NATURE OF ANAEMIA IN RHEUMATOID ARTHRITIS*

IX. FOLATE METABOLISM IN PATIENTS WITH RHEUMATOID ARTHRITIS

BY

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Moderate anaemia of a hypochromic, normocytic type is a common feature of rheumatoid arthritis (Friereich, Ross, Bayles, Emerson, and Finch, 1957). Macrocytic anaemia has been reported less frequently. Macrocytosis was regarded as co-incidental by Short, Bauer, and Reynolds (1957) or due to anticonvulsant drugs (Chanarin, Laidlaw, Loughridge, and Mollin, 1960). In a study of the bone marrow in 64 patients with rheumatoid arthritis, Richmond, Gardner, Roy, and Duthie (1956) found

no consistent abnormality apart from a moderate increase in plasma cells. Reviewing the literature, these authors found that megaloblastosis had been reported only once in patients with rheumatoid arthritis (Marmont, 1948).

Partridge and Duthie (1963) found an incidence of macrocytic anaemia attributed to vitamin B₁₂ deficiency in 1.38 per cent. of 2,544 patients with rheumatoid arthritis, as compared to an incidence of 0.27 per cent. in controls. Other authors (Forshaw, 1963; Mollin, Waters, Gough, and Read, 1963) suggested that folic acid deficiency may have been a causal factor. Gough, McCarthy, Read, Mollin,

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CHARACTERISTICS OF 37 PATIENTS

Case No.	Age (yrs)	Sex	Grade of Disease Activity	Duration of Disease (yrs)	Hb (g. per cent.)	ESR (mm./1 hr)	Blood Film	Bone Marrow Morphology
1	53	M	II	—	12.4	70	Normal	Early megaloblastic
2	64	F	II	6/12	12.7	32	Normal	Normoblastic
3	62	F	II	30	11.5	48	Normal	Normoblastic
4	78	F	II	1	8.7	52	Normal	Normoblastic
5	42	F	II	7	9.6	50	Normal	Normoblastic
6	65	F	II	14	10.5	30	Normal	Normoblastic
7	73	F	III	2	7.3	107	Normal	Normoblastic
8	71	M	II	4/12	9.8	58	Hypochromic	Normoblastic
9	42	M	II	9	13.2	70	Normal	Normoblastic
10	57	M	I	13	12.8	20	Normal	Normoblastic
11	51	F	II	8	9.4	70	Normal	Normoblastic
12	64	M	III	7/12	12.4	43	Normal	Normoblastic
13	70	M	II	8	14.6	22	Normal	Early megaloblastic
14	42	M	II	5/12	12.8	28	Normal	Normoblastic
15	74	F	II	3	8.4	53	Normal	Normoblastic
16	52	F	III	2	12.0	81	Normal	Early megaloblastic
17	64	F	III	15/12	8.2	112	Normal	Early megaloblastic
18	69	F	III	3/12	10.6	112	Normal	Early megaloblastic
19	35	M	III	4	9.4	72	Normal	Early megaloblastic
20	35	M	III	8	9.4	75	Normal	Normoblastic
21	51	F	II	1	9.7	30	Normal	Normoblastic
22	40	F	II	18/12	8.6	48	Normal	Normoblastic
23	57	F	III	1	9.0	77	Normal	Normoblastic
24	33	F	III	6/12	9.0	74	Normal	Normoblastic
25	63	M	I	7	12.8	22	Normal	Early megaloblastic
26	49	M	II	25	14.0	32	Normal	Normoblastic
27	23	M	II	1	11.8	34	Normal	Normoblastic
28	62	M	I	6	12.1	22	Normal	Normoblastic
29	16	F	II	6	9.4	52	Normal	Normoblastic
30	46	F	II	10	8.6	62	Normal	Normoblastic
31	73	F	II	3	8.4	40	Hypochromic	Normoblastic
32	26	F	I	4	13.3	39	Normal	Normoblastic
33	63	F	II	8	9.4	36	Occasional macrocyte	Early megaloblastic
34	62	F	II	22	7.8	26	Hypochromic	Normoblastic
35	54	M	II	18/12	11.8	50	Normal	Normoblastic
36	39	M	II	6	12.2	67	Normal	Normoblastic
37	73	F	II	18/12	8.4	40	Hypochromic	Normoblastic

and Waters (1964), in a study of 46 patients with rheumatoid arthritis, reported that 65 per cent. had subnormal serum folate levels, and that in 33 per cent. the levels were within the range encountered in megaloblastic anaemia due to folate deficiency. Only six of these patients had megaloblastic erythropoiesis due to folate deficiency. Of the patients with subnormal serum folate levels, 73 per cent. excreted abnormal quantities of formiminoglutamic acid (FIGLU) in the urine after a histidine load. Similar results were reported by Deller, Urban, Ibbotson, Horwood, Milazzo, and Robson (1966).

It was decided to undertake a more detailed investigation of folate metabolism in patients with rheumatoid arthritis. Various tests of folate metabolism, including plasma and erythrocyte folate values, were carried out, and comparisons were made with the results in normal subjects and in patients with megaloblastic anaemia due to folate deficiency.

Material

Subjects

Three groups were investigated:

Group I.—The control group consisted of 59 healthy

subjects who were either members of the laboratory staff or blood donors; 24 of them were females and 35 males, and their ages ranged from 19 to 57 years.

