Human milk folate and folate status in lactating mothers and their infants[1-4]

Tsunenobu Tamura, M.D., Yohzo Yoshimura,[5] M.D., and Tsuneo Arakawa,[6] M.D.

ABSTRACT Plasma and red blood cell folate levels of healthy, well-nourished lactating mothers were measured. Folate levels in their breast-fed infants were significantly higher than in the mothers. No abnormal hematological findings were observed in either mothers or infants. Folate levels in breast milk and in the infants' plasma were significantly correlated. The mean breast-milk folate level was 141.4 ng/ml. The total daily folate intake for breast-fed infants was assessed at 14 to 25 μg/kg body weight. The plasma and red blood cell folate levels of the lactating mothers were significantly increased after oral administration of 1 mg pteroylmonoglutamate daily for 4 weeks. However, milk folate levels did not change. *Am. J. Clin. Nutr.* 33: 193–197, 1980.

Many studies have suggested that critical folate deficiencies occur in lactating mothers. Also, macrocytic anemia or megaloblastic anemia has been reported in malnourished lactating women (1–3). The folate deficiency of lactating mothers was successfully correlated by oral supplementation of folate (1). Metz (3) reported that the smallest dose of folate that produces hematological response in lactating mothers with folate deficiency is higher than that in nonlactating patients (3). In breast-fed infants, however, the incidence of megaloblastic anemia due to folate deficiency has been reported to be extremely rare. Folate levels in blood from breast-fed infants were higher than those in artifically-fed infants (4). This fact may indicate that breast milk is the best source of folate for young infants.

This study was carried out to investigate the effects of lactation on the folate status of lactating mothers and the relationship between folate content in mother's milk and blood folate levels of their breast-fed infants. The recommended allowances of folate in infants were estimated from their daily folate intake based on the milk folate levels and amount of milk consumed.

Materials and methods

Subjects

Healthy, breast-fed infants (3 to 25 weeks old, weighing 6.32 ± 1.09 kg; mean ±SD, n = 25) were chosen from babies receiving regular monthly checkups at the pediatric clinic of a hospital located in the northern part of Japan. Their mothers were apparently healthy and had no history of any serious diseases at the time of this study. All subjects belonged to the same socioeconomic group and were from one geographic area. The selection of the subjects was based on the agreement and cooperation of their mothers. After the purpose and methods of the present study were explained to the mothers, consent forms were obtained. During the study, mothers were asked to continue with their normal dietary habits. However, dietary histories were not taken.

Collection of samples

Blood samples were obtained from both mothers and infants in early afternoon (2:00 to 3:00 PM). Subsequently, milk samples (approximately 10 ml) were collected by manual expression directly into test tubes containing ascorbic acid (5 to 10 mg) and were stored at −20 C until assayed. Milk samples were collected just before the babies were fed. Ramasastri (5) reported that milk folate levels (*Lactobacillus casei*, ATCC 7469, activity without pteroyl-γ-polyglutamylhydrolase (folate conjugase)) did not change during the course of emptying of the mammary glands (5).

Folate assay

Folate levels were determined by microbiological assay using *L. casei* (6). Plasma folate levels were measured

[1] From the Department of Pediatrics, Tohoku University School of Medicine, Sendai, 980, Japan.

[2] Supported in part by grant from the United States-Japan Medical Cooperation Program.

[3] Presented at International Symposium on the Influence of Environmental and Host Factors on Nutritional Requirements for Health in Asian Countries, 1974, Mt. Fuji, Japan.

[4] Address reprint requests to: Dr. T. Tamura, TB 192, Section of Gastroenterology, Department of Internal Medicine, University of California, Davis, California 95616.

