

Studies on Folic Acid in Infancy

III. Folates in Breast Fed Infants and Their Mothers

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IN A PREVIOUS publication in this series¹ it was shown that folate activity in whole blood was lower in infants than in adults. Although increased demand, dictated by rapid growth, undoubtedly is an important factor in determining blood folate levels in this age group, dietary intake may also be of importance. An indication of the effect of the infant's diet on its blood folate level was provided by the high values observed in a small group of breast fed infants included in the population studied previously,¹ which consisted largely of artificially fed infants.

In the present study this observation has been extended. Folate levels were assayed in the blood of a further group of breast fed infants and in their mothers' blood. In addition, the folate content of the mothers' milk and of cow's milk was determined.

MATERIALS AND METHODS

Thirty-five healthy, entirely breast fed infants attending well-baby clinics and their mothers were studied. The infants were six weeks to six months old and came from families of various ethnic origin and socioeconomic status. Capillary blood was obtained from infants and mothers for hemoglobin and folate determinations and a sample of milk was

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This investigation was aided by a grant from the Ford Foundation.

obtained from each mother for folate assay. All blood and milk samples were obtained between 8 and 10 A.M.

Data on infants were pooled with those obtained on a group of thirty-nine breast fed infants of comparable age included in the previous study referred to. All seventy-four infants were in apparent good health at the time of examination. They were all full term babies who thrived well. Their weight was well within the normal range for their ages. Only eight had experienced mild infections in the past. Their hemoglobin concentration varied between 8.1 and 15.1 gm. per cent, with a mean of 10.9 gm. per cent.

Samples of milk assayed for folate content included native fresh milk obtained from different farms, bottled pasteurized milk sampled on different days, samples of bottled sterilized milk and canned powdered whole milk. All milk samples were assayed for folate activity before and after boiling.

Folate activity was determined microbiologically on whole blood, according to the method of Grossowicz et al.,² adapted for small quantities of blood as previously described.¹ *Lactobacillus casei* was used for determining "total" folate activity and *Pedococcus cerevisiae* for folic acid and related reduced forms.

RESULTS

In Table I folate activity values of whole blood of seventy-four infants (combined group) and thirty-five lactating mothers are compared with those found in a group of 146 artificially fed infants aged six weeks to six months included in a series of healthy infants studied previously.¹

The mean value of 98.8 μg . per ml. for "total" folate (L. casei assay) in breast fed infants is significantly higher than the corresponding value of 65.5 μg . per ml. in artificially fed infants ($p < 0.001$). The mean



TABLE I
Folate Levels in Blood of Infants and Lactating Mothers

Subjects Studied	Total Folate (mµg./ml.)		Reduced Folate (mµg./ml.)	
	Mean	±S.D.	Mean	±S.D.
Breast fed infants . . .	98.8	40.3	10.9	6.2
Artificially fed infants	65.5	33.2	8.6	4.8
Lactating mothers . . .	56.5	27.0	6.9	3.6

value of 10.9 mµg. per ml. for reduced forms of folate (*P. cerevisiae* assay) is likewise significantly higher than the value of 8.6 mµg. per ml. found for artificially fed infants ($p < 0.001$). In neither group was there a consistent tendency for folate levels to rise or fall with age (Table II).

"Total" folate in blood of nursing mothers, 56.5 mµg. per ml., was significantly lower ($p < 0.001$) than the value of 89 mµg. per ml. found by Izak et al.³ for normal adults, whereas the mean value of 6.9 mµg. per ml. for reduced folates in nursing mothers did not differ materially from the mean of 6.3 mµg. per ml. found in normal adults.³

Breast fed infants showed higher folate activity than their mothers with both assays. The differences between the means of 98.8 and 10.9 mµg. per ml. with the *L. casei* and *P. cerevisiae* assays, respectively, in the infants and the corresponding means of 56.5 and 6.9 mµg. per ml. in the mothers is highly significant ($p < 0.001$). Figure 1 illustrates the relationship of total folate values in each of thirty-

four individual mother-infant pairs. It can be seen that in each but six cases folate levels are considerably higher in the infant than in its mother. The case on the extreme right of the graph, in which the values were exceptionally high (205 and 243 mµg. per ml. for infant and mother, respectively), deserves special mention. The mother, who was a public health nurse and knew about our work, on her own initiative took folic acid tablets regularly during lactation.