Group II.—This group comprised 23 patients with megaloblastic anaemia due to folic acid deficiency. There were 16 females and 7 males, aged between 23 and 87. The diagnosis was made on the basis of low plasma folate values, normal serum vitamin B₁₂ values and a megaloblastic bone marrow.

Group III.—This consisted of 37 patients (Table I) with classical or definite rheumatoid arthritis according to the diagnostic criteria of the American Rheumatism Association (Ropes, Bennett, Cobb, Jacox, and Jessar, 1959). There were 21 females and 16 males, and their ages ranged from 16 to 78 years. All patients were admitted to the Rheumatic Diseases Unit, Northern General Hospital, Edinburgh, and a full history, including details of dietary habits, duration of disease, and drugs used in treatment, was taken. Patients on drugs other than aspirin or corticosteroids were excluded and during the period of study all received the same basic therapy of rest in bed, splintage, and physiotherapy. The disease activity was classified as Grade I (inactive), Grade II (moderately active), and Grade III (very active). This classification was based on the criteria of Duthie, Thompson, Weir, and Fletcher (1955), except that the degree of anaemia was not taken into account; this was thought advisable in a study investigating the deficiency of a haemopoietic factor.

WITH RHEUMATOID ARTHRITIS

Plasma Folate (μg./ml.)	Erythrocyte Folate (μg./ml. packed cells)	Serum Vitamin B ₁₂ (μg./ml.)	Urinary Excretion (mg./8 hrs)		Sensitized Sheep Cell Titre	Diet + -	Folic Acid Absorption
			FIGLU	Urocanic Acid			
1.3	120	168	35	184	1/512	+	Normal
1.4	183	560	25	13	1/128	+	Normal
1.5	103	256	12	12	1/1024	+	Normal
4.4	209	308	16	210	1/16	+	Normal
0.7	121	488	70	62	1/32	+	Normal
2.0	88	424	9	11	1/1024	+	Normal
3.6	194	220	99	9	1/16	+	Normal
2.4	290	448	69	39	1/256	+	Normal
3.7	282	672	42	91	1/1024	+	Normal
3.0	199	568	13	4	1/32	+	—
2.3	225	566	14	25	1/32	+	—
6.1	251	472	14	25	1/256	+	—
2.3	189	288	12	2	1/128	+	—
1.9	109	448	28	41	1/1024	+	—
2.9	175	300	32	0	1/256	+	—
1.1	103	306	54	2	1/16	+	—
1.4	153	472	60	60	1/128	+	—
2.0	126	240	67	615	1/512	+	—
2.5	163	148	138	15	1/32	+	—
1.9	172	320	33	13	1/256	+	—
3.0	208	392	335	280	1/32	+	—
2.7	259	251	46	0	1/1024	+	—
3.9	135	552	60	100	1/16	+	—
4.3	304	386	273	0	1/512	+	—
2.8	208	244	58	26	1/256	+	—
1.6	113	473	0	4	1/512	+	—
4.6	175	443	21	0	1/128	+	—
2.2	205	168	—0	—0	1/1024	+	Normal
1.3	91	1,000	—	—	1/64	+	Normal
2.3	113	168	35	75	1/512	+	Normal
1.7	111	392	37	55	1/16	+	Normal
2.2	192	220	23	5	1/64	+	Normal
1.5	55	212	28	17	1/16	—	Normal
2.9	232	291	314	3	1/256	+	Normal
1.8	99	272	21	9	1/256	—	Normal
1.6	99	496	49	140	1/256	+	Normal
1.8	215	132	15	18	1/128	+	Normal

Effect of Adrenocorticotrophic Hormone (ACTH) Therapy

Folic acid metabolism was studied in five rheumatoid patients on admission and after a remission in disease activity had been induced by a 4-week course of ACTH (20 i.u. intramuscularly daily for 2 weeks and 10 i.u. daily for the remaining 2 weeks). All patients had generalized joint involvement and systemic manifestations of the disease on admission, and none received folic acid during the period in hospital.

Follow-up Study

Seven rheumatoid patients, none of whom received folic acid during the period of study, were recalled 3 months or more after discharge from hospital and their plasma and erythrocyte folate levels were re-estimated.

Methods**Collection of Blood and Preparation of Samples**

40 ml. blood were obtained by venepuncture from fasting subjects. 2 ml. were delivered into a sequestrene bottle for determination of the haematological indices, and 18 ml. were put into a universal container and allowed to clot for 2 hours at room temperature before the serum was separated and stored at -20°C . for vitamin B_{12} estimation. The remaining 20 ml. were added to a heparinized container from which plasma was separated by centrifugation and stored at -20°C . with 10 mg. dry ascorbic acid/ml. for folate estimation.

The packed erythrocytes were washed three times with acid citrate dextrose (ACD) and 0.8 ml. samples were added to 1.5 ml. aliquots of patient and control plasma respectively. The control plasma was obtained from the same donor throughout the study and stored at -20°C . Three 0.4 ml. volumes of each product, with 10 mg. dry ascorbic acid added, were stored at -20°C . On the day of the assay samples were thawed at room temperature, 1.6 ml. distilled water was added to each, and they were left for a further 15 minutes at room temperature. 18 ml. of 0.1 M phosphate buffer pH 6 containing 150 mg. per cent. ascorbic acid were then added and the proteins precipitated by autoclaving at 15 lb./sq. in. for 5 minutes.

