

**PROCEEDINGS OF
THE FLORIDA
SYMPOSIUM ON
MICRONUTRIENTS
IN HUMAN
NUTRITION**

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ON

MICRONUTRIENTS IN HUMAN NUTRITION

EDITORS

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PREFACE

The Florida Symposium on Micronutrients in Human Nutrition, held on the University of Florida campus in Gainesville on February 17 & 18, 1981, provided a unique opportunity for interaction among individuals interested in human nutrition research. Symposium participants included dietitians, physicians and scientists in nutrition and nutrition-related disciplines. More than 110 persons attended this exciting two-day conference which addressed issues and problems relevant to human nutrition.

The symposium emphasized current research in iron, zinc, folacin and vitamin B₆. The important role of these nutrients in human nutrition and the potential for inadequate intakes among certain subsets of the American population are now widely recognized. Symposium papers published in this Proceedings approach these four micronutrients from a human nutrition perspective with particular emphasis on current research questions and controversies. Throughout the Symposium, speakers and participants recognized the need for much more research to define human micronutrient requirements, their bioavailability from an increasingly complex food supply and the health consequences of marginal intakes.

Proceedings of the Florida Symposium on Micronutrients in Human Nutrition will be provided free-of-charge to persons who registered for the Symposium. Others may purchase the Proceedings at a cost of \$7.50 each from Dr. James Kirk, Chairman; Food Science and Human Nutrition Department, Institute of Food & Agricultural Sciences; University of Florida, Gainesville, FL 32611.

The program was developed by Symposium Chairman, James R. Kirk, in cooperation with the Micronutrients Symposium Planning Committee: James S. Dinning, Chairman; Patricia A. Wagner; Lynn B. Bailey; George K. Davis; Ray L. Shirley and Jesse F. Gregory III. Appreciation is expressed to all those who assisted in the preparation and organization of the Symposium.

Patricia A. Wagner and James R. Kirk

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CONTENTS

Preface.....	iii
Contents.....	v
List of Contributors.....	vii

I. VITAMIN B₆ , Session Chairman, James R. Kirk

Relationship of Vitamin B ₆ Nutriture During Pregnancy and, Lactation to Vitamin B ₆ Adequacy in the Breast-fed Infant, Avanelle Kirksey.....	1
Vitamin B ₆ Metabolism in Liver and Blood Plasma, Lawrence Lumeng.....	11
Food Composition and Processing Effects on Vitamin B ₆ Bioavailability, Jesse F. Gregory, III.....	23

II. ZINC, Session Chairman, George K. Davis

Zinc in Human Nutrition, Kenneth M. Hambidge.....	35
Zinc in Prenatal and Neonatal Nutrition, Lucille S. Hurley.....	47
Interaction Among the Micronutrients: Zinc, Iron and Vitamin B ₆ , Gary W. Evans.....	55
Zinc Status of Elderly and Adolescent Populations, Patricia A. Wagner.....	65
The Relationship of the Pancreas to Zinc Metabolism: Evidence For Zinc Deficiency and Zinc-Responsive Retinal Dysfunction in Patients with Chronic Pancreatitis, Phillip P. Toskes.....	77

