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Folate metabolism
intestinal absorption
newborns
plasma clearance
premature infants
pteroylglutamic acid

Folate Metabolism in Newborns and during Early Infancy

I. Absorption of Pteroylglutamic (Folic) Acid in Newborns

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Extract

Folic acid (PGA) absorption (40 μg/kg of body weight) was studied in normal newborns and adults using *Lactobacillus casei* and *Streptococcus faecalis* microbiological assays for folate determination. The PGA was given orally or intramuscularly to the subjects after either saturating their tissues with folate or without prior saturation of their tissues. Without prior saturation of the tissues the folic acid absorption curves in newborns were much lower than those found in adults (figs. 1 and 2). Saturation of the tissues of the newborns with folic acid, prior to administration of the oral test dose, raised the absorption curves of the folic acid, but these were still lower than those seen in adults (fig. 5). The folate curve of the newborns after an intramuscular test dose of PGA was also lower than that observed in adults (fig. 3); however, after saturating the tissues of the newborns with folic acid, the folate curve became comparable to that of the unsaturated adults (fig. 4).

After the oral test dose, newborns excreted a much smaller percentage of the administered dose in their urine than did the adults (table II). When the tissues in newborns were saturated with folic acid, however, the excretion of folate in the urine became comparable to that of unsaturated adults (table II). In newborns, the daily elevation of folate in the serum following 40 μg PGA/kg/24 h *per os* was comparable to that after intramuscular injection of 30 μg PGA/kg/24 h (fig. 6).

These data indicate that: *1)* plasma clearance of folate in newborns is more rapid than that in adults and that large saturating doses of folic acid are needed to reduce this clearance rate; and *2)* the folate absorption curve in the newborn is lower than in the adult. With the criteria set for adults, this could be interpreted as evidence of impaired folate absorption in newborns. The proper interpretation of the data indicates, however, that folate absorption by newborns is slower than seen in adults and that the net absorption is comparable to that of adults.

Speculation

In newborns, the absorption curve for orally administered folate is lower than in adults. Our study indicates that this is due to the slower absorption of folates and more rapid tissue uptake of folates by newborns. Further studies should be carried out to investigate the absorption of folate conjugates which are the main forms of folate found in food, to determine how much of the folate in food is available to newborns and small infants, and to evaluate the need for supplementary folate in small infants.

Introduction

Infants, especially those who are born prematurely, are prone to develop folic acid deficiency [18, 32, 37–39, 41, 51, 56, 59, 63, 64]. Although advanced megaloblastic anemia is not common in infants without predisposing factors, the available data demonstrate laboratory evidence of mild folate deficiency in a high proportion of such infants in the first few months of life [32, 38, 46, 51]. Infants are born with very high folate levels in serum and whole blood [12, 22, 38, 46, 56], but by 2–3 months of age the serum and blood folate levels fall to concentrations comparable to those seen in folate-deficient adults [38, 46]. Rapid rates of growth and dietary factors have been thought to be responsible for this fall [38, 46, 56]. No data are available in the literature with regard to folate absorption in the newborn. In this and a companion paper [47] we are reporting findings concerning the absorption, plasma clearance, and excretion of folic acid in urine. We also point to some of the pitfalls that may lead to false interpretation of the data obtained from absorption studies in newborns.

Materials and Methods

Normal adult volunteers (hospital personnel) and normal newborns were studied [67]. The newborns were 3–12 days old and, unless otherwise stated, were generally normal full-term infants broading in the hospital. Older infants, born prematurely, were used for urinary excretion studies. All blood samples from adults were taken from the antecubital vein; blood from newborns was obtained by heel prick by the same person, AMS. The samples were collected in siliconized capillary tubes (3.5 mm id) and transferred to folate-free glass tubes for separation of the serum. About 1–1.5 ml of blood were taken by heel prick for assay of folate activity. The serum folate (SFA) was determined by *L. casei* and *S. faecalis* microbiological assays. These assays were modified to take 0.1 ml of serum for absorption or clearance studies. Serum *L. casei* activity was determined by the method of WATERS and MOLLIN [57] with minor modifications. Normal range for adults in our laboratory is 3–15 ng/ml. Serum *S. faecalis* activity was determined by the JUKES modification [31] of the TEPLY and ELVEHJEM method [52], which in turn, was further modified to use a smaller amount of serum. The *S. faecalis* assays were carried out in tubes containing 2 ml of double strength culture media (Bacto Folic T. E. Medium) [65] and 2 ml of standard or diluted serum. Serum was diluted in water 1 : 50–1 : 800 depending on the expected folate activity. The folic acid used as standard was obtained commercially [66] and its folate content was checked against highly purified PGA [67]. Folic acid for parenteral use was prepared by diluting Folvite (15 mg sodium folate/ml) [67] to obtain concentrations of about 50, 100, 200, and 2,000 μg PGA/ml. The actual concentration of these samples was then checked against the standard folic acid. The diluted PGA for oral or parenteral use was placed in small sterile vials and kept at –20°. For each experiment one new vial was thawed and any remainder discarded.

