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Folate metabolism pteroylglutamic acid newborns

Folate Metabolism in Newborns and during Early Infancy

II. Clearance of Folic Acid in Plasma and Excretion of Folic Acid in Urine by Newborns

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Extract

Clearance in plasma of pteroylglutamic acid (PGA) was determined in human adults and newborns. The doses of PGA given to adults were 7.5 and 15 μg/kg and those given to newborns were 15 and 30 μg/kg of body weight, respectively. Microbiological assays using *Lactobacillus casei* determined the total folate in serum as well as the amount of unchanged PGA following intravenous administration of PGA. Excretion of folate was determined in adults and newborns receiving 40 μg PGA/kg intramuscularly.

Clearance of folic acid in plasma was found to be much more rapid in newborns than in adults (figs. 1 and 2). Less folate was excreted in the urine by the newborns (table I) which indicated that rapid clearance of folic acid in plasma in the newborns was due to rapid tissue uptake of folate.

Speculation

The rapid tissue uptake of PGA in newborns indicates the markedly increased demand for folic acid in the neonatal period and during the period of early infancy, when there is a rapid rate of cell replication and growth. This finding explains the rapid fall of serum [20] and blood folate levels [16] in the first few months of life when the increased demand for folate is not met by the dietary folate. Further studies are needed to determine folate stores in newborns, the dietary intake of folate by newborns, and the folate requirement of newborns and infants.

Introduction

Reported studies [21] have shown that the peak folate level in serum after an intramuscular dose of PGA was lower in newborns than in adults, suggesting a more rapid clearance of folic acid in the newborn infant. The experiments reported in this present paper were designed to study the clearance of folic acid in plasma in the newborn and to see whether the rapid clearance was due to rapid tissue uptake or increased loss in the urine. They were also designed to see whether there was any block in the utilization of folic acid in the newborn period when the folate level in serum was very high [5, 9, 16, 20].

Materials and Methods

Subjects were 12 normal adult volunteers (hospital personnel) and 9 normal full-term newborns (6–12 days old) [25]. The technique of blood collection and the source of folic acid for standard and test doses have

been described [21]. The PGA was administered to the adults through an antecubital vein; all blood samples for study were taken from the opposite arm. The PGA was administered to the newborns through a scalp vein and samples for study were taken by heel prick. One–1.5 ml of blood were taken by heel prick for assay of folate in serum. The duration for collection of blood was generally about 60 sec.

Excretion of folate was determined in a 6-h urine sample following intramuscular administration of 40 μg PGA/kg. A period of 6-h was chosen because it was easier and more practical in the newborns. The intramuscular route was chosen in preference to the intravenous route because of ease of administration and because data for intramuscular PGA were needed for another project [21]. Adults emptied their bladder and then received the test dose of PGA. Urine was collected 6 h after administration of the test dose. Test doses were given to the newborns shortly after they had voided; individual 6-h urine samples were collected in brown bottles by external drainage. All urine and serum samples were kept at –20° and were assayed within 3 weeks.

Serum folate was determined by *L. casei* microbiological assay using a minor modification of the technique of WATERS and MOLLIN [23]. Serum was stored at –20° and reconstituted with ascorbic acid powder (5 mg/ml of serum) on the day of assay. The folic acid assay medium was obtained commercially [26].

Stable serum folate was assayed in the same manner as the folate, using *L. casei* except that no ascorbic acid was added to the serum, buffer, or culture media. The major part of the folate in serum is heat labile and unless protected by ascorbic acid will be destroyed during the process of autoclaving for deproteinization and subsequent sterilization [6, 11, 12, 23]. The amount of stable folate determined in all subjects before PGA injection was less than 2 ng/ml (mostly less than 1 ng/ml). After intravenous administration of PGA, the detectable amount of stable folate is almost identical to that obtained by assay with *Streptococcus faecalis* [22]. The assay for stable folate in serum was used in preference to *S. faecalis* assay because it was easier and more economical and would allow the simultaneous determination of total, stable, and labile serum folate in the same run of assay (using the same standard, culture media, and organism).