III. IRON, Session Chairman, Ward D. Noyes

Iron Requirements in Man: An Overview,

George H. Beaton.....91

Dietary Inhibitors of Iron Absorption in Man,

James D. Cook.....99

Iron Overload: Clinical and Therapeutic Aspects,

Richard R. Streiff.....107

Folacin and Iron Status and Hematological Findings in

Adolescents, Lynn B. Bailey.....113

IV. FOLACIN, Session Chairman, James S. Dinning

Folic Acid in Human Nutrition,

Victor Herbert.....121

The Metabolic Relationship Between Folic Acid,

Vitamin B₁₂, Methionine and Thyroxine,

E. L. Robert Stokstad.....139

Folacin and Iron Status and Hematological Findings in an

Elderly Population,

Patricia A. Wagner.....149

Intestinal Absorption and Malabsorption of Folic Acid,

James J. Cerda.....155

RELATIONSHIP OF VITAMIN B-6 NUTRITURE DURING PREGNANCY AND
LACTATION TO VITAMIN B-6 ADEQUACY IN THE BREAST-FED INFANT

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The importance of vitamin B-6 for normal central nervous system (CNS) function has been known since the classical study of Snyderman et al. (1953) where the essentiality of the vitamin was determined by feeding a vitamin B-6 free diet to two infants. One of the infants developed seizures, clonic and tonic in character, which were preceded by shrill cries, accompanied by cyanosis and eventually comatosis. However, the infant recovered within 24 h after the intravenous administration of 50 mg pyridoxine·HCl. Coursin (1954) in another study, described the type of seizure observed in an infant fed a formula in which most of its vitamin B-6 had been destroyed by improper autoclaving. The eyes, fixed and staring with pupils contracted, showed no reaction to light while the body, held rigidly with the head thrown back, exhibited repetitive convulsive movements with an occasional outburst of an abnormally high-pitched cry. Marked EEG alterations accompanying the seizures returned to normal within 4 to 5 min after an intramuscular injection of 100 mg pyridoxine·HCl. The rapidity of response to vitamin B-6 therapy seen in these studies suggests a neurochemical lesion.

Bessey et al. (1957) reported that nine infants receiving less than 85 µg vitamin B-6/l of milk developed grand mal seizures. When vitamin B-6 intake was increased, the convulsions ceased; however, it is not known if a CNS lesion remained. These workers concluded that infants need at least 200 µg vitamin B-6 daily. West and Kirksey (1976), in this laboratory, observed that the mother of a breast-fed infant who suffered mild periodic seizures the first weeks of life had a low vitamin B-6 intake (1.5 mg/day). Analysis of the

mother's milk for vitamin B-6 showed that it contained only 67 $\mu\text{g/l}$ at one month postpartum. After the introduction of cereal at this time, the infant became asymptomatic. Cereal could contribute an additional 20 to 60 μg of vitamin B-6/day. We postulate that the mother's low intake of vitamin B-6 in conjunction with low tissue reserves following long-term (3 1/2 yr) use of oral contraceptives (OC) resulted in poor maternal vitamin B-6 nutriture. This was reflected in inadequate levels of vitamin B-6 in her milk for her infant.

Recently, Kirksey and Roepke (1981) determined levels of vitamin B-6 in the biological fluids of a 36-year-old primigravida and her breast-fed infant who developed symptoms of a CNS disorder at 7 days of age (Table 1). The symptoms included hyperactivity, shrill cries and rigid opisthotonos with transient cyanosis in the absence of other signs of illness. The mother had used OC for 10 yr prior to pregnancy and had discontinued OC for only a few weeks prior to conception. At delivery, vitamin B-6 levels in maternal and cord plasma were approximately one-half the expected levels and at 7 days postpartum, vitamin B-6 content in milk was very low. Also at this time, the level of plasma pyridoxal phosphate (PLP) of the infant was extremely low and neurological symptoms were evident. The mother's pyridoxine supplement was increased immediately from 5 to 20 mg/day. Subsequently the infant became asymptomatic without medication. At 17 days postpartum, both maternal and infant parameters of vitamin B-6 were improved markedly.

TABLE 1. LEVELS OF VITAMIN B-6 IN BIOLOGICAL FLUIDS OF A MOTHER AND HER BREAST-FED INFANT WHO SHOWED SYMPTOMS OF A CENTRAL NERVOUS SYSTEM DISORDER AT 7 DAYS OF AGE

	5 MONTHS GESTATION ¹	DELIVERY	POSTPARTUM		
			7 DAYS ²	17 DAYS	6 WEEKS
MOTHER ³		PLP, NG/ML			
PLASMA	24.9	3.4	-	33.5	51.0
		VITAMIN B-6, $\mu\text{G/ML}$			
MILK	-	-	16	204	255
INFANT		PLP, NG/ML			
PLASMA	-	20.3 ⁴	2.8	51.5	-

¹MOTHER SUPPLEMENTED WITH 5 MG PYRIDOXINE·HCL/DAY FROM 3 MONTHS GESTATION

²MOTHER'S SUPPLEMENT CHANGED FROM 5 TO 20 MG PYRIDOXINE·HCL/DAY

³USED ORAL CONTRACEPTIVES FOR 10 YEARS PRIOR TO PREGNANCY

⁴CORD PLASMA

EFFECT OF MATERNAL VITAMIN B-6 DEFICIENCY ON CENTRAL NERVOUS SYSTEM DEVELOPMENT IN RATS

Neurological disorders, similar to those reported for human infants, have been observed frequently in our laboratory in the offspring of rats fed vitamin B-6 deficient diets throughout growth, gestation and lactation (Thomas and Kirksey, 1976; Kirksey and Susten, 1978; and Morré and Kirksey, 1978). The characteristic symptoms develop between 10 to 18 days postnatally and include high pitched cries, tremors, abnormal running movements and epileptiform convulsions followed by coma, and in most cases, death. The critical metabolic pathways and/or morphological changes affected primarily by vitamin B-6 deficiency and resulting in the neurological symptoms remain unidentified. Our studies are part of a series of long term investigations concerned with the resolution of this problem and are the first to use a correlative biochemical-morphological approach to examine specific areas of developing brain and spinal cord in vitamin B-6 deficiency.