Folate activity varied considerably in breast milk and in cow's milk, but tended to be higher

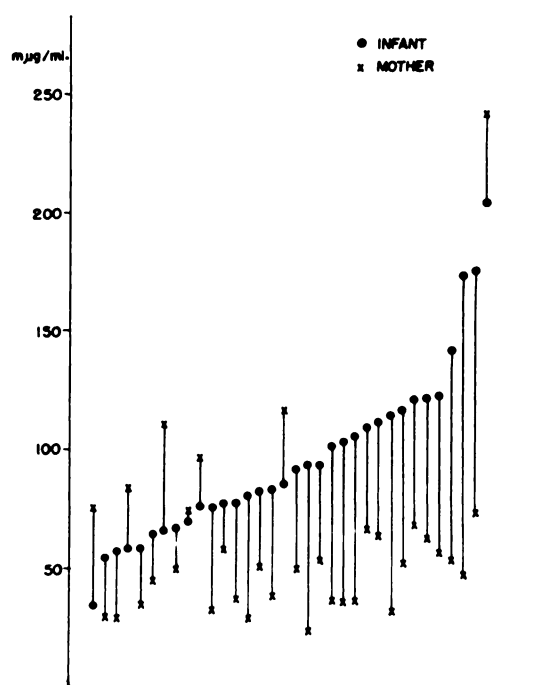


FIG. 1. Folate levels in blood of thirty-four infants and their mothers.

TABLE II
Mean Folate Blood Levels Related to Age of Infants

Age (wk.)	Total Folate (mµg./ml.)		Reduced Folate (mµg./ml.)	
	Breast Fed	Artificially Fed	Breast Fed	Artificially Fed
6-9	84.8	68.0	11.0	8.2
10-13	101.1	70.6	11.1	10.4
14-17	116.4	65.0	12.1	8.0
18-21	74.4	59.1	5.8	7.8
22-26	125.6	63.2	8.3	7.7

in the latter (Table III). However, since cow's milk in its native state is never used for infant feeding, data on the effect of heat treatment on folate activity in fresh milk and in various modifications of cow's milk in common use in infant feeding have been included in the table. It can be seen that pasteurization reduces the folate activity of milk only to a mild degree, whereas there is considerable loss of activity following heating to higher temperatures as is the case in sterilization. Reheating causes a practically complete loss of whatever folate

TABLE III
Folate Levels in Breast Milk and in Cow's Milk

Type of Milk	No. of Samples	Total Folate ($\mu\text{g./ml.}$)				Reduced Folate ($\mu\text{g./ml.}$)			
		Before Boiling		After Boiling		Before Boiling		After Boiling	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
Breast milk	35	24.0	7.4-61.0	7.3	2.4-17.6
Cow's milk									
Fresh	5	37.7	16.8-63.2	28.9	9.2-47.6	12.6	2.8-43.6	1.3	0.7-2.2
Pasteurized, bottled	3	35.3	28.0-43.0	11.8	10.5-13.2	4.8	3.8-5.9	0.9	0.7-1.2
Sterilized, bottled	2	9.3	8.6-10.0	very low		0.75	0.7-0.8	very low	
Powdered whole milk	2	14.6	13.2-16.1	9.3	5.6-13.1	1.0	0.8-1.2	0.7	0.6-0.8

activity is left following the initial heat treatment. Reduced folates are particularly affected by heating.

COMMENTS

Blood folate activity in the group of breast fed infants studied by us compared favorably with that found in normal adults. On the other hand, there was a sharp difference between breast fed infants and their artificially fed age mates, with blood folate activity being some 50 per cent higher in the former than in the latter. In a previous publication,¹ based on a study of a population consisting largely of artificially fed infants, it was concluded that blood folate levels tended to be low in infants. This generalization now has to be modified to exclude breast fed infants.