Folic Acid Absorption Test

The test dose of folic acid was 40 μg PGA/kg of body weight. This dose was given to subjects who either were, or were not, previously loaded with folic acid. The test dose was not given sooner than 3 h after the last feeding and subsequent feeding was not given earlier than 1 h after the test dose. The infants received the oral test dose in 2–3 ml of glucose in water and the adults received the dose in a glass of ginger ale. For a period of 3 days different loading doses of folate were given orally to the newborns; test doses were given 24 h after the last loading dose. Blood samples for the folate assay were obtained before, 1, 2, and 5 h after administration of the test dose. Serum folate was determined by *L. casei* and *S. faecalis* assay.

Excretion of Folate in Urine

The amount of folate excreted in a 6-h urine sample after an oral dose of 40 μg PGA/kg was determined. Adults were not loaded with folic acid prior to the test but excretion studies in newborns were carried out in those with or without prior loading doses of folic acid. As indicated previously, there were more premature infants (males only) used in the excretion studies because it was technically easier to get a satisfactory urine collection in male prematures. The 6-h urine samples were collected in brown bottles through external drainage tubing. Adults emptied their bladders before taking the test dose of PGA and then retained the urine for 6 h before voiding. Urine was kept at –20° until assayed. Folate in urine was determined in the same manner as *L. casei* activity of serum.

Results

Folic Acid Absorption

Absorption in newborns was studied by using *S. faecalis* assay. The results are shown in figure 1. It can be seen that the folate absorption curve in the newborn was almost flat. Subsequently, assays using both *S. faecalis* and *L. casei* activities were conducted. Figure 2 shows that with the *L. casei* assay the folate absorption curve of newborns was still much lower than that of adults, even though the original folate level in serum

was higher than that in adults. When newborns were given a test dose of 100 μg PGA/kg orally, the absorption curve was lower than that of adults receiving 40 μg PGA/kg orally (table I, fig. 2).

Four possibilities had to be considered to explain this relatively flat folate absorption curve in newborns: *a)* this could be due to rapid tissue uptake of folate in newborns; *b)* there could be a lower renal threshold for folic acid in newborns; *c)* folate absorption could be a slower process in newborns than in adults; and *d)* folate absorption could actually be impaired in newborns.

To study the possibility of rapid plasma clearance, the folate absorption curve following an oral test dose of 40 μg PGA/kg was compared with that following a similar dose given intramuscularly. The results are shown in figure 3. The fact that the peak level of folate

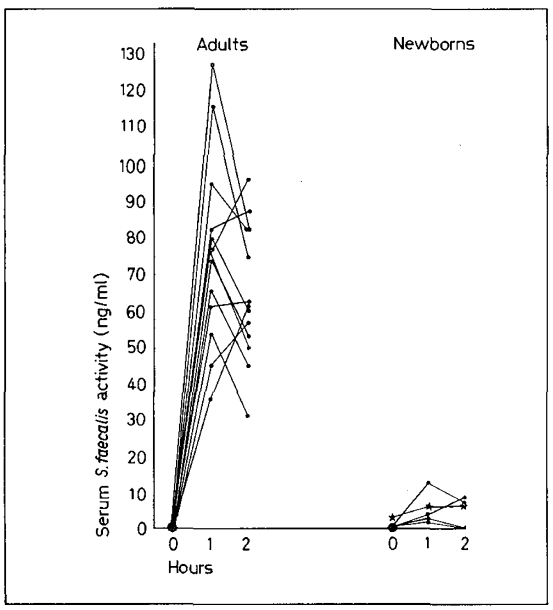


Fig. 1. Serum *S. faecalis* activity in normal adults and newborns after a test dose of 40 μg PGA/kg orally. The curve with asterisks (*) is from a 7-day-old infant, born prematurely.