Our findings (Kirksey et al. 1979) showed that regional levels of PLP, the active form of vitamin B-6, in CNS of progeny generally paralleled the level of pyridoxine intakes of dams. By 15 days of age, PLP content was significantly decreased in cerebellum, corpus striatum and upper cervical spinal cord in progeny of vitamin B-6 deficient mothers compared to controls. These findings suggest that regional differences in PLP content within the CNS may operate to potentiate the expression of deficiency symptoms. The content of myelin lipids, gangliosides and cerebroside in brain of progeny during the suckling period paralleled maternal intakes of vitamin B-6 (Thomas and Kirksey, 1978). Electron micrographs of the medio-dorsal portion of the pyramidal tract showed fewer myelinated axons in progeny of vitamin B-6 deficient rats (Morré et al., 1978). Quantitative electron microscopy of axon diameters in the ventral funicular portion of cervical spinal cord of 15 day-old progeny of vitamin B-6 deficient rats showed fewer large axons (Kirksey et al., 1980) with a decreased lamellae number in relation to axon size compared to controls (Fig 1 A,B). These changes were correlated with changes in the specific activity of 2',3'-cyclic nucleotide 3'-phosphohydrolase, a myelin marker enzyme. Additionally, the monolayer arrangement of Purkinje cells in cerebellum was abnormal (Morré et al., 1978) and Purkinje cell dendritic trees were stunted in vitamin B-6 deficiency (Fig. 1 C,D). Dendritic fields of

Purkinje cells were smaller in width, height and area in the deficiency and stereological measurements showed that dendritic branching density was less in progeny of vitamin B-6 deficient mothers (Chang et al., 1981).

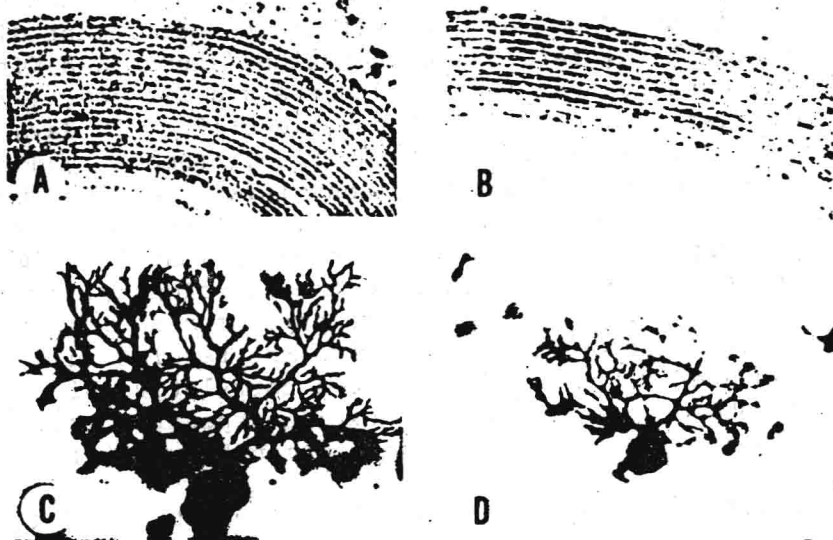


Fig. 1. Myelin lamellae of axons (2.0 μ diameter) in cervical spinal cord (X144,000) and Purkinje cell dendritic trees (X250) in 15-day old progeny of vitamin B-6 control (A,C) and deficient (B,D) rats.

The far-reaching consequences of vitamin B-6 inadequacy during early infancy on the developing CNS are evident from both human and animal studies. In view of the American Academy of Pediatric's (1978) recommendation that human milk be used as the sole source of nutrients for infants during the first four to six months, our efforts are directed toward the assessment of the vitamin B-6 content and volume of human milk as it relates to infant needs.