Plasma and Erythrocyte Folate

The folate content of plasma and erythrocytes was determined by the method of Waters and Mollin (1961), except that autoclaved ascorbate-phosphate buffer was added to standard tubes.

Serum Vitamin B_{12}

The vitamin B_{12} content of serum was determined by the method of Anderson (1964). The range of serum vitamin B_{12} concentration in normal controls is 150-1000 $\mu\text{g.}/\text{ml.}$

Folic Acid Absorption and Clearance

The absorption of folic acid was measured by the method of Girdwood (1953, 1960), and its clearance from the plasma by the method of Chanarin, Mollin, and Anderson (1958).

Bone Marrow Examination

May-Grunwald-Giemsa stained slides of bone marrow were examined by an experienced haematologist (S. H. Davies) and one of us (A.O.), and were classified as normoblastic, early megaloblastic, or frankly megaloblastic. Classification was based on the overall marrow picture particularly affecting the erythroid series. Patients whose marrow showed only the presence of a few giant metamyelocytes were not included in the megaloblastic group.

Urinary Formiminoglutamic Acid (FIGLU) and Urocanic Acid

Combined urinary FIGLU and urocanic acid was measured by the spectrophotometric method of Chanarin and Bennett (1962) in an 8-hour collection of urine after an oral dose of 15 g. histidine. Urocanic acid was also determined separately after destroying FIGLU by autoclaving the urine at an alkaline pH.

Sensitized Sheep Cell Titre

The sensitized sheep cell titre (SSCT) for rheumatoid factor was performed by the method of Ball (1952), modified by the use of M.R.C. haemagglutination plates in place of test tubes.

Other Haematological Indices

The haematological methods were those described by Dacie and Lewis (1963).

Results**Plasma Folate Levels**

The plasma folate levels for healthy controls, patients with megaloblastic anaemia due to folic acid deficiency, and patients with rheumatoid arthritis are compared in Fig. 1 (opposite). The range for normal controls is 2.6 to 10.4 $\mu\text{g.}/\text{ml.}$ (mean of 4.7). Patients with megaloblastic anaemia had levels of less than 2.0 $\mu\text{g.}/\text{ml.}$ In the rheumatoid arthritis group, 24 cases (65 per cent.) had subnormal levels, and in seventeen of these (46 per cent.) the folate values fell within the range found in megaloblastic folate deficiency.

Erythrocyte Folate Values

It was found that the release of assayable folate from erythrocytes by normal plasma and by the plasma of patients with megaloblastic folate deficiency did not differ significantly from that obtained by the use of control plasma. Release by rheumatoid plasma, however, was significantly reduced compared to control plasma. This finding will be discussed in a future communication. For the purposes of the present investigation, the results obtained by the use of control plasma were used in all three groups.

The erythrocyte folate values found for the three

Fig. 1.—Plasma folate levels ($\mu\text{g./ml.}$) in 59 controls, 23 cases of megaloblastic folate deficiency, and 37 cases of rheumatoid arthritis.

Fig. 2.—Erythrocyte folate levels ($\mu\text{g./ml.}$ packed cells).

groups are shown in Fig. 2. All megaloblastic folate deficient cases had subnormal levels (less than $130 \mu\text{g./ml.}$ of packed red cells) and in nineteen (83 per cent.) the values were less than $60 \mu\text{g./ml.}$ Fourteen patients (37 per cent.) with rheumatoid arthritis had subnormal levels ranging between

55 and $120 \mu\text{g./ml.}$ of packed red cells, but only one had a level of less than $60 \mu\text{g./ml.}$

(a) *Relationship to Plasma Folate Level.*—There was a good correlation between the plasma and erythrocyte folate levels in rheumatoid arthritis (Fig. 3). The Figure also shows that, whereas all

Fig. 3.—Plasma folate and erythrocyte folate in cases of rheumatoid arthritis.

fourteen cases with subnormal erythrocyte folate levels had reduced plasma folate values, there were ten in which a subnormal plasma folate value was associated with a normal erythrocyte level.

(b) *Relationship to Haemoglobin Concentration.*—No relationship was shown in rheumatoid arthritis between haemoglobin concentration (g. per cent.) and erythrocyte folate level.

Bone Marrow Morphology

The bone marrow was normoblastic in 29 (78 per cent.) of the rheumatoid patients. The remaining eight cases showed early megaloblastic change. Table II shows that only five of these cases had plasma folate levels within the range found in megaloblastic anaemia due to folate deficiency, and that four of these also had subnormal erythrocyte folate levels.

Folic Acid Clearance

This was measured in eight of the rheumatoid patients (Fig. 4). Erythropoiesis was normoblastic in all patients studied (Table III, opposite). Cases 3, 5, and 30 showed a rapid clearance of injected folic acid, and also had subnormal plasma and erythrocyte folate levels, and two of them (Cases 5 and 30) excreted high quantities of FIGLU and urocanic acid in the urine. A rapid clearance (especially at 15 and 30 minutes) was also obtained in Case 4, in which the only other abnormality was excessive excretion of urocanic acid, and in Case 20 which had a subnormal plasma, but normal erythrocyte, folate level.

Urinary FIGLU and Urocanic Acid Excretion

The range for FIGLU excretion in thirteen normal subjects was 0.20 mg./8 hrs, and for urocanic acid 0.4 mg./8 hrs. The upper limit for excretion

of both histidine metabolites, or of FIGLU alone, was taken as 25 mg./8 hrs, while that for urocanic acid was considered as 5 mg./8 hrs.