Blood folate values in breast fed infants were uniformly high irrespective of economic status. This is in contrast to artificially fed infants, in whom there was a positive correlation between folate level and economic status.¹

The demand for folates is equally high in both groups but the dietary supply differs. The high folate level in breast fed infants could be accounted for on the basis of an adequate dietary intake. An infant five to six months of age, weighing 6 kg., would have a blood volume of about 500 ml. With a folate blood level of 100 $\mu\text{g.}$ per ml., the total amount of folate in his blood would be about 50 $\mu\text{g.}$ A daily intake of 800 ml. breast milk, with a folate concentration of 24 $\mu\text{g.}$ per ml.,

would supply the infant with some 20 $\mu\text{g.}$, a quantity sufficient to allow a complete turnover of blood folates in two and a half days. Such a calculation, however, may be misleading, since it is known that folates in blood represent a relatively small fraction of total body folate, the main stores being found in organs, such as liver and kidneys.^{4,5}

A better approach to the problem of adequacy of dietary supply would be an estimate of the infant's daily folate requirement. The minimal daily requirement for adults has been estimated by Herbert⁶ to be about 50 $\mu\text{g.}$ By simple extrapolation to infants on the basis of body weight, a daily requirement of 5 $\mu\text{g.}$ for a baby weighing 6 kg. would be arrived at. Considering the large demands for growth, this is obviously underestimated. A daily intake of some 20 $\mu\text{g.}$, as supplied by breast milk, on the other hand, seems to be sufficient to cover the infant's needs, since with this intake an adequate folate blood level is maintained. It should be remembered, however, that quantitative differences in folate intake may not necessarily be the sole factor responsible for the higher blood folate levels in breast fed infants. Other factors, such as folate absorption, may be involved. Furthermore, the role played by the intestinal flora as a producer or a consumer of folates in either breast fed or artificially fed infants is not known.

In the artificially fed infant the dietary supply of folate is inadequate. This is particularly true of the infant being fed boiled pasteurized

or sterilized milk. The use of bottled pasteurized milk for infant feeding and the practice of boiling it for increased safety are widespread in this and other countries.

The drain on folate reserves imposed by lactation is reflected in the low blood folate levels found in nursing mothers, which are grouped around a mean of 56.5 m μ g. per ml. This value is about two-thirds the normal adult mean and is somewhat lower than the mean values found in other groups with a similar physiologic predisposition to a "strained" folate metabolism, such as pregnant women³ and rapidly growing infants.¹

Low folate levels in our group of mothers could not be correlated with economic status, nutritional history or duration of lactation. An average normal diet is obviously not sufficient, in most cases, to compensate for the daily loss of some 20 μ g. of folate in milk. There was no correlation between the folate levels in blood and milk of individual mothers. It should be noted, however, that the level of folate in whole blood consists largely of folate in blood cells, whereas the level in serum would be of importance if a blood:milk filtration gradient was to be demonstrated.

The high total folate blood levels in infants, even at the expense of their mothers, deserve emphasis. They indicate that, when the folate intake of the mother is inadequate, folate depletion is more apt to occur in the mother than in the infant. The high levels of reduced folate in infants, compared to those in their mothers, probably reflects the general trend for a higher ratio of reduced to total folate characteristic of infancy.¹

SUMMARY

Total folate (*Lactobacillus casei* assay) and folic acid (*Pediococcus cerevisiae* assay) have been assayed in blood of breast fed infants, in

blood and milk of lactating mothers and in samples of cow's milk.

Total folate activity in breast fed infants was significantly higher than in a comparable group of artificially fed infants. Total folate activity in the mothers was considerably lower than in their babies or in normal adults.

Reduced folate levels were significantly higher in breast fed infants than in their mothers, in artificially fed infants or in normal adults.

Native cow's milk is at least as rich in folates as breast milk. However, following heat treatment, a considerable proportion of folate activity is lost. Since heat in various forms is applied to cow's milk prior to its use for infant feeding, dietary intake of folate is lower in artificially fed infants than in breast fed infants.

The low folate levels in the mothers can be accounted for at least in part by the drain on folate reserves imposed by lactation, which is of the order of 20 μ g. per day.

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