Results

Plasma Clearance of Intravenously Administered PGA

The clearance of PGA was determined in 12 adults and 9 full-term newborns. Originally, samples of blood were taken at zero time (just before injection of PGA), 5, 15, 30, and 60 min after the test dose of 15 μg PGA/kg. In subsequent experiments, however, since the clearance curve of plasma in newborns was very low, samples were taken at 5, 10, and 20 min and an attempt was made to give smaller doses to the adults (7.5 μg/kg) and larger doses to the newborns (30 μg/kg), to see whether we could obtain comparable curves. Figure 1 shows the clearance of stable folate in serum (unchanged PGA) and figure 2 shows the rise in serum *L. casei* activity after

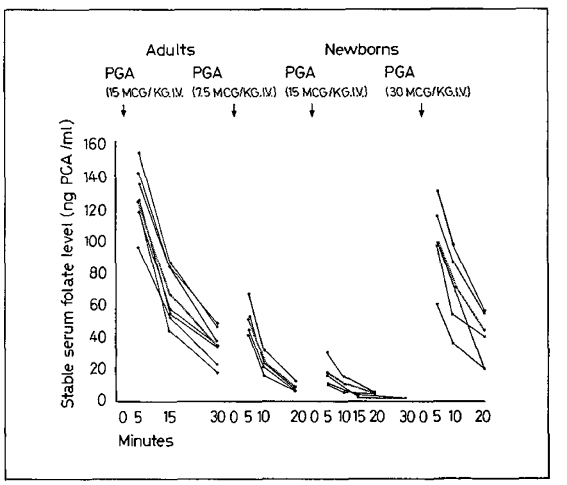

Fig. 1. Clearance rates of intravenously administered doses of PGA in plasma. The stable folate in serum represents the amount of unchanged PGA. Dotted lines represent the mean.

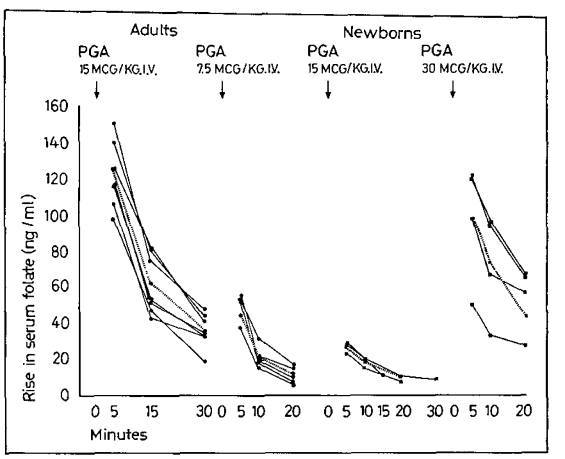

Fig. 2. Clearance rates of intravenously administered doses of PGA in plasma. The original folate levels in serum have been deducted from the levels obtained after intravenous administration of PGA to show the rise in folate levels in serum attributed to PGA. Dotted lines represent the mean.

intravenous administration of PGA. Clearance rates of PGA in the plasma of newborns were much faster than that seen in adults. The mean rise of serum folate in adults, 5 min after the test dose (15 μg/kg) was 122.7 ng/ml and in newborns, 26.5 ng/ml. The levels for stable folate (unchanged PGA) in adults and newborns were 124.7 and 16.5 ng/ml, respectively. At 5 min, after a 15-μg PGA/kg test dose, the mean rise in the level of folate in serum of newborns was 26.5 ng/ml, which was much less than that found in adults (45.2 ng/ml) after a test dose of 7.5 μg/kg PGA. In turn, the mean value of 98 ng/ml found after administration of 30 μg PGA/kg in newborns was less than that found in adults (122.7 ng/ml) after administration of 15 μg PGA/kg. Sixty minutes after intravenous administration of 15 μg PGA/kg to adults the average rise of folate in serum was 21.7 ng/ml and the amount of unchanged PGA was 18.2 ng/ml (not shown in figures). The decay curves following administration of 15 μg PGA/kg to the newborn, shown in figures 1 and 2, appeared more flat than those in adults. This was because most of the folate had already been cleared from the plasma of the newborns by the time sample 1 was taken (at 5 min) (also the time intervals for sampling are different). Considering the average plasma volume of the adult as 45 ml/kg and that of the newborn as 41 ml/kg [18], then the mean rise of folate in serum of adults receiving 15 μg PGA/kg intravenously at 5 min (122.7 ng/ml), 15 min (62.9 ng/ml), 30 min (36.5 ng/ml), and 60 min (21.7 ng/ml) represents 36.8, 18.8, 10.9, and 6.5% of the injected dose, respectively. In the newborns receiving 15 μg PGA/kg, the mean rise in serum folate at 5 min (26.5 ng/ml), 10 min (17.5 ng/ml), and 20 min (9.5 ng/ml) represents only 7.5, 4.8, and 2.6% of the injected dose, respectively. The mean level of stable folate in serum of adults receiving 15 μg PGA/kg, at 5, 15, 30, and 60 min represents 37.2, 19.5, 10.2, and 5.5% of the injected dose, respectively. The mean level in the newborn at 5, 10, and 20 min represents 4.5, 2.8, and 1% of the injected dose, respectively.

