Folic Acid Metabolites in Whole Blood and Serum in Anemia of Pregnancy

G. Izak, M.D.,* M. Rachmilewitz, M.D.,† A. Sadovsky, M.D.,‡ B. Bercovici, M.D.,§
J. Aronovitch, M.Sc.,‖ and N. Grossowicz, Ph.D.¶

Anemia of pregnancy is prevalent in tropical[1,2] as well as nontropical countries.[3,4] The mechanism responsible for the development of this anemia is not clearly understood despite the numerous studies reported in recent years.[3–6] The classification of this anemia is usually based on morphologic criteria and the response to treatment. The development in recent years of microbiologic assays for the quantitative determination of substances necessary for normal hematopoiesis permits a new appraisal of these anemias. The microbiologic assays for vitamin B_{12} activity in body fluids have contributed to the understanding of the role of this vitamin in anemia of pregnancy. Few data, however, exist concerning the role of folic acid in anemia of pregnancy.

This report deals with folic and folinic acid determinations in whole blood and serum of anemic pregnant women, using three microbiologic assays simultaneously.

MATERIAL AND METHODS

Fifteen hundred women underwent hematologic examination at the time of delivery in the Obstetrics Department of the Hadassah University Hospital. They ranged in age from sixteen to forty-two years. Forty-three per cent were multipara women with six or more previous pregnancies, 24 per cent with two to four previous pregnancies, 26 per cent with one to two previous pregnancies and 7 per cent were primipara. The majority of these women (78 per cent) came to Israel from Middle Eastern§§ and Mediterranean†† countries, while 22 per cent originated from Central and Eastern Europe.

Among the 1,500 women examined, 420 (28 per cent) were found to be anemic at the time of delivery (i.e., hemoglobin levels below 10 gm. per 100 ml.). In sixty-four of these, detailed folic acid studies were also carried out, using three different assays for the determination of the various forms of the folic acid group in whole blood and serum. A group of forty-three healthy subjects served as controls.

In anemic women the serum concentration of vitamin B_{12} was determined as well as a complete blood count including hemoglobin (cyanmethemoglobin), red cell, reticulocyte, platelet, white cell counts and hematocrit, according to standard procedures. Serum iron and unsaturated iron binding capacity were determined according to the method of Davis et al.[7] Serum vitamin B_{12} values were determined microbiologically, using a mutant strain of Escherichia coli as the test organism.[8] Whole blood and serum folic and folinic acid (citrovorum factor) were determined microbiologically. Lactobacillus casei was used for the determination of conjugated and free folic and folinic acids (which will be referred

From the Departments of Medicine B, Obstetrics and Gynecology, Hadassah University Hospital, and the Department of Bacteriology, Hebrew University-Hadassah Medical School, Jerusalem, Israel.

* Lecturer in Medicine; † Professor of Medicine; ‡ Associate Professor of Obstetrics and Gynecology; § Instructor in Obstetrics and Gynecology; ‖ Research Assistant, Department of Bacteriology; ¶ Associate Professor of Bacteriology.

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§§ Syria, Iraq, Lebanon, Iran, Yemen.
†† Tunis, Algeria, Morocco.

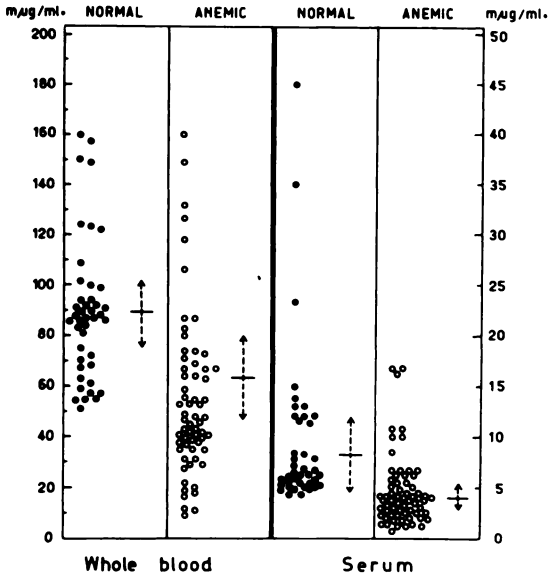

FIG. 1. Whole blood and serum total folic acid values in normal subjects and in anemic pregnant women.

to as "total folic acid"). Streptococcus fecalis was used for the determination of free folic and folinic acids and Pediococcus cerevisiae (Leuconostoc citrovorum) was employed to determine the free and conjugated folinic acid (citrovorum factor). The methods of the assay methods used are modifications of the procedures described by Toennies et al.[9] and will be published separately.[10]

RESULTS

The serum iron levels were found to be low, between 0 and 59 μg. per 100 ml., in fifty-two of the sixty-four patients (about 80 per cent) included in this study. The unsaturated iron binding capacity in these patients ranged from 290 to 476 μg. per 100 ml.

