

Serum and erythrocyte folates in combined iron and folate deficiency^{1, 2}

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ABSTRACT A high incidence of iron and folate deficiency was found in 80 female subjects living in a private institution. Iron therapy in individuals with low serum iron values resulted in a significant increase in hemoglobin levels. An improvement in serum and RBC folate levels was also found following iron therapy but this could not be attributed to treatment since a similar increase was observed in untreated control subjects, probably due to an increased dietary intake of folates during the study period. In subsequent studies small amounts of pteroylglutamic acid were given to all patients and their response to therapy was related to initial serum and RBC folate values. No correlation between serum folate levels and response to folate therapy could be demonstrated. Red cell folate levels on the other hand correlated well with response to therapy. A significant increase in hemoglobin was found following folate therapy in patients with low RBC folates, but no increase in subjects with normal RBC folates. Conversely, the increase in hemoglobin following iron therapy in subjects with normal RBC folates was three times as much as in patients with low RBC folates. Thus, unlike serum folate determinations, RBC folate measurements are a reliable index of tissue folate stores, and useful in the prediction of response to folate therapy in both the iron-deficient and iron-replete states. *Am. J. Clin. Nutr.* 28: 1217-1222, 1975.

The correlation of folate measurements with hemoglobin levels in nutritional anemia due to combined deficiency is poor (1, 2). It has been suggested that iron deficiency might influence the distribution of folates between stores and plasma and thus complicate the interpretation of serum and red cell folate measurements (3-6). The present study was undertaken following the admission to the hospital of a patient from a private institution for chronic mental disease with megaloblastic anemia due to severe folate deficiency. Examinations performed on 80 patients living in the same institution disclosed a high incidence of both iron deficiency and low serum folate levels. In subsequent studies the effect of iron therapy on serum and red cell folates was examined in treated and untreated control subjects. The effect of folate therapy on subjects with different levels of serum and red cell folates was also studied to determine the predictive value of initial folate measurements in the response of patients to this therapy.

Patients and methods

The study was performed on a group of 95 female patients with chronic mental disease living in a private institution in Jerusalem. Their ages ranged from 28 to 76,

with a mean of 53 years. Eighty-three were schizophrenic, 8 mentally retarded, 2 had senile dementia, and 2 were epileptics. All patients were on permanent medication including various phenothiazine derivatives, amitriptyline and imipramine. The two epileptic patients received primidone and phenobarbital. They all shared a common dining room and were all supplied with the same diet. The study was started in June 1974 and terminated in September 1974.

Hemoglobin (Hb), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were determined by a Model S Coulter counter (Coulter Electronics, Fla.) calibrated daily using the 4C Reference Control supplied by the company. Serum iron and total iron binding capacity (TIBC) were determined by the ICSH panel method (7). Whole blood and serum folate, and serum vitamin B₁₂ were determined by microbiological assays (8, 9). Erythrocyte folate was calculated from whole blood folate using the formula of Cooper and Lowenstein (10).

Results

The first patient to be studied in the group reported was a 75-year-old woman admitted

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to the Hadassah University Hospital because of megaloblastic anemia. Since the findings suggested reduced folate intake as the cause of anemia, a survey of the patient population in her institution was undertaken.

Eighty of the 95 patients residing in the institution have been examined. The initial hemoglobin of the patients was 13.0 ± 0.2 g/dl (mean ± 1 SE), the MCV $86.7 \pm 0.8 \mu^3$, the MCH 29.7 ± 0.05 pg, the serum iron $84 \pm 3 \mu\text{g/dl}$, the TIBC $305 \pm 9 \mu\text{g/dl}$, and the serum vitamin B₁₂ 414 ± 30 pg/ml. The serum folic acid was 3.08 ± 0.17 ng/ml and the RBC folate 366.2 ± 15.4 ng/ml. The frequency distribution of these two measurements is given in Fig. 1. The peak incidence of serum folate in patients was between 2 to 3 ng/ml and 45 of the 75 measurements were below 3 ng/ml. In comparison, the mean serum folate in a group of 50 normal blood donors consisting of 32 males and 18 females (mean age 33 and 29 years, respectively) was 6.87 ± 0.63 ng/ml ($P < 0.001$) with a peak incidence between 6 to 7 ng/ml, and none of the 50 determinations were less than 3 ng/ml. The mean RBC folate in normal donors was 473 ± 26 ng/ml, significantly higher than in patients ($P < 0.001$), but the peak incidence

was similar in both groups, between 300 and 400 ng/ml. Twenty six of the 77 patients and 9 of 50 normals had RBC folates of 300 ng/ml or less. Throughout the duration of the present study no consistent changes in serum vitamin B₁₂ were observed, and the final measurement of 468 ± 33 pg/ml was not significantly different from the initial values.