Fig. 4.—Folic acid clearance in cases of rheumatoid arthritis.

TABLE II
CHARACTERISTICS OF EIGHT CASES OF RHEUMATOID ARTHRITIS WITH MEGALOBlastic ANAEMIA

Case No.	Haemoglobin (g. per cent.)	Bone Marrow	Plasma Folate (mug./ml.)	Erythrocyte Folate (mug./ml. packed cells)	Urinary Excretion (mg./8 hrs)		Serum B ₁₂ (μg./ml.)
					FIGLU	Urocanic Acid	
1	12.4	Early megaloblastic	1.3	120	35	184	168
13	14.6	Early megaloblastic	2.3	189	12	2	288
16	12.0	Early megaloblastic	1.1	109	54	2	306
17	8.2	Early megaloblastic	1.4	153	60	0	472
18	10.6	Early megaloblastic	2.0	126	67	615	240
19	9.4	Early megaloblastic	2.5	163	138	15	148
25	12.8	Early megaloblastic	2.8	208	58	26	244
33	9.4	Early megaloblastic	1.5	55	28	17	212

TABLE III
FOLIC ACID CLEARANCE STUDIES IN EIGHT PATIENTS WITH RHEUMATOID ARTHRITIS

Case No.	Folic Acid Clearance	Plasma Folate (mµg./ml.)	Erythrocyte Folate (mµg./ml. packed cells)	Urinary Excretion (mg./8 hrs)		Bone Marrow
				FIGLU	Urocanic Acid	
3	Fast	1.5	103	12	12	Normoblastic
4	Fast	4.4	209	16	210	Normoblastic
5	Fast	0.7	121	70	62	Normoblastic
6	Normal	2.0	88	9	11	Normoblastic
20	Fast	1.9	172	33	13	Normoblastic
21	Normal	3.0	208	335	280	Normoblastic
28	Normal	2.2	205	0	0	Normoblastic
30	Fast	2.3	113	35	75	Normoblastic

(a) *Relationship to Plasma Folate.*—24 of 36 (66 per cent.) rheumatoid patients excreted abnormal quantities of FIGLU in the urine. Eight of these had normal plasma folate levels. Conversely, FIGLU excretion was normal in eight cases with subnormal plasma folate levels (Fig. 5).

Fig. 5.—FIGLU excretion related to plasma folate levels in rheumatoid arthritis.

(b) *Relationship to Erythrocyte Folate Content.*—Fig. 6 shows that thirteen of those who excreted abnormal quantities of FIGLU had normal erythrocyte folate levels and that the highest quantities were excreted in this group. Three cases with subnormal erythrocyte folate levels excreted normal quantities of FIGLU.

Fig. 6.—FIGLU excretion related to erythrocyte folate levels in rheumatoid arthritis.

Fig. 7.—Urocanic acid excretion related to erythrocyte folate levels in rheumatoid arthritis.

(c) *Urocanic Acid Excretion.*—Fig. 7 shows that 23 cases (66 per cent.) excreted excess urocanic acid. Excessively high levels (more than 150 mg./8 hrs) were obtained in four cases, two of which had normal bone marrow morphology and erythrocyte folate levels.

(d) *Excretion of Histidine Metabolites in Rheumatoid Patients with a Normal Folate Status.*—Table IV shows that ten out of twelve patients with normal plasma and erythrocyte folate levels and in whom erythropoiesis was normoblastic excreted abnormal quantities of FIGLU or urocanic acid or both in the urine.

TABLE IV
EXCRETION OF HISTIDINE METABOLITES IN TWELVE RHEUMATOID PATIENTS WITH NORMAL FOLATE VALUES AND BONE MARROW

Case No.	Duration of Disease (yrs)	Grade of Disease Activity	Urinary Excretion (mg./8 hrs)		
			FIGLU	Urocanic Acid	Total
4	1	II	16	210	226
7	2	III	99	9	108
9	9	II	42	91	133
10	13	I	13	4	17
12	7/12	III	14	25	39
15	3	II	32	0	32
21	1	II	335	280	615
22	18/12	II	46	0	46
23	1	III	60	100	160
24	6/12	III	273	0	273
27	1	II	21	0	21
34	22	II	314	3	317

Relationship of SSCT to Folate Status

There was no correlation between the SSCT and plasma folate values, erythrocyte folate values, or excretion of histidine metabolites.

Relationship of Various Parameters to Activity and Duration of Disease in Rheumatoid Arthritis

(a) Disease Activity

There was no relationship between either plasma or erythrocyte folate levels and disease activity. Thus, of the 24 cases classified as Grade II, 50 per cent. had subnormal erythrocyte folate levels, and 70 per cent. had subnormal plasma folate levels; the corresponding percentages in Grade III cases were 22 and 44 respectively.

A comparison was made of the excretion of FIGLU and urocanic acid in patients with different grades of disease activity. The number with Grade I activity is too small to allow comparison with the other groups. All patients with Grade III activity excreted abnormal quantities of histidine metabolites in the urine as compared to 80 per cent. of those classified as Grade II.

(b) Duration of Disease

There was no correlation between plasma or erythrocyte folate levels and duration of disease.

Similarly, there was no correlation between the excretion of histidine metabolites and duration of disease. Four patients who had had the disease for more than 10 years excreted normal quantities, while two (Cases 18 and 8) who had had the disease for 3 and 4 months excreted 682 and 108 mg./8 hrs respectively.

