NATURE OF ANAEMIA IN RHEUMATOID ARTHRITIS

X. FOLATE-RELEASING PROPERTIES OF PLASMA IN HEALTH AND DISEASE, WITH SPECIAL REFERENCE TO RHEUMATOID ARTHRITIS

BY

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The availability of erythrocyte folate in an assayable form depends on the interaction of a plasma factor with inactive precursors in the red cell (Toennies, Usdin, and Phillips, 1956). Low levels of erythrocyte folate have been reported in cases of megaloblastic erythropoiesis due to either folate or vitamin B₁₂ deficiency (Hansen and Weinfeld, 1962; Cooper and Lowenstein, 1964; Hoffbrand, Newcombe, and Mollin, 1966), and these low levels have been attributed to a lack of erythrocyte folate precursors rather than to a decrease in plasma factor activity. Thus, Cooper and Lowenstein (1964) found no significant alteration in the folate-releasing properties of plasma from two patients with pernicious anaemia. Similar findings were reported by Hoffbrand and others (1966) in megaloblastic anaemia due to both folate and vitamin B₁₂ deficiency.

Omer and Mowat (1968), in a study of folate metabolism in patients with rheumatoid arthritis, found sub-normal erythrocyte folate values in fourteen of 37 cases (37 per cent.). It was found that erythrocyte folate release by rheumatoid plasma was significantly reduced compared to the release by control plasma.

The present paper reports further investigations undertaken to compare the erythrocyte folate-releasing properties of plasma from patients with rheumatoid arthritis, patients with megaloblastic erythropoiesis due to either folate or vitamin B₁₂ deficiency, and normal controls.

Material

Four groups of subjects were investigated:

Group 1.—32 healthy subjects who were either members of the laboratory staff or blood donors.

Group 2.—Nineteen patients with megaloblastic anaemia due to folate deficiency. The diagnosis was made on the basis of a plasma folate of less than 2 mμg./ml., a normal serum vitamin B₁₂, and a megaloblastic bone marrow.

Group 3.—Nineteen patients with megaloblastic anaemia due to vitamin B₁₂ deficiency. The diagnosis was made by the finding of a serum vitamin B₁₂ of less than 100 μμg./ml., a normal plasma folate, a megaloblastic bone marrow, histamine-fast achlorhydria, and impaired absorption of radioactive vitamin B₁₂ (Schilling test).

Group 4.—25 patients with classical or definite rheumatoid arthritis according to the diagnostic criteria of the American Rheumatism Association (Ropes, Bennett, Cobb, Jacox, and Jessar, 1959). Full details of these patients have been published previously (Omer and Mowat, 1968).

Methods

Collection of Blood and Preparation of Samples

The control plasma was obtained from the same donor throughout the study and stored without ascorbic acid at -20°C.

40 ml. blood were obtained by venepuncture from fasting subjects. The serum from 20 ml. was used for vitamin B₁₂ estimation. The remaining 20 ml. were added to a heparinized container from which plasma was separated by centrifugation. Some of the plasma was stored at -20°C. with 10 mg. dry ascorbic acid/ml. for folate estimation, and the remainder was used for recombination with the red cells.

Release of Folate from Donor Erythrocytes by Control and Donor Plasma

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 subject and control plasma respectively. 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. of distilled water was added to each, and they were left for a further 15 minutes

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at room temperature. 18 ml. of 0.1 M phosphate buffer pH 6 containing 150 mg. per cent. ascorbic acid was then added, and the proteins were precipitated by autoclaving at 15 lb./sq. in. for 5 minutes.

Plasma and Erythrocyte Folate

The folate content was determined by the method of Waters and Mollin (1961), except that autoclaved ascorbate-phosphate buffer was added to standard tubes. The erythrocyte folate content was considered normal if greater than 135 m μ g./ml. of packed cells.

Serum Vitamin B₁₂

The vitamin B₁₂ content of serum was determined by the method of Anderson (1964) using *E. gracilis*.

Results

Release of Folate from Donor Erythrocytes by Control and Donor Plasma

The amounts of folate released from donor erythrocytes by control plasma and donor plasma are shown in the Table. The values are not significantly different in normal subjects and in patients with megaloblastic anaemia due either to folate or

vitamin B₁₂ deficiency. However, the folate released by the donor plasma is significantly reduced when compared with that released by control plasma in the group of patients with rheumatoid arthritis ($P < 0.001$).

Discussion

The present study shows that the levels of assayable folate released from donor erythrocytes by control and donor plasma are not significantly different in normal individuals and in patients with megaloblastic anaemia due to either folate or vitamin B₁₂ deficiency. These results confirm those of Hoffbrand and others (1966). However, the folate released from the erythrocytes of patients with rheumatoid arthritis by autologous plasma is significantly less than that released by control plasma. Thus, in five patients (Cases 11, 13, 15, 16, and 17 in the Table) the folate released by the donor plasma is less than 85 per cent. of that released by the control plasma, and in four patients (Cases 15, 17, 18, and 23) the folate released was within the deficiency range of less than 135 m μ g./ml. of packed cells.