In thirty-six patients (about 50 per cent) the serum vitamin B_{12} level was found to be below 200 μμg. per ml. as compared to the normal average of 350 μμg. per ml. The serum vitamin B_{12} values ranged from 50 to 100 μμg. per ml. in fourteen of these patients, 100 to 150 μμg. per ml. in eleven and 150 to 200 μμg. per ml. in the remainder.

Folic Acid Studies

Normal Values. As seen from Table I the highest folic acid values were obtained in the whole blood of normal subjects when the microorganism used for the determination was L. casei (mean value 89 mμg. per ml.), as this organism utilizes in addition to pteroyl glutamic acid and folinic acid, di- and triglutamates of pteroyl glutamic acid (conjugated forms of folic acid). Much lower values (mean value 12 mμg. per ml.) were obtained with Strep. fecalis, as this organism responds to pteroylglutamic acid and folinic acids, whereas it cannot utilize pteroylglutamic acid conjugates. The values obtained with L. citrovorum, which measures only folinic acid and its conjugates, were about half of those found with Strep. fecalis (mean value 6.34 mμg. per ml.).

It can be seen that the bulk of folic acid is found in the erythrocyte. Employing L.

TABLE I

Mean Normal Folic and Folinic Acid Values in Normal Subjects and in Women Found Anemic at Time of Delivery

Subjects	Total Folic Acid (L. casei) (mμg./ml.)				Pteroylglutamic Acid and Folinic Acid (Strep. fecalis) (mμg./ml.)				Folinic Acid (L. citrovorum) (mμg./ml.)			
	Blood	Serum	Blood	Serum	Blood	Serum	Blood	Serum	Blood	Serum	Blood	Serum
	Mean	S.D ±	Mean	S.D. ±	Mean	S.D. ±	Mean	S.D. ±	Mean	S.D ±	Mean	S.D. ±
43 normal subjects.	89.0	13.4	8.26	3.51	12.0	7.31	0.75	0.60	6.34	4.83	0.30	0.22
64 anemic women..	62.4	16.2	4.16	1.14	9.7	8.87	0.70	0.89	5.2	4.99	0.35	0.24

casei the whole blood contained 89 mμg. per ml. folic acid, while less than 10 per cent of this amount was found in the serum with the same microorganism. An even higher proportion of intraerythrocytic folic acid was found with the other two microorganisms, where the ratio of whole blood folic acid over the serum folic acid amounted to about 20.

Folic Acid Values in Anemic Pregnant Women. Total folic acid values in whole blood and serum are significantly lower in this group than those of the control subjects (mean value 62.4 mμg. per ml. for whole blood and 4.16 mμg. per ml. for serum) (Fig. 1). This difference was not apparent when the organisms used in the assay procedures were Strep. fecalis or L. citrovorum (Table I). The whole blood and serum total folic acid values varied widely in the group with anemia, hence the relatively high calculated mean value. In thirty-five of the sixty-four anemic women (about 50 per cent) whole blood and serum total folic acid values were very low and did not reach the lowest values obtained in any of the normal control subjects (i.e., 50 mμg. per ml.). Of these thirty-five women, seven had whole blood total folic acid values below 20 mμg. per ml., fifteen had values below 40 mμg. per ml. and thirteen below 50 mμg. per ml. Correspondingly low values were found in the serum of these women.

As can be seen from Table II, the simultaneous finding of low blood and serum total folic acid, low serum iron and low vitamin B_{12} was frequently encountered. In fourteen women these values were definitely below normal. In some patients only serum vitamin B_{12} and serum iron values were low, whereas folic acid levels were normal. The most frequent single deficiency was that of iron, encountered in thirteen women, whereas low values of folic acid only or vitamin B_{12} only were found in a few instances.

TABLE II

The Distribution of "Total Folic Acid," Serum Iron and Serum Vitamin B_{12} Levels in Sixty-Four Pregnant Anemic Women

Women (no.)	Total Folic Acid (mμg./ml.)	Serum Iron (μg./per cent)	Serum Vitamin B_{12} (μμg./ml.)
14	9.6– 48.0	13- 52	50–185
6	39.0– 49.0	66–109	50–200
13	12.0– 48.0	0- 49	248-450
12	74.0–132.0	10- 52	50–200
13	79.0–149.0	20- 47	200–421
4	81.0–106.0	70–109	50–145
2	11.0– 28.0	65–110	260–390