Results of iron therapy

Serum iron levels of $70 \mu\text{g/dl}$ or less and a transferrin iron saturation below 18% (mean 15.9 ± 0.7) were found in 25 of the 80 patients. The mean Hb level of this group was 11.3 ± 0.3 g/dl (Table 1), about 2 g less than the entire group of 80 patients ($P < 0.001$). The MCV and MCH of the entire group with low serum iron were only slightly reduced, but in the 4 patients with Hb below 10 g/dl the MCV was $74.2 \pm 5.1 \mu^3$, and the MCH 22.4 ± 2.8 pg, significantly lower than the entire group of 80 patients ($P < 0.01$).

Oral iron therapy in the form of ferrous sulfate tablets twice daily, providing 120 mg elemental iron was given continuously for 6 weeks to the 25 patients with low serum iron. At the end of this period all examinations were repeated in the same patients. The hemoglobin level of the group rose from 11.3 to 13.5 g/dl ($P < 0.001$) and the serum iron level increased from 59 to 115 $\mu\text{g/dl}$. A slight increase in serum folate ($P < 0.05$) and RBC folate was also found following iron therapy.

Another group of 16 patients with normal serum iron was examined simultaneously to serve as untreated controls. No significant changes in Hb, red cell indices or serum iron were found in this group during the 6-week study period. However, similar to the treated iron-deficient group a significant increase in serum folate and RBC folate ($P < 0.02$) was also found in this untreated control group.

To establish whether the response to iron therapy was modified by a coexistent folate deficiency, the increment in hemoglobin of the iron-treated patients was related to their initial serum and RBC folate levels (Table 2). Patients with an RBC folate of 300 ng/ml or less, which is more than 1 SD below the mean of normal controls had an increment in Hb that was only one-third of the response to iron therapy of patients with 500 ng or more RBC folate ($P < 0.025$). In contrast, no such

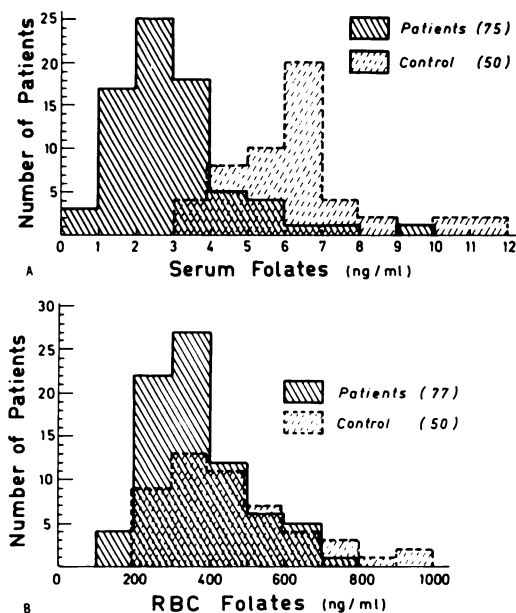


FIG. 1. A: distribution of serum folate measurements in patients, and in a group of normal blood donors (control). B: distribution of RBC folate measurements in patients and in a group of normal blood donors (control).

relation to serum folate was found, and the response of patients with a serum folate of 3 ng/ml or less did not differ significantly from the response of patients with higher serum folate levels.

Results of folate therapy

Thirty-six patients were given pteroylglutamic acid in a daily dose of 150 μ g for 10 consecutive days. Ten of these patients have been treated previously with iron in the first part of the study, and the rest were untreated patients with normal serum iron. The mean Hb level of this group was 13.5 ± 0.2 g/dl at the outset and 13.8 ± 0.2 g/dl at the end of folate treatment (Table 3). Their MCV, MCH and serum iron were within normal limits. None of these mean values changed significantly following folate administration. On the other hand, serum folate levels were doubled from a mean of 3.0 to 6.0 ng/ml ($P < 0.001$). A slight increase in RBC folate from 389 to 425 ng/ml was also found

following folate therapy, but this was not statistically significant.