[5] Present address: Department of Pediatrics, Yamagata University, Yamagata, Japan [6] Professor Emeritus, Department of Pediatrics, Tohoku University School of Medicine.

by the method of Waters and Mollin (7). The red blood cell (RBC) folate determination was based on those of Hoffbrand et al. (8) and Bird et al. (9). Whole blood (1 ml) was mixed with distilled water (2 ml) and the hemolysate was diluted with 0.2 M sodium phosphate buffer pH 6.0 (3 ml) containing ascorbic acid (1 g/100 ml), and autoclaved at 121 C for 10 min. After cooling to room temperature, the contents were mixed and centrifuged. The clear supernatants were diluted with 0.05 M sodium phosphate buffer pH 6.1 containing ascorbic acid (0.1 g/100 ml) and treated with hog kidney conjugase (9). Milk folate levels were estimated by the method of Hurdle et al. (10) with slight modifications (11).

Folic acid administration

Sixteen lactating mothers were given folic acid (pteroylmonoglutamate, PteGlu, 1 mg per day) for 4 weeks after the collection of the first samples. The second samples were collected at the end of this administration period. Some mothers failed to take the folic acid tablets every day. However, the minimal amount of folic acid taken by any mother was 15 mg, and more than 90% of them ingested 23 to 28 mg folic acid in the 4-week period.

Results

In the present study, no abnormal hematological findings were observed in either lactating mothers or their infants (Table 1). As shown in Table 2, folate levels in the plasma and RBC of the mothers were within the normal range (8, 12). However, the plasma and RBC folate levels in their infants were much higher. Significant correlations between plasma and RBC folate levels were found in both mothers and infants with correlation coefficients of 0.69 ($P < 0.01$) and 0.48 ($P < 0.01$), respectively (Table 2).

In our study, we found that breast milk contained 141 ng/ml of "total folate" ranging from 62 to 280 ng/ml. "Free folate" levels, calculated at the half-maximal point of the *L. casei* response curve (6) accounted for approximately 40% of total folate in breast milk. This finding indicates that polyglutamate forms of folate derivatives are present in human milk.

Ramasastri (5) reported that folate levels in breast milk increased with the progress of lactation. In the present study, however, no significant changes were found in folate levels during lactation from 3 to 25 weeks after parturition.

Plasma folate levels in infants were significantly correlated with the breast milk folate levels of their mothers (Fig. 1). This suggests that the plasma folate level of breast-fed infants, which is an index of their folate nutritional status, is directly proportional to the folate content of their mother's milk.

TABLE 1
Hematological findings in lactating mothers and breast-fed infants

	Hemoglobin	Mean corpuscular volume	Lob average of neutrophils[a]
	g/100 ml	μ^3	
Mothers	12.5 ± 1.1 (15)[b]	94.0 ± 5.3 (15)	2.23 ± 0.23 (21)
Infants	12.3 ± 1.3 (30)	92.3 ± 8.9 (30)	2.60 ± 0.34 (30)

[a] The number of nuclear lobes in each of 100 neutrophils, stained with Wright-Giemsa, was counted and expressed as the average number of lobes per cells. [b] Mean ± SD (number of cases).

TABLE 2
Folate status in lactating mothers and breast-fed infants

	Plasma	Red blood cells[a]	Milk[a]
		ng/ml	
Mothers	5.9 ± 3.5 (39)[b]	232.7 ± 83.6 (39)	141.4 ± 47.9 (39)
Infants	29.0 ± 13.6 (25)	429.1 ± 185.5 (25)	

[a] *L. casei* activity after conjugase treatment. [b] Mean ± SD (number of cases).