Effect of ACTH Therapy on Folate Levels

The haematological indices and the folate levels in five patients before and after a remission in disease activity had been induced by ACTH are shown in Table V. The differences in the folate values are not significant for the number of subjects studied.

Follow-up Study

The folate levels in seven patients were assessed 3 months or more after discharge from hospital and compared with the original levels (Table VI, opposite).

None of them received folic acid. The follow-up levels in three were lower than the initial levels, but the differences are not statistically significant.

Discussion

The finding of reduced plasma folate levels in the majority of patients with rheumatoid arthritis

TABLE V
FOLATE VALUES IN FIVE RHEUMATOID PATIENTS BEFORE AND AFTER ACTH THERAPY

Case No.	Time of Estimation	Haemoglobin (g. per cent.)	ESR (mm./1 hr.)	Grade of Disease Activity	Plasma Folate (m μ g./ml.)	Erythrocyte Folate (m μ g./ml. packed cells)
17	Before ACTH	8.2	112	III	1.4	153
	After ACTH	14.6	10	I	1.3	125
19	Before ACTH	9.4	72	III	2.5	163
	After ACTH	11.4	25	I	2.2	155
22	Before ACTH	8.6	48	II	2.7	259
	After ACTH	13.5	4	I	2.3	245
23	Before ACTH	9.0	77	III	3.9	135
	After ACTH	15.4	6	I	4.0	177
24	Before ACTH	9.0	74	III	4.3	304
	After ACTH	10.2	18	I	4.5	286

TABLE VI
INITIAL AND FOLLOW-UP FOLATE VALUES IN
SEVEN PATIENTS WITH RHEUMATOID ARTHRITIS

Case No.	Plasma Folate (m μ g./ml.)		Erythrocyte Folate (m μ g./ml. packed cells)	
	Initial	Follow-up	Initial	Follow-up
8	2.4	1.5	290	205
9	3.7	2.7	282	222
10	3.0	3.5	199	204
11	2.3	1.9	225	233
12	6.1	4.7	251	185
13	2.3	2.3	189	144
14	1.9	2.0	109	160

investigated in the present study is in agreement with previous reports (Gough and others, 1964; Deller and others, 1966). Both experimental (Herbert, 1962a) and clinical studies (Hellman, Iannotti, and Bertino, 1964; Mollin and Hoffbrand, 1965) indicate that the significance of a low serum folate is best assessed by estimating the erythrocyte folate content and by looking for changes in the bone marrow and haematological indices.

The plasma folate level in rheumatoid patients correlated well with the erythrocyte folate content, but the mean erythrocyte/plasma folate ratio in this condition (72:1) is higher than in normal subjects (42:1), indicating a proportionately greater reduction in the plasma folate level compared to that of the erythrocytes. Further, normal erythrocyte folate levels were found in ten cases with reduced plasma folate values ranging from 1.5-2.5 m μ g./ml. The reduction in the plasma folate is genuine, and possible inhibitors to *L. casei* growth, including salicylates, were excluded by experimental trials in this and other studies (Cowan, Hoffbrand, and Mollin, 1966). This indicates that the plasma folate activity in this condition does not have the same significance as it does in other folate deficient states. Thus, in a series of patients with subnormal serum folate levels due to conditions other than rheumatoid arthritis, Hoffbrand, Newcombe, and Mollin (1966c) found that levels in the unequivocal deficiency range were almost invariably associated with subnormal erythrocyte folate values and with variable degrees of megaloblastic change in the bone marrow.

There is a striking paucity of megaloblastic change in patients with rheumatoid arthritis, who should theoretically have a significant degree of folate deficiency as manifested by subnormal levels of both plasma and erythrocyte folate. Thus, of fourteen such cases only four showed bone marrow changes suggestive of megaloblastosis, and in all of them the changes were classified as early. Of the remaining ten normoblastic cases, three also showed a rapid

clearance of folic acid from the plasma. In the series reported by Gough and others (1964), mild to moderate megaloblastic change was found in only six out of fifteen rheumatoid patients with serum folate levels in the unequivocal deficiency range. Comparison with the other folate deficiency states in this study, and with the series reported by Hoffbrand and others (1966c) shows that megaloblastic change in rheumatoid arthritis is less frequent and, when present, less severe than would be expected from the degree of folate deficiency. It is possible that this is due to the hypochromic nature of the anaemia of rheumatoid arthritis, since it is known that a co-existent impairment of haemoglobin synthesis, *e.g.* in iron deficiency anaemia or haemoglobinopathy, may mask the overt megaloblastic changes of folate deficiency (Herbert, 1965).

A relationship has been shown in folate deficiency between FIGLU excretion and serum folate levels. Mollin and Hoffbrand (1965) and Kershaw and Girdwood (1964) found excess FIGLU in the urine of all patients with subnormal serum folate values. However, a dissociation between FIGLU excretion and serum folate values has been shown in vitamin B₁₂ deficiency (Zalusky and Herbert, 1961; Kohn, Mollin, and Rosenbach, 1961; Kershaw and Girdwood, 1964); in conditions with neither vitamin B₁₂ or folate deficiency, *e.g.* liver disease (Carter, Heller, Schnaffer, and Korn, 1961); and in malignancy (Noeypatimanond, Watson-Williams, and Israëls, 1966).