As in the case of iron therapy, results of folate therapy were also correlated with initial serum and RBC folate measurements (Table 4). The increment in hemoglobin within 10 days in patients with RBC folate of 300 ng or less was 0.83 g as compared to 0.08 in patients with more than 500 ng RBC folate ($P < 0.025$). Serum folate levels on the other hand had no predictive value in the response to folate therapy; patients with a serum folate of 3 ng/ml or less had a Hb increment of 0.30 g as compared to 0.48 in patients with higher serum folate levels (NS). Response to folate therapy was modified by coexistent iron requirements as shown by the failure of patients with a decreasing serum iron to improve their Hb level as compared with an increment of 0.65 g/dl in patients in whom serum iron increased within the 10-day period ($P < 0.01$). In the ten patients who received iron therapy in the first part of the study, folate

TABLE 1
Effects of iron therapy

	Time, days	No.	Hemoglobin, g/dl	MCV, μ^2	MCH, pg	Serum iron, μ g/dl	Serum folate, ng/ml	RBC folate, ng/ml
Iron treatment	0	25	11.31 ± 0.34^a	86.9 ± 1.9	27.6 ± 0.8	59.2 ± 2.7	3.04 ± 0.21	443.1 ± 44.5
	42	25	13.52 ± 0.16	90.2 ± 1.0	29.5 ± 0.5	115.4 ± 7.2	3.99 ± 0.37	498.3 ± 44.1
Untreated control	0	16	12.98 ± 0.34	89.2 ± 1.3	29.4 ± 0.9	97.1 ± 4.8	2.84 ± 0.20	330.3 ± 29.9
	42	16	13.29 ± 0.31	92.1 ± 0.8	30.3 ± 0.4	116.9 ± 9.7	4.68 ± 1.07	445.7 ± 40.2

^a Mean \pm 1 SE.

TABLE 2
Folate levels and response to iron therapy

Initial RBC folate, ng/ml	No.	<300	No.	>500	
Δ Hb, g/dl	6	1.21 ± 0.24^a	5	3.58 ± 0.95	$P < 0.025$
Initial serum folate, ng/ml	No.	<3	No.	>3	
Δ Hb, g/dl	15	2.04 ± 0.46	10	1.36 ± 0.33	P NS

^a Mean \pm 1 SE.

TABLE 3
Effects of folate therapy

Time, days	No.	Hemoglobin, g/dl	MCV, μ^2	MCH, pg	Serum iron, μ g/dl	Serum folate, ng/ml	RBC folate, ng/ml
0	30	13.45 ± 0.20^a	91.7 ± 0.9	29.8 ± 0.5	109.0 ± 6.1	3.04 ± 0.25	388.7 ± 24.6
10	36	13.84 ± 0.23	93.4 ± 1.0	30.9 ± 0.3	101.5 ± 4.6	5.98 ± 0.42	425.1 ± 24.6

^a Mean \pm 1 SE.

TABLE 4
Factors affecting response to folate therapy

Initial RBC folate, ng/ml Δ Hb, g/dl	No. (8)	< 300 0.83 ± 0.30^a	No. (6)	> 500 0.08 ± 0.21	$P < 0.025$
Initial serum folate, ng/ml Δ Hb, g/dl	No. (20)	< 3 0.30 ± 0.19	(4)	> 3 0.48 ± 0.22	P NS
Final serum iron, μ g/dl Δ Hb, g/dl	No. (19)	decreasing 0.02 ± 0.15	No. (16)	increasing 0.65 ± 0.19	$P < 0.01$

^a Mean \pm 1 SE.

treatment resulted in an additional increase of 0.54 g hemoglobin/dl.