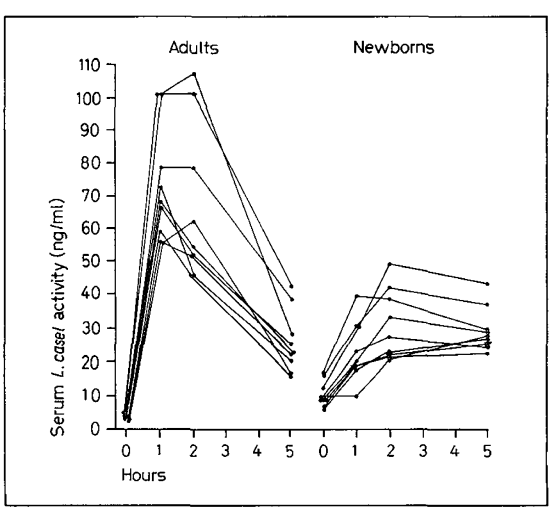


Fig. 2. Serum *L. casei* activity in normal adults and newborns after a test dose of 40 μg PGA/kg orally. The curve with asterisks (*) is from a 7-day-old infant, born prematurely.

Table I. Serum *L. casei* activity after an oral test dose of 100 μg PGA/kg[1]

Birth wt, g	Age, day	Serum *L. casei* activity, ng/ml			
		0 h[2]	1 h	2 h	5 h
2,200	4	12.5	32.5	47.5	—
3,560	11	21.3	—	71.0	72.7
3,650	11	12.3	37.5	—	51.7
3,680	10	6.0	42.0	59.0	71.2
4,640	8	8.2	18.0	21.0	23.5

[1] Newborns were not previously loaded with folic acid.
[2] Time after administration of challenge.

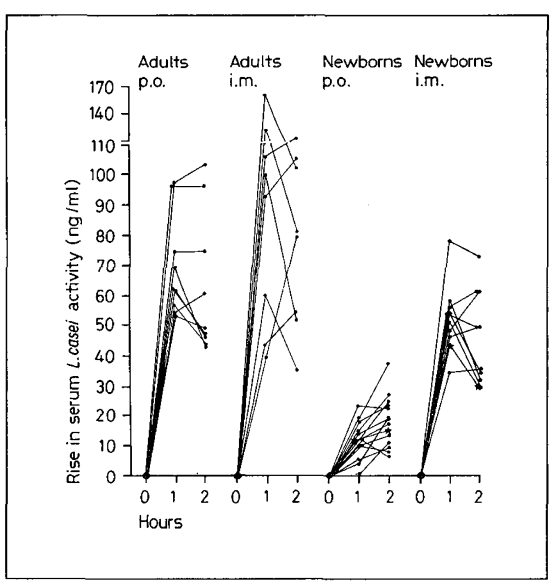


Fig. 3. Comparison of the rise in serum folate (*L. casei* activity) in adults and newborns following an oral or intramuscular dose of PGA (40 μg/kg). The initial folate level in serum is deducted from subsequent serum folates to show the actual rise of serum folate related to the test dose. The curves with asterisks (*) are from infants born prematurely.