In patients with rheumatoid arthritis in whom vitamin B₁₂ deficiency, liver disease, and malignancy had been excluded, no clear relationship was shown between FIGLU excretion and plasma folate values, and no association was shown between FIGLU excretion and erythrocyte folate values. Although there was no relationship between the excretion of histidine metabolites and duration of disease, there was, however, some correlation between the excretion of histidine metabolites and disease activity.

Most observers would agree that increased FIGLU excretion indicates disordered folate metabolism due to either lack, or interference with the function, of the folate co-enzymes or of FIGLU transferase. Folate deficiency may therefore be responsible for the excretion of large quantities of FIGLU in the urine by those rheumatoid patients who had other criteria of the deficiency state, *e.g.* reduced folate levels and bone marrow changes suggestive of megaloblastic erythropoiesis. The occurrence of a positive FIGLU test, on the other hand, in patients who have a normal folate status may indicate a deficiency of FIGLU transferase, possibly related to disease activity.

Urocanic acid may be the major or only histidine metabolite in the urine of folate deficient subjects (Chanarin and Bennett, 1961; Mohamed and Roberts, 1966). Since folate co-enzymes are not directly involved in the breakdown of urocanic acid, the mechanism whereby folate deficiency causes a high excretion of this metabolite is obscure. Allen and Whitehead (1965) suggested that the excessive excretion of urocanic acid in protein malnutrition is caused by the disturbed protein metabolism rather than by folate deficiency. Similarly, Hoffbrand, Neale, Hines, and Mollin (1966b) found that patients with low serum albumin levels excreted large quantities of urocanic acid, and that this was returned to normal by intravenous albumin therapy. It is possible, therefore, that increased urocanic acid excretion is related to the reduced serum albumin levels frequently found in rheumatoid patients (Gutman, 1948).

Edozien and Obasi (1965) found a correlation between the excretion of urocanic acid after a histidine load and of xanthurenic acid after a tryptophan load in patients with protein malnutrition. They argued that, since the excretion of xanthurenic acid after a tryptophan load is a test of pyridoxine deficiency, and since the enzyme urocanase has pyridoxal requirements (Gupta and Robinson, 1961), the excretion of excessive urocanic acid in these patients reflected pyridoxine deficiency. Abnormalities of tryptophan metabolism in patients with rheumatoid arthritis have been reported (Bett, 1962a, 1962b), and a relative deficiency of pyridoxine suggested as a cause (Bett, 1966). This provides a further possible explanation for the increased urocanic acid excretion and the poor correlation with erythrocyte folate levels in rheumatoid arthritis.

Herbert (1962b) and Gough and others (1964) suggested that dietary deficiency might be responsible for the abnormal folate status of patients with rheumatoid arthritis, but they were not able to show any significant difference in the diet of their patients compared to controls. Gough and others (1964) also showed that intestinal absorption of the vitamin was normal in the six megaloblastic cases in their series.

Histories of adequate dietary intake were obtained in all but two cases in the present study, and the intestinal absorption of folic acid was shown to be normal in all of the eighteen cases studied. Further, preliminary results have shown that the urinary loss of folic acid in rheumatoid patients is not different from that in controls.

It is known that barbiturates and anticonvulsants can cause abnormalities in folic acid metabolism,

but patients on these drugs were excluded from the present study. It is possible that the gastrointestinal blood loss which occurs in rheumatoid patients on salicylates (Scott, Porter, Lewis, and Dixon, 1961) and the mild haemolytic process in rheumatoid arthritis (Richmond, Alexander, Potter, and Duthie, 1961) may contribute to the folate deficiency.

Increased utilization of folic acid during cellular proliferation in the joints of rheumatoid arthritis was suggested by Gough and others (1964). Hoffbrand, Kremenchukzy, Butterworth, and Mollin (1966a) reported raised serum lactate dehydrogenase (LDH) activity in a number of haematological disorders with some correlation to an associated low serum folate level. It was suggested that increased cell turnover was responsible. In this context it may be relevant that Chalmers (1967) reported an LDH activity in rheumatoid synovial fluid of seven times the serum level. This concept of increased utilization is very possible, but difficult to prove.

Neither the incidence nor the degree of folate deficiency could be related to the duration or severity of the disease. The plasma and erythrocyte levels remained unchanged in five patients in whom a remission of disease activity and a significant rise in haemoglobin concentration were produced by ACTH therapy, and in seven patients who were re-assessed a short time after discharge from hospital and whose disease activity might be expected to have lessened.

Summary

- (1) Folate metabolism of 37 patients with rheumatoid arthritis was assessed and the occurrence of folic acid deficiency confirmed.
- (2) Subnormal plasma folate levels were found in 65 per cent. of cases, but subnormal erythrocyte folate levels were found in only 37 per cent. of cases, indicating that a reduced plasma folate value is not necessarily associated with a significant degree of folate deficiency in this disease.
- (3) Megaloblastic bone marrow changes were found in 22 per cent. of cases. Both the incidence and severity were less than would be expected from the degree of folate deficiency.
- (4) Histidine metabolism is abnormal in rheumatoid arthritis and in many cases unrelated to the folate status of the patients. Possible causes for this are discussed.
- (5) Possible aetiological factors in the production of folate deficiency in rheumatoid arthritis are discussed.