Discussion

Serum and erythrocyte folate determinations are commonly used indicators of folate status in nutritional deficiency. In combined iron, folate, and vitamin B₁₂ deficiencies, however, deviations from normal of serum and erythrocyte folates are often dissociated. Thus, in vitamin B₁₂ deficiency increased serum folate and reduced RBC folate levels were observed (10, 11). Conversely, in iron deficiency increased RBC folates (3) as well as reduced serum folate levels have been reported (4-6). The effect of iron deficiency on folate metabolism is poorly understood and the data reported are contradictory. In iron deficiency a megaloblastic marrow, as well as hypersegmentation of neutrophilic granulocytes attributed to secondary folate deficiency were noted by several investigators (2, 12, 13). However, hypersegmentation is poorly correlated with megaloblastic changes in the marrow, or low blood folate measurements (14) and claims for a low serum folate in man (4, 5) and rats (6, 15) with iron deficiency were not confirmed by others (3, 16-18). A reduced activity of hepatic glutamate formyltransferase in iron-deficient rats was reported by Vitale et al. (15, 19), but this was not confirmed in a later study by Burns and Spray (17). Likewise, iron therapy was claimed to result in an increase in serum folates by some (3, 4, 6), a reduction by others (16), or no effect at all (2, 18). Some of these discrepancies might be explained by the scarcity of simultaneous studies of untreated control patients to exclude variations in dietary folate intake and other factors unrelated to iron therapy.


In the present study repeated examinations were performed on a group of patients some of whom were first treated with iron and then with pteroylglutamic acid. The existence of significant iron deficiency in part of the patient population is proved by the correction of anemia by iron therapy, whereas no change in hemoglobin levels was observed in the untreated control group throughout the same period. Taking 3 ng/ml as the lower limit of normal (20) the majority of iron-deficient patients had subnormal serum folate levels which increased moderately following iron therapy. However, these findings cannot be taken as confirmation of a cause and effect relation between iron deficiency and low serum folate levels, since the presence of even lower initial serum folate levels in the untreated control group, and the subsequent spontaneous increase in serum folate in this group indicate that the improvement was unrelated to iron therapy and is most probably explained by an increased dietary intake of folates during the ensuing 6 weeks' study period. This conclusion is supported by a simultaneous increase in erythrocyte folates in both the iron-treated and control groups reflecting a genuine improvement in folate status rather than redistribution of stores.

The diagnostic value of serum and RBC folate measurements was examined in the present study by their correlation with response to therapy. It was assumed that subjects with sufficient folate stores would respond better to iron therapy than those with significant folate deficiency and conversely, that folate therapy would be more effective in folate-deficient patients than in those with normal folates. Results of both studies have clearly demonstrated that erythrocyte folate measurements were a better indicator of



folate status than serum folate. No correlation between serum folate levels and response to folate or iron therapy could be demonstrated. Red cell folate levels on the other hand correlated well with response to therapy. A significant increase in hemoglobin was found following folate therapy in patients with low RBC folates, but no increase in subjects with normal RBC folates. Conversely, the increase in hemoglobin following iron therapy in subjects with normal RBC folates was three times as much as in patients with low RBC folates.

In patients with low RBC folates whose response to iron therapy was limited, a further increase in hemoglobin levels was elicited by additional folate therapy. This positive response indicated that folate and iron deficiency in the population studied were not causally related, and reinforced the suggestion that in groups with a high incidence of folate and iron deficiency, both hematinics should be supplied in order to prevent anemia (21).

The advantage of RBC folate measurements over those of serum folate in the diagnosis of folate deficiency is explained by their relative rates of change with subnormal dietary intake. Low folate intake is followed by a rapid fall of serum folate within a few weeks, but many weeks are needed for a similar reduction of RBC folate (22). Thus, low serum folate may indicate a negative folate balance caused by a temporary reduction of intake or increased folate requirements, but it is of limited value in the diagnosis of depleted folate stores (23). As shown in the present study, the folate content of erythrocytes is unaffected by coexistent iron deficiency and thus, its measurement remains a useful clinical aid in predicting response and determining indications for folate treatment in both the iron-deficient and iron-replete states. 