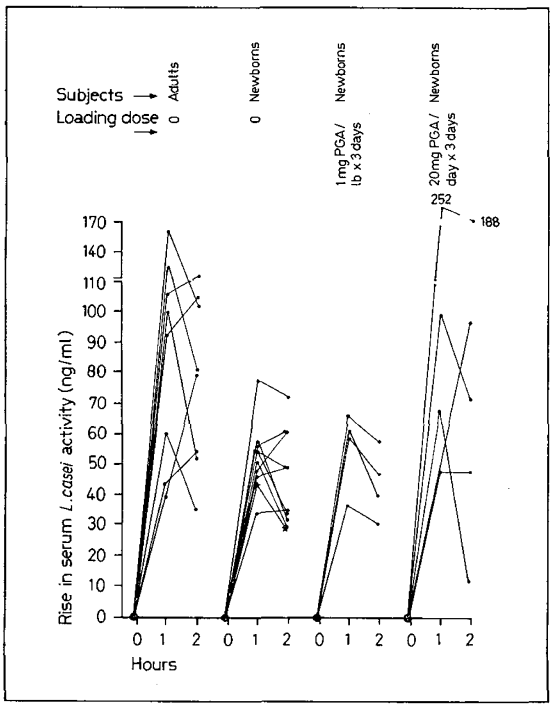
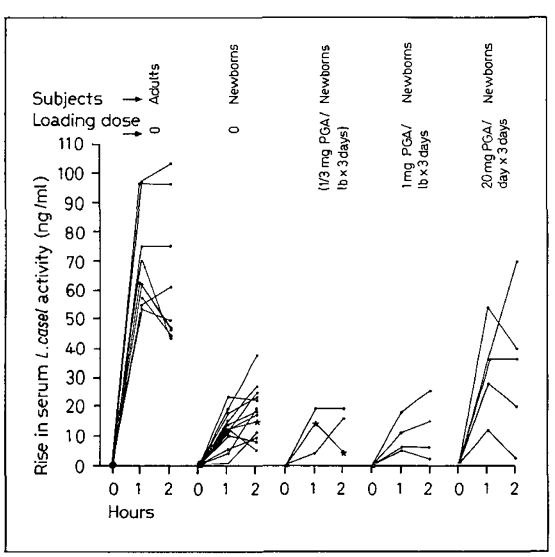


Fig. 4. Comparison of the rise in levels of folate in serum following an intramuscular dose of 40 μg PGA/kg in adults (not loaded) and in newborns loaded with different loading doses of folic acid. The levels of folate before giving the test doses are deducted from the subsequent levels of folate in serum to show the actual rise. The loading doses of PGA were given orally. The loading dose of 20 mg/24 h was given as 5 mg 4 times/24 h. The curves with asterisks (*) are from infants born prematurely.



in serum following intramuscular test doses was lower in the newborns than in the adults, suggests that plasma clearance of folate in the newborns was faster than in the adults. Comparison of the folate curve following an oral dose with that following an intramuscular dose in each of the two groups, however, indicated that the difference between the absorption curve of newborns and adults given folate orally could not be explained by rapid plasma clearance alone.

To eliminate the factor of rapid plasma clearance, oral and intramuscular test doses of PGA were given to the newborns after their body tissues were loaded with varying doses of folic acid. Figure 4 shows that with very large loading doses of folic acid (5 mg,4 times/ 24 h × 3 days, or the equivalent of about 20 times the loading dose generally given to adults), the folate curve after intramuscular test doses became comparable to that of unloaded adults. As shown in figure 5, however, irrespective of the loading dose of folic acid, after an oral test dose the folate absorption curve in newborns never reached the adult curve. This indicates that the difference between the absorption curves of adults and of newborns who have received folate orally was not due to rapid plasma clearance alone. The difference between the adult and the newborn was even more striking when *S. faecalis* assay was used (data not shown).

Studies of excretion of folate showed that newborns excrete a much smaller percentage of the administered dose of folate in the urine and that only when the tissues are saturated with folic acid and the folate level in serum was above 50 ng/ml could they have folate excretion in urine comparable to the normal unloaded adult (table II). A full-term infant, 5 days old, having received 40 μg PGA/kg intramuscularly, excreted 13.5 % of the folate dose in his 6-h urine; then when the same dose was given orally, at 10 days of age, excreted only 0.6 % of the folate dose in his urine. Similarily, an infant born prematurely excreted 0.2 % of the oral dose at day 21 of life and at day 26, excreted 8.7 % of the same dose of folate in his urine when the folate was given intramuscularly (see [47] for urinary excretion after intramuscular test doses in adults and newborns.)

Excretion studies ruled out the possibility that the increased urinary excretion of absorbed folate could be

Fig. 5. Comparison of the rise in levels of folate in serum following an oral dose of 40 μg/PGA/kg in adults (not loaded) and in newborns loaded with different loading doses of folic acid. The levels of folate before giving the test doses are deducted from the subsequent folate levels in serum to show the actual rise. The loading doses of PGA were given orally. The loading dose of 20 mg/ 24 h was given as 5 mg 4 times/24 h. The curves with asterisks (*) are from infants born prematurely.