Table I. Excretion of folate in urine 6 h after intramuscular administration[1]

	% Excreted		
	Adults	Newborns	
		Full-term	Premature
	31.1	13.5 (4.0, 9)[2]	8.3 (2.0, 9)[2]
	31.7	20.4 (2.9, 9)	16.3 (2.0, 7)
	32.2	29.6 (3.4, 7)	17.7 (2.1, 9)
	34.1	31.8 (3.0, 11)	—
	26.1	—	—
Mean	31.0	23.8	14.1

[1] Dose was 40 μg PGA/kg.

[2] Numbers in parentheses indicate, respectively, the birth weight, in kilograms, and the age of the subject, in days, at the time of the experiment.

Excretion of Folic Acid in the Urine

Table I shows the amount of folate (*L. casei* active) excreted by adults and newborns in urine after an intramuscular dose of 40 μg PGA/kg body weight. Although the number of subjects studied was too small for statistical evaluation, it appeared that the newborns tended to excrete a lesser percentage of the administered folate in their urine. Table I also shows excretion rates in three premature infants for comparison with those of full-term infants and adults and also for comparison with the reported data [21].

Discussion

The clearance rates in plasma of intravenously injected PGA change depending on the dose of PGA, the time of sampling, and the status of the body tissue saturated with folate [4, 15, 24]. The smaller the dose the more rapid the initial plasma clearance. It was difficult to obtain an accurate clearance rate of PGA in plasma in the newborn from the data shown in figures 1 and 2. It can be seen that with a high dose of PGA (30 μg/kg) most of the PGA was cleared from the blood at the time of the first sampling (only 13.5% of the injected dose remained in the plasma after 5 min). When we compared the data of the two groups receiving 15 μg PGA/kg, we calculated that 36.8% of the injected PGA remained in the plasma of adults 5 min after administration, whereas only 7.5% was present in the plasma of the newborns. The amount remaining in the plasma of the newborns 5 min after injection of PGA (7.5% total folate and 4.5% stable folate) was almost equal to that remaining in the plasma of adults 60 min after injection of the same dose of PGA (6.5% total folate and 5.5% stable folate), indicating a remarkably rapid clearance rate of PGA in plasma in the newborn. Since the amount of folate excreted in the urine after an intramuscular injection of PGA was no greater in newborns than in adults (table I), we must assume that the very rapid clearance of PGA in plasma in newborns was due to rapid tissue uptake of folate.

Considering the rapid rate of cell replication and growth in newborns, the discovery of rapid tissue uptake of folate in newborns is not unexpected. Its magnitude and the very large amount of folate required to slow this rapid clearance, however, was surprising.

The rapid clearance of folate in plasma in newborns, when the serum folate is higher than in adults [2, 5, 9, 20], eliminates the possibility of any blockage in the

utilization of folate in the newborn period. If this rapid tissue uptake of folate by the newborn is taken as an indication of tissue need, it could explain the subsequent fall of folate levels in serum and blood [16, 20] in early infancy when the folate supply in the infant's diet cannot meet the tremendous demand.

Clearance of folic acid in plasma in man has been studied by many investigators [1, 3, 7, 8, 10, 13, 14, 17, 19, 24]; however, these have generally been in adults. HIRATA and ARAKAWA [13] have reported clearance of folic acid in plasma in children, but their study involved, for the most part, patients and older children. We do not have any data regarding clearance of folate in older infants and children and we do not know when the clearance rate becomes comparable to that of adults or older children. It is safe, however, to assume this would occur gradually.

This rapid clearance of folate in plasma should always be considered when interpreting the data in regard to folate clearance, absorption, or excretion in infancy. No meaningful interpretation of these data can be made unless data from normal controls of similar age and weight are available for comparison.

Summary

The clearance of varying doses of pteroylglutamic (folic) acid in plasma was determined in adults and newborns. Folic acid that was administered intravenously was cleared much faster in newborns than in adults. Lower excretion of folate in the urine of the newborns indicated that this rapid clearance was due to rapid tissue uptake of folate in the newborns.

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25. Informed consent was obtained for all subjects in this study.
26. PGA Medium, Baltimore Biological Laboratories.
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