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REFERENCES

- Allen, D. M., and Whitehead, R. G. (1965). *Blood*, **25**, 283 (The excretion of urocanic acid and formiminoglutamic acid in megaloblastosis accompanying kwashiorkor).
- Anderson, B. B. (1964). *J. clin. Path.*, **17**, 14 (Investigations into the Euglena method for the assay of the vitamin B₁₂ in serum).
- Ball, J. (1952). *Ann. rheum. Dis.*, **11**, 97 (Sheep cell agglutination test for rheumatoid arthritis).
- Bett, I. M. (1962a). *Ibid.*, **21**, 63 (Metabolism of tryptophan in rheumatoid arthritis).
- (1962b). *Ibid.*, **21**, 388 (Effect of pyridoxine on tryptophan metabolism in rheumatoid arthritis).
- (1966). *Ibid.*, **25**, 556 (Urinary tryptophan metabolites in rheumatoid arthritis and some other diseases).
- Carter, F. C., Heller, P., Schnaffer, G., and Korn, R. J. (1961). *Arch. intern. Med.*, **108**, 41 (Formiminoglutamic acid (FIGLU) excretion in hepatic cirrhosis).
- Chalmers, T. M. (1967). *Scot. med. J.*, **12**, 125 (Lactate dehydrogenase (LDH) activity in synovial fluid).
- Chanarin, I., and Bennett, M. C. (1962). *Brit. med. J.*, **1**, 27 (A spectrophotometric method for estimating formimino-glutamic acid and urocanic acid).
- , Laidlaw, J., Loughridge, L. W., and Mollin, D. L. (1960). *Ibid.*, **1**, 1099 (Megaloblastic anaemia due to phenobarbitone. The convulsant action of therapeutic doses of folic acid).
- , Mollin, D. L., and Anderson, B. B. (1958). *Brit. J. Haemat.*, **4**, 435 (The clearance from the plasma of folic acid injected intravenously in normal subjects and patients with megaloblastic anaemia).
- Cowan, J. D., Hoffbrand, A. V., and Mollin, D. L. (1966). *Lancet*, **1**, 11 (Effect of serum-factors other than folate on the *Lactobacillus casei* assay).
- Dacie, J. V., and Lewis, S. M. (1963). "Practical Haematology", 3rd ed. Churchill, London.
- Deller, D. J., Urban, E., Ibbotson, R. N., Horwood, J., Milazzo, S., and Robson, H. N. (1966). *Brit. med. J.*, **1**, 765 (Folic-acid deficiency in rheumatoid arthritis: relation of levels of serum folic-acid activity to treatment with phenylbutazone).
- Duthie, J. J. R., Thompson, M., Weir, M. M., and Fletcher, W. B. (1955). *Ann. rheum. Dis.*, **14**, 133 (Medical and social aspects of the treatment of rheumatoid arthritis).
- Edozien, J. C., and Obasi, M. E. (1965). *Clin. Sci.*, **29**, 1 (Protein and amino acid metabolism in kwashiorkor).
- Forshaw, J. (1963). *Brit. med. J.*, **1**, 328 (Macrocytic anaemia in rheumatoid arthritis).
- Friereich, E. J., Ross, J. F., Bayles, T. B., Emerson, C. P., and Finch, S. C. (1957). *J. clin. Invest.*, **36**, 1043 (Radioactive iron metabolism and erythrocyte survival studies of the mechanism of the anaemia associated with rheumatoid arthritis).
- Girdwood, R. H. (1953). *Lancet*, **2**, 53 (A folic-acid excretion test in the investigation of intestinal malabsorption).
- (1960). *Scot. med. J.*, **5**, 10 (Microbiological methods of assay in clinical medicine with particular reference to the investigation of deficiency of vitamin B₁₂ and folic acid).
- Gough, K. R., McCarthy, C., Read, A. E., Mollin, D. L., and Waters, A. H. (1964). *Brit. med. J.*, **1**, 212 (Folic-acid deficiency in rheumatoid arthritis).
- Gupta, N. K., and Robinson, W. G. (1961). *Fed. Proc.*, **20**, 4 (Urocanase and pyridoxal-5-phosphate).
- Gutman, A. B. (1948). *Advanc. Protein Chem.*, **4**, 155 (The plasma proteins in disease).
- Hellma, S., Iannotti, A. T., and Bertino, J. R. (1964). *Cancer Res.*, **24**, 105 (Determinations of the levels of serum folate in patients with carcinoma of the head and neck treated with methotrexate).
- Herbert, V. (1962a). *Trans. Ass. Amer. Phycns*, **75**, 307 (Experimental nutritional folate deficiency in man).
- (1962b). *Arch. intern. Med.*, **110**, 649 (Minimal daily adult folate requirements).
- (1965). *Ann. Rev. Med.*, **16**, 359 (Folic acid).
- Hoffbrand, A. V., Kremenchukzy, S., Butterworth, P. J., and Mollin, D. L. (1966a). *Brit. med. J.*, **1**, 577 (Serum lactic dehydrogenase activity and folate deficiency in myelosclerosis and other haematological diseases).