Table II. Percentage of folate dose excreted in urine 6 h following oral administration of 40 μg PGA/kg

	Adults, not loaded	Newborns	
		Not loaded	Loaded with 1 mg PGA/kg/24 h × 3
	18.4 (4.7)[1]	0.2 (1.6, 21, 2.5)[2]	16.2 (2.1, 8, 54)[2]
	19.1 (2.7)	0.3 (2.1, 14, 5.4)	21.6 (2.3, 5, 82)
	20.0 (4.2)	0.4 (2.8, 20, 11.5)	29.4 (1.9, 20, 94)
	20.0 (6.5)	0.6 (4, 10, 8.0)	45.0 (2, 8, 110)
	21.2 (3.7)	4.8 (1.9, 8, 4.4)	
Mean	19.7 (4.3)	1.2 (6.3)	28.0 (85)

[1] The numbers in parentheses indicate the folate level in serum, in nanograms per milliliter, before giving the test dose.

[2] Numbers in parentheses represent, respectively, birth weight, in kilograms; age at the time of experiment, in days; and folate level in serum, in nanograms per milliliter.

responsible for flat absorption curves. The very low excretion of folate by the newborns is in keeping with the finding of a low folate absorption curve, since the amount of folate excreted in the urine is dependent on the folate level in serum [62]. Table II shows that when newborns were loaded with folic acid, the higher the serum folate, the more folate was excreted in the 6-h urine.

In an attempt to eliminate one of the two remaining possibilities, folic acid was given to the newborns either as a 40-μg/kg oral dose daily or as a 30-μg/kg intramuscular dose daily for 4 days. Samples of blood were taken before and 24 h after each dose. Based on tritiated folic acid studies and the measurement of fecal excretion, the percentage of absorption of orally administered folic acid is reported to be 60–98.5% [30, 35, 60]. Accepting 75% absorption as normal absorption, we compared the rise in the serum folate following 40 μg PGA/kg orally with that following 30 μg PGA/kg intramuscularly. If the gastrointestinal (GI) absorption of PGA in newborns was normal one would expect a comparable rise of serum folate in those receiving 40 μg PGA/kg orally and those receiving 30 μg PGA/kg intramuscularly. Figure 6 shows that the average daily rise of serum folate following 40 μg PGA/kg orally was comparable to that seen after 30 μg PGA/kg intramuscularly, indicating a normal net absorption of PGA in newborns.

The markedly low folate absorption curve and low excretion of folate in the urine in newborns could then be explained by the rapid tissue uptake of folate and the slow but adequate GI absorption of PGA in the newborn. Figure 6 shows that after four daily oral doses of 40 μg PGA/kg the initial mean folate level in serum doubled in the newborn group (from 6.6 to 13.6 ng/ml after four doses). Similar doubling of the mean initial folate concentration was obtained in six normal adults with a much smaller dose of folate. With 10 μg PGA/kg/24 h for 4 days, the initial mean SFA of 4.2 ng/ml rose to the mean of 8.9 ng/ml. This indicates a markedly increased volume of distribution of folic acid in the newborns.

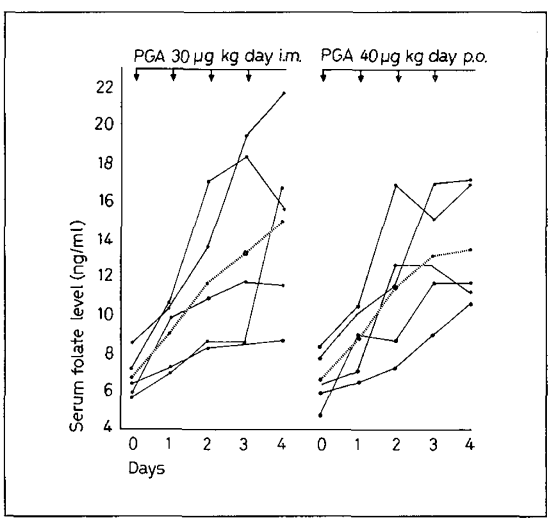


Fig. 6. Comparison of the rise in serum folate (*L. casei* activity) in newborns receiving 40 μg PGA/kg/24 h orally to those receiving 30 μg PGA/kg/24 h intramuscularly. All samples from each subject were run simultaneously and in two different runs of assays. Each curve is the average of two determinations. The dotted lines represent the means.

