticotrophin would suggest a relationship between hypoferraemia and active inflammation.

The results of this study would support the suggestion made by Weinstein (1959) that in rheumatoid arthritis there is a diminished availability of iron from effete red cells to the plasma and hence to the

marrow for erythropoiesis. They would also support the conclusion of Freireich and others (1957b) that the hypoferraemia in rheumatoid arthritis is the result of a failure to release endogenous iron derived from senescent red cells at a normal rate from the reticulo-endothelial cells concerned in extravascular haemolysis.

Summary

(1) The changes in plasma iron after intravenous injection of nicotinic acid and aged autologous blood have been compared.

(2) The use of nicotinic acid has been shown to be a simple and satisfactory method of inducing a significant increase in endogenous iron derived from red blood cells.

(3) The metabolism of endogenous iron has been studied in five controls, seven cases of rheumatoid arthritis, and one of polymyalgia rheumatica.

(4) The smaller rise and more rapid fall in plasma iron after nicotinic acid and autologous aged blood in these patients would suggest a diminished release of endogenous iron from the reticulo-endothelial system.

(5) The administration of prednisolone and corticotrophin tends to correct these abnormalities.

We wish to express our thanks to Dr. J. J. R. Duthie and Dr. W. R. M. Alexander for their help and encouragement throughout this study, and also to other members of the staff of the Rheumatic Diseases Unit, Northern General Hospital, Edinburgh, for their co-operation.

We should also like to thank Dr. R. A. Cumming, Blood Transfusion Service, Edinburgh Royal Infirmary, who supplied the equipment used to collect and store the autologous blood.

During the period when this work was performed, the Rheumatic Diseases Unit was in receipt of grants from the Medical Research Council, the Arthritis and Rheumatism Council, the Nuffield Foundation, and Boots Pure Drug Company Limited.

While this study was carried out one of the authors (E.T.O.) was supported by a Geigy Fellowship awarded by the Australian Rheumatism Council.

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Nature de l'anémie de l'arthrite rhumatismale VI. Métabolisme du fer endogène

RÉSUMÉ

(1) On compara les modifications du taux du fer plasmatique après injection intraveineuse d'acide nicotinique et de sang autologue conservé.

(2) L'utilisation de l'acide nicotinique s'avéra être une méthode simple et satisfaisante pour produire une augmentation significative de la quantité de fer endogène formé à partir des globules rouges.

(3) On étudia le métabolisme du fer endogène chez cinq sujets témoins, sept cas d'arthrite rhumatismale et un cas de pseudo-polyarthrite rhizomélisque.

(4) On observa chez ces patients une élévation moins importante et une chute plus rapide du fer plasmatique après injection d'acide nicotinique et de sang autologue conservé; cela suggérerait une réduction de la libération de fer endogène par le système réticulo-endothélial.

(5) L'administration de prednisolone et de corticotrophine tend à corriger ces anomalies.

Naturaleza de la anemia en la artritis reumatoide VI. Metabolismo del hierro endógeno

SUMARIO

(1) Las variaciones de las cifras del hierro plasmático después de inyección intravenosa de ácido nicotínico y de sangre autóloga conservada han sido comparadas.

(2) La utilización del ácido nicotínico ha sido demostrada ser un método simple y satisfactorio de producir un aumento significativo de hierro endógeno derivado de los eritrocitos.

(3) El metabolismo del hierro endógeno ha sido estudiado en cinco testigos, siete casos de artritis reumatoide y uno de polimialgia reumática.

(4) Se han observado en estos enfermos un pequeño aumento y una caída más rápida de hierro plasmático después de la inyección de ácido nicotínico y de sangre autóloga conservada. Esto sugiere que el sistema reticulo-endotelial libera menos hierro endógeno.

(5) La administración de prednisolona y corticotropina tiende a corregir estas anomalías.