- , Neale, G., Hines, J. D., and Mollin, D. L. (1966b). *Lancet*, **1**, 1231 (The excretion of formiminoglutamic acid and urocanic acid after partial gastrectomy).
- , Newcombe, B. F. A., and Mollin, D. L. (1966c). *J. clin. Path.*, **19**, 17 (Method of assay of red cell folate activity and the value of the assay as a test for folate deficiency).
- Kershaw, P. W., and Girdwood, R. H. (1964). *Scot. med. J.*, **9**, 201 (Some investigations of folic acid deficiency).
- Kohn, J., Mollin, D. L., and Rosenbach, L. M. (1961). *J. clin. Path.*, **14**, 345 (Conventional voltage electrophoresis for formiminoglutamic acid determination in folic acid deficiency).
- Marmont, A. (1948). *Arch. E. Maragliano Pat. Clin.*, **3**, 1289 (Il sangue ed il midollo osseo nella poliartrite cronica primaria).
- Mohamed, S. D., and Roberts, M. (1966). *J. clin. Path.*, **19**, 37 (Relative importance of formiminoglutamic and urocanic acid excretion after a histidine load).
- Mollin, D. L., and Hoffbrand, A. V. (1965). *Scand. J. Haemat.*, Series Haematologica, **3**, 1 (Diagnosis of folate deficiency).
- , Waters, A. H., Gough, K. R., and Read, A. E. (1963). *Brit. med. J.*, **1**, 609 (Macrocytic anaemia in rheumatoid arthritis).
- Noeypatimanond, S., Watson-Williams, E. J., and Israëls, M. C. G. (1966). *Lancet*, **1**, 454 (Excretion of formiminoglutamic acid in reticulosis and carcinoma).
- Partridge, R. E. H., and Duthie, J. J. R. (1963). *Brit. med. J.*, **1**, 89 (Incidence of macrocytic anaemia in rheumatoid arthritis).
- Richmond, J., Alexander, W. R. M., Potter, J. L., and Duthie, J. J. R. (1961). *Ann. rheum. Dis.*, **20**, 133 (Nature of anaemia in rheumatoid arthritis. IV. Red cell survival measured by radioactive chromium).
- , Gardner, D. L., Roy, L. M. H., and Duthie, J. J. R. (1956). *Ibid.*, **15**, 217 (Nature of anaemia in rheumatoid arthritis. III. Changes in the bone marrow and their relation to other features of the disease).
- Ropes, M. W., Bennett, G. A., Cobb, C., Jacox, R., and Jessar, R. (1959). *Ibid.*, **18**, 49 (Diagnostic criteria of rheumatoid arthritis, 1958 revision).
- Scott, J. T., Porter, I. H., Lewis, S. M., and Dixon, A. St. J. (1961). *Quart. J. Med.*, **30**, 167 (Studies of gastrointestinal bleeding caused by corticosteroids, salicylates, and other analgesics).
- Short, C. L., Bauer, W., and Reynolds, W. E. (1957). "Rheumatoid Arthritis". Harvard Univ. Press, Cambridge, Mass.
- Waters, A. H., and Mollin, D. L. (1961). *J. clin. Path.*, **14**, 335 (Studies on the folic acid activity of human serum).
- Zalusky, R., and Herbert, V. (1961). *J. clin. Invest.*, **40**, 1091 (Failure of formiminoglutamic acid (FIGLU) excretion to distinguish vitamin B₁₂ deficiency from nutritional folic acid deficiency).

La nature de l'anémie dans la polyarthrite rhumatoïde IX. Le métabolisme du folate chez des malades atteints de polyarthrite rhumatoïde

RÉSUMÉ

(1) On a étudié le métabolisme du folate chez 37 malades atteints de polyarthrite rhumatoïde et on a confirmé que la carence en acide folique s'observe chez certains d'entre eux.

(2) On a trouvé un taux d'acide folique dans le plasma au-dessous de la normale dans 65 pour cent des cas, mais dans le hématis ce taux n'était bas que dans 37 pour cent des cas. Il s'en suit qu'un chiffre bas de folate plasmatique n'est pas nécessairement associé à une carence prononcée dans cette maladie.

(3) Des altérations mégalo-blastiques dans la moelle osseuse furent trouvées dans 22 pour cent des cas. La fréquence et la gravité de ces altérations étaient moins prononcées que ne l'aurait laissé prévoir l'intensité de la carence en folate.

(4) Le métabolisme de l'histidine est anormal dans la polyarthrite rhumatoïde et, dans beaucoup de cas, indépendamment du taux de folate. On en discute les causes possibles.

(5) On discute les facteurs étiologiques pouvant intervenir dans la production de la carence en folate dans la polyarthrite rhumatoïde.

La naturaleza de la anemia en la poliartritis reumatoide IX. El metabolismo del folato en enfermos con poliartritis reumatoide

SUMARIO

(1) Se estudió el metabolismo del folato en 37 enfermos con poliartritis reumatoide y se confirmó el acontecimiento de la carencia de ácido fólico.

(2) Niveles subnormales de folato en el plasma se hallaron en un 65 por ciento de los casos, mientras que niveles subnormales de folato en los eritrocitos se encontraron sólo en un 37 por ciento de los casos, indicando que cifras bajas de folato no se ven necesariamente asociadas con una carencia importante de folato en esta enfermedad.

(3) Alteraciones megaloblásticas en la médula ósea se vieron en un 22 por ciento de los casos. Tanto la incidencia como la severidad de estas alteraciones fueron menores cuando se toma en consideración la intensidad de la carencia de folato.

(4) El metabolismo de la histidina es anormal en la poliartritis reumatoide y, en muchos casos, independiente de las cifras de folato. Se discuten las causas posibles de este hecho.

(5) Se discuten los factores etiológicos pudiendo obrar en la producción de la carencia de folato en la poliartritis reumatoide.