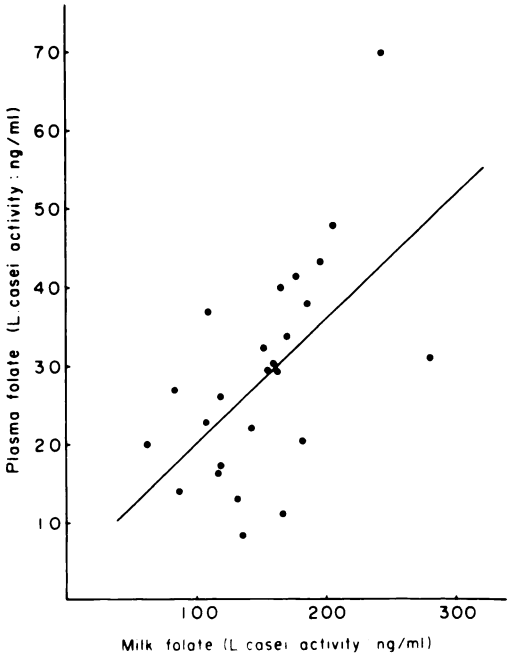

FIG. 1. Relationship between folate levels in breast milk and infant plasma (correlation coefficient 0.58: $P < 0.01$).

TABLE 3
Plasma, RBC, and milk folate levels in lactating mothers before and after folate administration

	Plasma	Red blood cells[a]	Milk[a]
		ng/ml	
Before PteGlu administration[b]	6.0 ± 2.0 (16)[c]	250.8 ± 85.2 (16)	130.2 ± 45.9 (16)
After PteGlu administration[b]	41.8 ± 53.8 (15)	389.9 ± 85.5 (15)	136.6 ± 41.2 (16)

[a] *L. casei* activity after conjugase treatment. [b] Oral administration of pteroylmonoglutamate (1 mg/day) for 4 weeks. [c] Mean ± SD (number of cases).

In the present study, the daily folate intake for healthy infants can be calculated to be around 14 to 25 μg/kg body weight, assuming an infant consumption of 100 to 180 ml breast milk per kilogram daily. This would indicate that the average daily intake of folate in breast-fed infants ranges from 57 to 156 μg/day, since the mean body weight of infants was 6.32 kg.

Synthetic PteGlu (1 mg per day) was given orally for 4 weeks to sixteen random lactating mothers. After administration of PteGlu, maternal plasma, RBC, and milk folate levels were estimated and lobe averages of neutrophils were counted. As shown in Table 3, there were significant increases in plasma and RBC folate levels, but not in milk folate levels ($P > 0.6$). No significant changes in the lobe averages were found. Also, no significant correlations were found between breast milk folate levels and plasma or RBC folate levels from lactating mothers before and after PteGlu administration.

Discussion

As reviewed by Metz (3), macrocytic anemia was not infrequently found in lactating mothers, and this was considered to be due to lactation which might drain a significant amount of folate from maternal body stores. Abnormal hematological findings were not observed in the lactating mothers in this study, and their plasma and RBC folate levels were within the normal range (Table 2). In contrast, the plasma and RBC folate levels in their infants were higher than those in normal adults. These observations are identical with findings previously reported (13–18). Blood folate levels were high in early infancy, but after several months of life, had fallen to the normal range of adults (15, 17, 18).

It has been reported that free folate levels in breast milk range from 0.3 to 32 ng/ml (*L. casei* activity without conjugase treatment) (4, 5, 16, 19, 20), and total folate from 52 to 64 ng/ml (4, 21). Other workers, using *Pediococcus cerevisiae* and *Streptococcus faecalis* as test organisms, which do not respond to methyltetrahydrofolate, reported free folate levels of 7.3 and 0.71 ng/ml, respectively (4, 22). In our study, we found considerably higher levels of total folate in milk (Table 2), ranging from 62 to 280 ng/ml.

It is generally agreed that breast milk from healthy, well nourished mothers is the best food for young infants. It is also believed that the assessment of the recommended allowances of infants can be based on the composition of milk from well-nourished mothers (19). The values of folate intake (57 to 156 μg/day) presented in this report are slightly higher than the level of 50 μg/day for infants in Recommended Dietary Allowances (23), and in other reports (4, 21, 24–28). The requirement of folate, as compared with pure folic acid PteGlu, for healthy infants remains undetermined. It could be suggested that the daily nutritional requirement of pure folic acid for healthy infants is lower than the values presented in this study, since the availability of breast milk folate might be lower than synthetic folate compounds. The availability of food folate varies from food to food (11) and the availability of breast milk folate for infants is not known. Further studies are needed to investigate the availability of milk folate, with special reference to folate-binding protein(s) that might have a considerable influence on the vitamin economy and on the composition of the intestinal flora (4, 29).