RESPONSE OF HUMAN MILK TO CHANGES IN VITAMIN B-6 INTAKE

Vitamin B-6 content in human milk responds rapidly to changes in vitamin B-6 intake since regulatory mechanisms apparently are not present for maintaining the concentration of the vitamin within definite limits. Karlin (1959) observed that within one hour after an intramuscular injection

of pyridoxine into five lactating women, the level of vitamin B-6 in milk rose to six times the initial level. The highest concentration, ten times the initial level, was reached 3 h after the injection. After 24 h, the concentration of vitamin B-6 in milk was more than three times the initial level and did not return to the original level for 5 to 7 days. Similarly, Karlin (1959) found that following the oral administration of a single large dose of pyridoxine (500 or 1000 mg) to three lactating subjects, the vitamin appeared rapidly in the milk. Approximately 3 h after the oral dose, a peak of 9,000 to 11,500 μg vitamin B-6/l milk, or about 100 times the initial level, was reached. The vitamin content decreased rapidly in the next 9 h but remained elevated for 2 to 7 days at about twice its original level. Findings from our laboratory (Kirksey et al. 1981) indicate that the vitamin B-6 content in human milk responds also to small oral doses of vitamin B-6. Approximately 5 h after lactating women took oral supplements of 2 or 20 mg pyridoxine·HCl levels of vitamin B-6 in milk doubled (200 to 425 $\mu\text{g}/\text{l}$) or tripled (450 to 1325 $\mu\text{g}/\text{l}$), respectively. Approximately 12 h after the doses, levels of vitamin B-6 in milk remained slightly elevated above initial levels.

In contrast to the elevated levels of vitamin B-6 in milk following oral doses of the vitamin, extremely low levels of the vitamin have been observed in human milk when the intake of vitamin B-6 is low. In this laboratory, West and Kirksey (1976) found less than 50 μg vitamin B-6/l in the milk of a subject whose intake of vitamin B-6 was estimated to be only 1.5 mg/day. Hence, less than 4% of this woman's daily intake of vitamin B-6 appeared in her milk, an inadequate amount of vitamin B-6 for her infant. These data indicate the inability of mammary gland to concentrate vitamin B-6 in milk when the intake of the vitamin is deficient. These findings are supported by rat studies. Following a short period (6 days) of vitamin B-6 deficiency during lactation in the rat, the vitamin B-6 content in milk decreased to less than 20% of control values (Felice and Kirksey, 1981). In muscle, vitamin B-6 concentration was not significantly altered by the short period of dietary inadequacy and in liver was only slightly decreased. The early failure of these tissues to supply vitamin B-6 to milk, when vitamin B-6 intake was inadequate, support our postulations that mammary gland does not concentrate the vitamin and that milk is an early indicator of impending vitamin B-6 inadequacy (Kirksey and Susten, 1978).

RELATIONSHIP OF MATERNAL TO INFANT INTAKES OF VITAMIN B-6

Kirksey et al. (1981) determined breast-fed infant's intakes of vitamin B-6 from milk volume measurements estimated by differences in infant weights prior to and after each feeding for a 24 h period. Increase in weight of the infant was assumed to represent the weight of milk consumed by the infant and was converted to volume by use of specific gravity measurements. Vitamin B-6 content was determined for samples of milk obtained at each feeding and multiplied by milk volume intake to estimate vitamin B-6 intakes of infants. Preliminary data indicated that intakes of vitamin B-6 by breast-fed infants paralleled maternal supplements of 2.5, 10.0 and 20.0 mg pyridoxine·HCl/day (Table 2). Infant intakes of vitamin B-6 were about doubled by maternal pyridoxine supplements of 10.0 mg/day and were quadrupled by supplements of 20.0 mg/day compared to values when maternal supplements were 2.5 mg/day. After a non-supplemented mother (Subject E) was given a supplement of 20 mg pyridoxine, her breast-fed infant's intake of vitamin B-6 increased about six-fold, 0.07 to 0.44 mg/day (Table 2). Similarly, when pyridoxine supplements for Subject F were increased from 2 to 20 mg/day, her infant's intake of vitamin B-6 was increased about three-fold, 0.12 to

TABLE 2. VITAMIN B-6 CONTENT IN MILK OF WOMEN WHO RECEIVED NONE OR VARIED LEVELS OF VITAMIN B-6 SUPPLEMENTS AND THE VOLUME OF MILK AND VITAMIN B-6 INTAKES OF THEIR INFANTS