TABLE
RELEASE OF FOLATE FROM DONOR ERYTHROCYTES BY (A) CONTROL PLASMA AND (B) DONOR PLASMA

Case No.	Normal Subjects		Megaloblastic Folate Deficiency		Megaloblastic Vitamin B ₁₂ Deficiency		Rheumatoid Arthritis	
	A	B	A	B	A	B	A	B
1	228	230	36	35	166	159	120	105
2	184	183	37	25	163	147	183	160
3	164	155	112	105	108	103	103	111
4	245	279	50	43	247	250	209	198
5	156	145	37	38	171	168	121	112
6	160	143	32	34	204	181	88	70
7	178	161	30	24	49	45	194	166
8	149	143	98	83	148	146	290	305
9	195	190	24	23	213	202	282	255
10	147	140	61	60	98	88	199	177
11	170	185	32	31	151	155	225	179
12	190	186	42	40	88	79	251	215
13	185	164	99	98	130	150	189	150
14	199	170	53	55	188	179	109	117
15	150	162	24	26	61	57	175	130
16	159	177	51	54	105	99	109	91
17	175	186	40	43	148	153	163	131
18	166	167	130	132	104	115	136	118
19	175	184	23	26	50	54	163	164
20	151	152					172	169
21	225	195					208	188
22	168	152					259	259
23	146	147					135	129
24	170	167					304	263
25	239	235					208	188
26	269	231						
27	200	176						
28	238	240						
29	139	138						
30	186	204						
31	152	174						
32	168	186						
Mean	182	179	53	51	137	134	183	166
SD $\pm$	34	34	32	31	56	55	61	59
P	0.8		0.1		0.1 > 0.2		< 0.001	

Assayable folate is released from erythrocytes by the interaction of erythrocyte folate precursors and a plasma factor (Toennies and others, 1956). These findings indicate that the low erythrocyte folate levels resulting from the use of whole blood from some patients with rheumatoid arthritis may indicate a reduced plasma factor activity rather than a decrease in the erythrocyte folate precursors. Using a different technique, a similar reduction in plasma factor activity in the megaloblastic anaemia of pregnancy has been reported by Grzesiukowicz, Jennison, and Gowenlock (1965).

Folate deficiency in terms of a low serum folate and occasional megaloblastic anaemia has been reported in patients with rheumatoid arthritis (Gough, McCarthy, Read, Mollin, and Waters, 1964; Dellar, Urban, Ibbotson, Horwood, Milazzo, and Robson, 1966). These findings were confirmed by Omer and Mowat (1968), who also showed a true erythrocyte folate deficiency in 37 per cent. of their patients using the method described in this paper.

The relationship of the reduced plasma factor activity to these findings is uncertain. The bulk of folate in the body is stored in erythrocytes as folate precursors. On release from effete erythrocytes the polyglutamic form is converted to a monoglutamic form, 5-methyl-tetrahydrofolic acid, by the action of plasma factor. Plasma folate activity is dependent chiefly and perhaps entirely on the monoglutamic form (Herbert, Larrabee, and Buchanan, 1962).

Grzesiukowicz and others (1965) suggested that the reduced plasma factor activity in the megaloblastic anaemia of pregnancy may lead to poorer utilization of the erythrocyte precursors, resulting in less folate being available in the circulation and contributing to the folate deficiency state. The presence of a reduced red cell mass in these patients could imply that the body stores of folate are depleted and that hence less is available for release by plasma factor. This would add further to the folate deficiency. The reduced plasma factor activity may also explain the altered erythrocyte/plasma folate

ratio of 72 : 1 in patients with rheumatoid arthritis compared with a normal ratio of 42 : 1 reported by Omer and Mowat (1968).

It is considered essential, therefore, when investigating the erythrocyte folate status of patients with possible folate deficiency states, to use a standard normal plasma against the patients' erythrocytes for a true estimate of the erythrocyte folate values as described in this paper.

Summary

(1) The release of folate by control and donor plasma from the donor's erythrocytes was compared in four groups of subjects.

(2) The release of erythrocyte folate by control and donor plasma was not significantly different in normal individuals and in patients with megaloblastic anaemia due to either folate or vitamin B₁₂ deficiency.

(3) The release of folate from the erythrocytes of patients with rheumatoid arthritis by autologous plasma was significantly less than the release by control plasma.

(4) The abnormality in plasma factor activity which occurs in patients with rheumatoid arthritis has been discussed.

(5) It is suggested that a standard normal plasma be used in the investigation of possible erythrocyte folate deficiency states, and a method is described.

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La nature de l'anémie dans la polyarthrite rhumatoïde

RÉSUMÉ

(1) La libération du folate érythrocytaire du donneur par le plasma des donneurs et des témoins a été comparée dans quatre groupes de sujets.

(2) La libération du folate érythrocytaire par le plasma des témoins et des donneurs n'était pas appréciablement différente chez les sujets normaux et chez les malades atteints d'anémie mégalo-blastique due à une carence de vitamine B₁₂ ou d'acide folique.

(3) La libération du folate érythrocytaire par le plasma autologue des cas de polyarthrite rhumatoïde était significativement plus faible que sa libération par le plasma des témoins.

(4) On discute l'anomalie de l'action du facteur plasmatique se manifestant chez les malades atteints de polyarthrite rhumatoïde.

(5) On recommande l'emploi d'un plasma normal standard lorsqu'on recherche une insuffisance d'acide folique dans les globules rouges et on décrit une méthode à cet effet.

Naturaleza de la anemia en la poliartritis reumatoide

SUMARIO

(1) La liberación de folato de eritrocitos por el plasma de control y de donante fue comparada en cuatro grupos de sujetos.

(2) La liberación de folato de eritrocitos por plasma de control y de donante no resultó significativamente distinta en individuos sanos y en pacientes con anemia megaloblástica debida a insuficiencia de vitamina B₁₂ o de ácido fólico.

(3) La liberación de folato por el plasma autólogo de pacientes con poliartritis reumatoide fue significativamente menor que la liberación por el plasma de control.

(4) Se discute la actividad anormal del factor plasmático que ocurre en pacientes con poliartritis reumatoide.

(5) Se sugiere el empleo de un plasma standard normal en la investigación de posibles estados de deficiencia de folato eritrocítico y se describe un método.