Discussion

We had previously reported that, at birth, the folate level in serum of infants born prematurely is higher than that seen in adults, but that this concentration

gradually falls in the subsequent 1–3 months of life, reaching levels comparable to those of folate-deficient adults [46]. We attributed this fall to the increased demand for folate due to rapid rate of growth, at a time when dietary folate is derived mainly from milk, a poor source of folate [37, 53]. Shortly thereafter, MATOTH *et al.* [38] reported a similar pattern of change in the blood folate level of full-term infants during year 1 of life. They indicated that the blood folate level of the lower socioeconomic group was lower than that of the higher socioeconomic group, and blood folate levels of breast-fed infants were higher than those of artificially fed infants. Thus, the diet of normal infants may play an important role in the development of biochemical evidence of folate deficiency in the first few months of life. Subsequent to these reports, however, NAIMAN and OSKI [42] reported that based on *L. casei* microbiological assay, the folate content of most of the commercial milk products used for infant formulas was above 50 or even above 100 μg/liter. Considering the *L. casei* activity of the milk formula, we realized that although some premature infants at 1–3 months of age were showing laboratory evidence of folate deficiency, actually they were taking in their diet an amount of folate equivalent to 5–10 times that of the minimal daily requirement reported for adults [27] (corrected for infants weight). This suggested that either the folate requirement of premature infants was so high that they became folate deficient even with large folate intakes, or there was impaired absorption of food folate in early infancy. We attempted to obtain the food folate equivalent value of infants formulas and other baby foods by observing the rise of folate levels in serum following food intake and comparing this elevation with the rise in folate after intake of different amounts of PGA. When we failed to notice any rise in serum folate of newborns after ingestion of formula or its equivalent amount of PGA, we studied the absorption and metabolism of PGA in newborns.

The absorption of PGA in adults has been studied by many investigators, either by microbiological assay, by observing the rise of folate in serum, by measuring excretion of folate in urine after a test dose [11, 13, 15, 17, 19, 20, 49, 50], or by the use of tritiated folic acid [23, 33–35, 60, 61]. Figures 1, 2, and 3, and table II meet all the criteria for the diagnosis of malabsorption of folate set for adults; i. e., low serum folate level after test dose of PGA, low excretion of folate in the urine, low ratio of the amount excreted in the urine following oral administration of PGA to that following intramuscular administration of PGA. The proper interpretation of the data, however, indicated that the very flat folate absorption curve in the newborns was due to rapid plasma clearance and slow but normal net absorption of PGA. The evidence for slow absorption of folic acid in newborns is contained in figures 2 and 6. Figure 2 shows that the peak SFA generally occurs in adults at 1 h and that there is a rapid fall of SFA between 2 and 5 h thereafter. In newborns, the peak SFA occurs at 2 h and there is little difference between the SFA at 2 h and at 5 h. This indicates that absorption of PGA has occurred fairly rapidly in the adults and that most of the folate is absorbed within 1–2 h, and that the rapid fall of SFA after 2 h is due to tissue uptake and excretion of folate in the urine. In the newborn, however, the continuous absorption of folate after 2 h prevents the rapid fall of SFA after 2 h (fig. 2, table I). When the folate level in serum was determined 24 h after test doses of PGA to allow maximum absorption of folate, as shown in figure 6, the average rise in folate after giving a dose of 40 μg PGA/kg by mouth was at least equal to the rise seen after giving 30 μg PGA/kg intramuscularly; these results indicate about 75% absorption of orally administered PGA (not corrected for the difference in urinary excretion of intramuscular and oral dose).

The mechanism and site of folic acid absorption has been studied in man [4, 26, 55] and in animals [5, 14, 25, 28, 48, 54, 61]. Most of the investigators have shown that folic acid is better absorbed in the proximal small intestine [25, 26, 28, 55, 61]. Whether this is an active or passive absorption is very controversial. TURNER and HUGHES [54], SPENCER and BOW [48], and YOSHINO [61] have shown evidence in favor of the passive absorption of folic acid in the rat and hamster. BURGEN and GOLDBERG [5], HERBERT and SHAPIRO [28], and HEPNER [25] have shown evidence in favor of the active transport of folic acid in the proximal small intestine of the rat. HEPNER's data suggest that the folate absorption is active in the jejunum and passive in the ileum of the rat. In man, HEPNER *et al.* [26] showed that the absorption of crystalline folic acid occurs principally in the proximal part of the jejunum. They showed poor absorption of folic acid in the distal jejunum and no absorption in the ileum. They considered their data in favor of an active transport process for folic acid. HELBOCK [24] and MATTY and BLAIR [40], however, have interpreted the data [26] to be more in support of the passive absorption of folic acid. The form in which absorbed folate enters into circulation has been studied by several authors and conflicting results have been obtained. BAKER *et al.* [2] suggested that folate is absorbed as a methylated monoglutamate form regardless of the form in which it is ingested. COOPERMAN and LUHBY [15] found polyglutamates in both the serum and urine of four normal subjects after oral ingestion of brewer's yeast. WHITEHEAD and COOPER [58] have shown that PGA is absorbed through the small intestine intact and methylation of folate occurs in the liver. BUTTERWORTH *et al.* [8] have shown that no significant

methylation of PGA occurs during the first 1–2 h after ingestion of PGA.