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References

1. COOK, J. D., J. ALVARADO, A. GUTNISKY, M. JAMRA, J. LABARDINI, M. LAYRISSE, J. LINARES, A. LORIA, V. MASPE, A. RESTREPO, C. REYNARFARJE, L. SANCHEZ-MEDAL, H. VELEZ AND F. VITERI. Nutritional deficiency and anemia in Latin America: A collaborative study. *Blood* 38: 591, 1971.
2. VELEZ, H., A. RESTREPO, J. J. VITALE AND E. E. HELLERSTEIN. Folic acid deficiency secondary to iron deficiency in man. Remission with iron therapy and a diet low in folic acid. *Am. J. Clin. Nutr.* 19: 27, 1966.
3. OMER, A., N. D. C. FINLAYSON, D. J. C. SHEARMAN, R. R. SAMSON AND R. H. GIRDWOOD. Plasma and erythrocyte folate in iron deficiency and folate deficiency. *Blood* 35: 821, 1970.
4. MICHOT, P. Das Verhalten der Serum folsäure beim blutungsbedingten Eisenmangel. *Schweiz. Med. Wochschr.* 100: 1971, 1970.
5. SARAYA, A. K., B. N. TANDON AND K. RAMACHANDRAN. Folic acid deficiency: Effects of iron deficiency on serum folic acid levels. *Indian J. Med. Res.* 59: 1796, 1971.
6. TOSKES, P. P., G. W. SMITH, T. A. BENSINGER, R. A. GIANELLA AND M. E. CONRAD. Folic acid abnormalities in iron deficiency: the mechanism of decreased serum folate levels in rats. *Am. J. Clin. Nutr.* 27: 355, 1974.
7. International Committee for Standardization in Hematology—Expert panel on iron. Proposed recommendations for measurement of serum iron in human blood. *Brit. J. Haematol.* 20: 451, 1971.
8. GROSSOWICZ, N., F. MANDELBAUM-SHAVIT, R. DAVIDOFF AND J. ARONOVITCH. Microbiologic determination of folic acid derivatives in blood. *Blood* 20: 609, 1962.
9. GROSSOWICZ, N., J. ARONOVITCH AND M. RACHMILEWITZ. Determination of vitamin B₁₂ in human serum by a mutant of *Escherichia coli*. *Proc. Soc. Exptl. Biol. Med.* 87: 513, 1954.
10. COOPER, B. A., AND LOWENSTEIN. Relative folate deficiency of erythrocytes in pernicious anemia and its correction with cyanocobalamin. *Blood* 24: 502, 1964.
11. HERBERT, V., AND R. ZALUSKY. Interrelation of vitamin B₁₂ and folic acid metabolism. Folic acid clearance studies. *J. Clin. Invest.* 41: 1263, 1962.
12. CHANARIN, I., D. ROTHMAN AND V. BERRY. Iron deficiency and its relation to folic-acid status in pregnancy: results of a clinical trial. *Brit. Med. J.* 1: 480, 1965.
13. BEARD, M. E. J., AND L. R. WEINTRAUB. Hypersegmented neutrophilic granulocytes in iron deficiency anemia. *Brit. J. Haematol.* 16: 161, 1969.
14. LOWENSTEIN, L., G. CANTLIE, O. RAMOS AND L. BRUNTON. The incidence and prevention of folate deficiency in a pregnant clinic population. *Can. Med. Assoc. J.* 95: 797, 1966.
15. VITALE, J. J., A. RESTREPO, H. VELEZ, J. B. RIKER AND E. E. HELLERSTEIN. Secondary folate deficiency induced in the rat by dietary iron deficiency. *J. Nutr.* 88: 315, 1966.
16. ROBERTS, P. D., D. J. B. ST. JOHN, R. SIMHA, J. S. STEWART, I. M. BAIRD, N. E. COGHILL AND J. O. MORGAN. Apparent folate deficiency in iron-deficiency anemia. *Brit. J. Haematol.* 20: 165, 1971.
17. BURNS, D. G., AND G. H. SPRAY. Normal folic acid metabolism in iron-deficient rats. *Brit. J. Nutr.* 23: 665, 1969.

18. SARAYA, A. K., V. P. CHOUDHRY AND O. P. GHAI. Interrelationships of vitamin B₁₂, folic acid, and iron in anemia of infancy and childhood; effect of vitamin B₁₂ and iron therapy on folate metabolism. *Am. J. Clin. Nutr.* 26: 640, 1973.
19. VITALE, J. J., R. R. STREIFF AND E. E. HELLERSTEIN. Folate metabolism and iron deficiency. *Lancet* 2: 393, 1965.
20. WHO Scientific group. Nutritional anemias. *Tech. Rept. Ser.* 405: 9, 1968.
21. IZAK, G., S. LEVY, M. RACHMILEWITZ AND N. GROSSOWICZ. The effect of iron and folic acid therapy on combined iron and folate deficiency anaemia: The results of a clinical trial. *Scand. J. Haematol.* 11: 236, 1973.
22. HERBERT, V. Experimental nutritional folate deficiency in man. *Trans. Assoc. Am. Physicians* 75: 307, 1962.
23. CHANARIN, I. *The Megaloblastic Anaemias*. Oxford and Edinburgh: Blackwell, 1969, p. 324.