COMMENTS

The data presented here indicate that significantly low total folic acid values, as determined by L. casei, were found in whole blood as well as in serum in about 50 per cent of women found anemic at the time of delivery. These low folic acid values were frequently associated with low serum iron and/or serum vitamin B_{12} concentrations. No correlation was evident between the decrease in these hematinics in the blood and the type of anemia. A characteristic hyperchromic macrocytic anemia was found in only seven of these patients. In the majority (56 per cent), however, a dimorphic (macrocytic, hypochromic) type of anemia was seen. This lack of correlation is not surprising in view of the frequent association of several deficiencies, and corroborates our findings on the combined vitamin B_{12} and iron deficiencies in anemia of pregnancy, in which the presence of iron deficiency masks the morphologic characteristics of vitamin B_{12} deficiency.[11] The examination of the bone marrow also fails to supply definite criteria for the classification of these anemias. In patients with very low folic acid or vitamin B_{12} concentrations megaloblasts, when present, were found only in small numbers and at times were atypical (so-called transitional megaloblasts[12]), not justifying the widely used term megaloblastic anemia.

The frequent development of iron deficiency in pregnancy is a universal finding. The factors responsible for the depletion of vitamin B_{12} in pregnant women have been studied in recent years[11,13,14] and it has been established that various degrees of depletion are more frequent than was considered on the basis of morphologic criteria. The so-called megaloblastic anemia of pregnancy is known to re-

spond more readily to treatment with folic acid than to vitamin B_{12}.[15,16] The reason for this observation is not clear. Folic acid deficiency in anemia of pregnancy as manifested by low blood folic acid values on direct determinations has been reported in only a few instances.[17,18] This may be due to the fact that in these studies[17,18] only serum folic acid was determined. However, Toennies et al.[9] and our own findings showed that the bulk of folic acid is present in the red cells and only about 10 per cent of this vitamin is found in the serum. It is not surprising, therefore, that low folic acid values were not found to be associated with clinically suspected folic acid deficiency states, particularly with anemia of pregnancy. Indirect methods to detect folic acid deficiency were developed by Girdwood[19] and later by Chanarin et al.[20] These methods are based on diminished excretion of folic acid in the urine following an oral load and rapid clearance of the vitamin from the serum following its intravenous injection to folic acid-deficient patients as compared to normal control subjects. Using the latter procedure, Chanarin and associates[20] concluded that anemia of pregnancy is frequently associated with folic acid deficiency.

Baker,[21] Herbert and their associates[22] recently reported results obtained on folic acid determinations in the serum using L. casei. The normal range found by them was 7.5 to 23 mμg. per ml. This range is comparable to that obtained in our laboratory, which was 4.5 to 24 mμg. per ml. serum. The same authors found low serum values of both vitamin B_{12} and folic acid in two patients with megaloblastic anemia of pregnancy.[23]

As to the cause of folic acid deficiency, it is difficult to believe that only insufficient intake is responsible for the development of folic acid deficiency in anemic women, in view of the abundance of foodstuffs rich in folic acid (fresh vegetables and citrus fruit) or in factors activating folic acid in this country. In search of another or an additional cause, folic and folinic acid values were compared in paired fetal and maternal blood samples and it was found that fetal blood contains eight times more folinic acid (citrovorum factor) than its maternal counterpart.[24] It seems of interest that while the difference was found to be marked between maternal and fetal folinic acid values, the total folic acid in fetal blood was only about twice that of the maternal blood. This finding together with the decrease in whole blood total folic acid values in anemic pregnant women suggests that conjugated folic acid may be mobilized from maternal red cells finding its way through the placenta to the fetus where it is found mainly in its metabolically active forms.[24] It seems reasonable to conclude, therefore, that large fetal demand is mainly responsible for a relative folic acid deficiency in the mother, thus contributing to the development of anemia of pregnancy. Baker et al.[23] in their last report arrived at similar conclusions which are based on serum folic acid determinations of blood from a group of pregnant women and an unmatched group of cord blood samples.

SUMMARY

Folic acid determinations were carried out on whole blood and serum by means of three microbiologic assays. The total folic acid (conjugated and free pteroylglutamic acid and folinic acid) was determined by L. casei, free pteroylglutamic acid and folinic acid were determined by the use of Strep. fecalis, and P. cerevisiae was employed for the determination of folinic acid (citrovorum factor).

In sixty-four pregnant women with anemia, folic acid as well as serum iron and serum vitamin B_{12} levels were determined. In thirty-five of them, significantly low folic acid values were found in whole blood and serum when compared with values in normal subjects. Low folic acid values were frequently associated with low serum vitamin B_{12} and low serum iron concentration. The mechanism of the development of folic acid deficiency in pregnancy is discussed.

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