Metz et al. (30) reported that there were appreciable increases in breast milk folate concentrations (less than 5 ng/ml to more than 60 ng/ml; *L. casei* activity without conjugase treatment) in two lactating patients with megaloblastic anemia, after 4 days of oral supplementation with 100 or 200 μg PteGlu daily (30). A possible reason for this

large increase in milk folate is that the mothers were extremely folate deficient at the beginning of the study, resulting in abnormally low folate concentrations in their milk. These authors mentioned that in lactating mothers with folate deficiency, orally administered PteGlu might be taken up by the mammary glands in preference to hematopoietic system.

In the present study, we observed that there was no significant increase in breast milk folate levels of the lactating mothers after the oral administration of 1 mg PteGlu per day for 4 weeks. This might be due to the fact that the folate nutritional status in the lactating mothers was sufficient to maintain their breast milk folate levels before folate supplementation. Metz et al. (30) reported that milk folate levels increased before the hematological responses occurred. Based on the results of our study and that of Metz et al. (30), it appears that a regulatory mechanism exists to maintain folate levels in human milk. Folate-binding protein in milk may play a role in this regulation, but it is unlikely to be the whole answer. Ford et al. (31) reported that the folate content of goat milk was not wholly explained by its folate-binding capacity. Further studies are needed to elucidate the role of folate-binding protein(s) in breast milk, in particular its influence on the availability of milk folate and its possible role in the maintenance of constant milk folate levels.

The authors are indebted to Dr. F. Chiba, Pediatric Clinic, Hiraga General Hospital, Yokote for his help in carrying out this study.