In our study of PGA absorption in adults it can be seen that serum *S. faecalis* activity in the first 2 h after ingestion of PGA was very close to the rise in serum *L. casei* activity (figs. 1 and 2). This indicates that in adults most of the test dose of PGA was absorbed intact. In the newborn, however, the rise in serum *L. casei* activity was much higher than the serum *S. faecalis* activity, indicating that a large portion of absorbed folate in the newborn was rapidly converted to a substance no longer active for *S. faecalis*, probably N_5-methyltetrahydrofolate. At the present time, we cannot state whether the slow absorption of folic acid in newborns is due to the slow gastric emptying process in the newborn [10] or due to the actual impairment of folate absorption in the jejunum requiring the further absorption of folic acid in the ileum of the newborn. In adults, because of a more rapid gastric emptying time, most of the test dose of PGA enters the jejunum and is absorbed within the first 2 h after ingestion. This was demonstrated by a rapid rise of folate in serum in the first 1–2 h after ingestion of PGA, followed by a rapid fall of SFA. In newborns, since gastric emptying time is prolonged [10], the ingested PGA enters the jejunum more gradually, demonstrated by a lower peak serum folate and an almost horizontal absorption curve between the 2- and 5-h sample.

The difference between the folate absorption curve of newborns and adults could also be explained by impaired absorption of PGA in the jejunum of the newborns with compensatory absorption of remaining folate in the ileum. This could explain the low peak and flatness of the absorption curve in the newborns. At the present time we have no data to rule in or out the latter possibility and we do not know of any practical way of studying this possibility. Since the folate absorption curve depends on the rate of folate absorption, tissue uptake, and the urinary excretion of folate, we did not think that direct administration of PGA in the duodenum could provide significant additional data to justify intubation of many normal adults and newborns.

The absorption of food folate is more complicated than that of PGA. The major portion of food folate is a polyglutamate [7, 9, 43, 45]. The polyglutamates have to be broken down to monoglutamates by deconjugating enzymes of the intestinal mucosa before they are absorbed [3, 6, 8, 29, 43, 44]. We have no data with regard to the efficiency of the absorption of food folate in newborns and have not been able to find any in the literature.

In the present report we have by no means answered the question of folate absorption in the newborn. We have merely shown that the absorption of PGA in newborns was slower than that found in adults, but that the net intestinal absorption of PGA in newborns was comparable to that of adults. We have also shown the very rapid plasma clearance of folic acid in newborns [47]. We have shown that this rapid plasma clearance was due to rapid tissue uptake of folate (large doses of folate were needed to saturate the tissues of the newborn) and to slow the folate clearance rate in newborns. These peculiarities should be considered in any study relating to folate absorption or to the metabolism of folic acid in newborns and during the period of infancy. These findings confirm that data obtained from the study of normal adults cannot be extrapolated to infants. For any group of infants studied there should be a suitable control group matched for age, weight, and state of growth before one can interpret the data and draw a meaningful conclusion.

Summary

The absorption and excretion of folic acid in urine were studied in adults and newborns. Based on the rise in the folate level in serum and excretion of folate in urine after an oral or intramuscular dose of 40 μg PGA/kg, it was shown that: *1)* the rise in serum folate following an oral test dose was much less in newborns than in adults; *2)* the excretion of folate in the urine following an oral dose of PGA was less by the newborns than by the adults; *3)* the plasma clearance of folic acid was more rapid in newborns than in adults; *4)* in newborns, the daily rise of serum folate following 40 μg PGA/kg/24 h orally was similar to that following 30 μg PGA/kg/24 h intramuscularly; and *5)* the absorption of PGA in newborns was slower than in adults, but the net absorption of folic acid by the newborn was comparable to adults.

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