References

1. Edelstein, T., K. Stevens, V. Brandt, N. Baumslag and J. Metz. Tests of folate and vitamin B_{12} nutrition during pregnancy and the puerperium in a population subsisting on a suboptimal diet. J. Obstet. Gynaecol. Brit. Commonwealth 73: 197, 1966.
2. Shapiro, J., H. W. Alberts, P. Welch and J. Metz. Folate and vitamin B_{12} deficiency associated with lactation. Brit. J. Haematol. 11: 498, 1965.
3. Metz, J. Folate deficiency conditioned by lactation. Am. J. Clin. Nutr. 23: 843, 1970.
4. Matoth, Y., A. Pinkas and C. Sroka. Studies on folic acid in infancy. III. Folates in breast fed infants and their mothers. Am. J. Clin. Nutr. 16: 356, 1965.
5. Ramasastri, B. V. Folate activity in human milk. Brit. J. Nutr. 19: 581, 1965.
6. Tamura, T., Y. S. Shin, M. A. Williams and E. L. R. Stokstad. *Lactobacillus casei* response to pteroylpolyglutamates. Anal. Biochem. 49: 517, 1972.
7. Waters, A. H., and D. L. Mollin. Studies on the folic acid activity of human serum. J. Clin. Pathol. 14: 335, 1961.
8. Hoffbrand, A. V., B. F. A. Newcombe and D. L. Mollin. Method of assay of red cell folate activity and the value of the assay as a test for folate deficiency. J. Clin. Pathol. 19: 17, 1966.
9. Bird, O. D., V. M. McGlohon and J. W. Vaitkus. Naturally occurring folates in the blood and liver of the rat. Anal. Biochem. 12: 18, 1965.
10. Hurdle, A. D. F., D. Barton and I. H. Searles. A method for measuring folate in food and its application to a hospital diet. Am. J. Clin. Nutr. 21: 1202, 1968.
11. Tamura, T., and E. L. R. Stokstad. The availability of food folate in man. Brit. J. Haematol. 25: 513, 1973.
12. Chanarin, I. The significance of the serum and red cell folate concentration. In: The Megaloblastic Anemias. Oxford: Blackwell Scientific Publications, 1969, p. 320.
13. Matoth, Y., A. Pinkas, R. Zamir, F. Mooallen and N. Grossowicz. Studies on folic acid in infancy. I. Blood levels of folic acid and folinic acid in healthy infants. Pediatrics 33: 507, 1964.
14. Vanier, T. M., and J. F. Tyas. Folic acid status in normal infants during the first year of life. Arch. Diseases Childhood 41: 658, 1966.
15. Roberts, P. M., D. E. Arrowsmith, S. M. Rau and M. E. Monk-Jones. Folate state of premature infants. Arch. Diseases Childhood 44: 639, 1969.
16. Landon, M. J., and A. Oxley. Relation between maternal and infant blood folate activities. Arch. Diseases Childhood 46: 810, 1971.
17. Landon, M. J., and E. N. Hey. Renal loss of folate in the newborn infant. Arch. Diseases Childhood 49: 292, 1974.
18. Hibbard, E. D., and A. P. Kenna. Plasma and erythrocyte folate levels in low-birth-weight infants. J. Pediat. 84: 750, 1974.
19. Macy, I. G., and H. J. Kelly. Human milk and cow's milk in infant nutrition. In: Milk: The Mammary Gland and its Secretion, edited by S. K. Kon and A. T. Cowie. New York: Academic Press, 1961, vol. 2, p. 265.
20. Burland, W. L., K. Simpson and J. Lord. Response of low birthweight infants to treatment with folic acid. Arch. Diseases Childhood 46: 189, 1971.
21. Ford, J. E., and K. J. Scott. The folic acid activity of some milk foods for babies. J. Dairy Res. 35: 85, 1968.
22. Collins, R. A., A. E. Harper, M. Schreiber and C. A. Elvehjem. The folic acid and vitamin B_{12} content of the milk of various species. J. Nutr. 43: 313, 1951.
23. Food and Nutrition Board, Committee on Dietary Allowances. Recommended Dietary Allowances (8th ed.). Washington, D.C.: National Academy of Sciences—National Research Council, 1974.
24. Sullivan, L. W., A. L. Luhby and R. R. Streiff. Studies of the daily requirement for folic acid in infants and the etiology of folate deficiency in goat's milk megaloblastic anemia. Am. J. Clin. Nutr. 18: 311, 1966.
25. Ghitis, J. The labile folate of milk. Am. J. Clin. Nutr. 18: 452, 1966.
26. Kamel, K., C. I. Waslien, Z. El-Ramly, S.

GUINDY, K. A. MOURAD, A. K. KHATTAB, N. HASHEM, V. N. PATWARDHAN AND W. J. DARBY. Folate requirements of children. II. Response of children recovering from protein-calorie malnutrition to graded doses of parenterally administered folic acid. Am. J. Clin. Nutr. 25: 152, 1972.

27. ASFOUR, R., N. WAHBEH, C. I. WASLIEN, S. GUINDI AND W. J. DARBY. Folacin requirement of children. III. Normal infants. Am. J. Clin. Nutr. 30: 1098, 1977.
28. WASLIEN, C. I. Folacin requirement of infants. In: Folic Acid, Biochemistry and Physiology in Relation to the Human Nutrition Requirement. Washington, D.C.: National Academy of Sciences—National Research Council, 1977, p. 232.
29. FORD, J.E. Some observations on the possible nutritional significance of vitamin B_{12}- and folate-binding proteins in milk. Brit. J. Nutr. 31: 243, 1974.
30. METZ, J., R. ZALUSKY AND V. HERBERT. Folic acid binding by serum and milk. Am. J. Clin. Nutr. 21: 289, 1968.
31. FORD, J. E., G. S. KNAGGS, D. N. SALTER AND K. J. SCOTT. Folate nutrition in the kid. Brit. J. Nutr. 27: 571, 1972.