SUBJECT	DAY	VIT B-6 SUPPL ¹	MILK VITAMIN B-6 CONTENT		INFANT INTAKE		
			MG/DAY	UG/L	AVG	VOLUME OF MILK ML/DAY	VITAMIN B-6 MG/DAY AVG
A	1	0	84			475	0.04
	2	0	130	107		660	0.09
B	1	2.5	130			616	0.08
	2	2.5	177	154		645	0.12
C	1	10.0	240			655	0.15
	2	10.0	247	244		896	0.22
D	1	20.0	575			820	0.46
	2	20.0	567	571		799	0.46
E	1	0	79			590	0.05
	2	0	141	110		580	0.08
	3	20.0	778			640	0.50
	4	20.0	656	717		615	0.37
F	1	2.0	249	249		465	0.12
	2	20.0	636			630	0.41
	3	20.0	577	607		670	0.39

¹ PYRIDOXINE·HCL

0.40 mg/day. Vitamin B-6 intakes of two breast-fed infants, whose mothers did not take pyridoxine supplements, ranged from 0.04 to 0.09 mg/day (Table 2), levels which were associated previously with grand mal seizures in infants (Bessey et al., 1957).

Milk volume was not altered by supplements ranging from 2.5 to 20 mg pyridoxine (Table 2). This is in contrast to Greentree's (1979) warning that vitamin B-6 is a lactation-inhibiting vitamin and should be excluded from multi-vitamin supplements for nursing mothers. Conflicting reports exist as to whether even pharmacologic doses of pyridoxine (200 to 600 mg) decrease prolactin levels. Presently, there is no evidence that physiologic doses of vitamin B-6, as contained in major prenatal formulations, have hypoprolactinemic or anti-lactogenic effects. This is supported by findings from our laboratory in which 2.0 to 20 mg pyridoxine supplements were used.

MATERNAL VITAMIN B-6 NUTRITURE AND INFANT PEDIATRIC RATINGS

Vitamin B-6 nutritional status appears to influence significantly the vitamin B-6 content of human milk. Our findings (Roepke and Kirksey, 1979) showed that maternal nutritional status, assessed by the level of vitamin B-6 in maternal serum at 5 months gestation, was significantly correlated ($r = 0.51$) with the level of vitamin B-6 in milk at 14 days postpartum. Previous long-term use (>30 months) of OC was associated with reduced levels of vitamin B-6 in maternal serum at 5 months gestation and at delivery in cord serum and milk (Roepke and Kirksey, 1979a). The mean level of vitamin B-6 in maternal serum at delivery for OC users was only 4.8 ng/ml approximately 30% that of nonusers while cord serum of OC users was about 50% that of nonusers (Table 3). The higher ratio of vitamin B-6 in cord to maternal serum for OC users (4.2) compared to nonusers (3.4) suggested a drain of maternal reserves of vitamin B-6 to the fetus. This was reflected in the marked decrease in vitamin B-6 levels in maternal serum of OC users at delivery. The shunting of vitamin B-6 to the fetus by OC users suggested an adaptive response to vitamin B-6 inadequacy in an attempt to protect the fetus.

Levels of vitamin B-6 in milk were similar in the early postpartum period (3 days) among women grouped according to OC use but were significantly different at 14 days postpartum (Table 3). At this time, the level of vitamin B-6/l milk was

TABLE 3. RELATION OF PREVIOUS USE OF ORAL CONTRACEPTIVES TO VITAMIN B-6 CONTENT IN SERUM AND MILK AT DIFFERENT STAGES OF PREGNANCY AND LACTATION

VITAMIN B-6 MEASUREMENTS	ORAL CONTRACEPTIVE USE		SIGNIFICANCE
	NONE	>30 MONTHS	
INTAKE, MG/DAY			
DIET AND SUPPLEMENTS	6.6 ± 1.1	6.7 ± 1.3	NS
MATERNAL SERUM, NG/ML			
5 MO. GESTATION	16.6 ± 1.9	8.1 ± 2.0	P<0.004
DELIVERY	15.7 ± 2.2	4.8 ± 1.4	P<0.001
CORD SERUM, NG/ML	26.0 ± 2.4	15.3 ± 3.6	P<0.02
MILK, UG/L			
3 DAYS POSTPARTUM	17.0 ± 4.5	18.2 ± 7.3	NS
14 DAYS POSTPARTUM	65.0 ± 10.9	26.6 ± 5.1	P<0.003
MILK, UG/G PROTEIN			
3 DAYS POSTPARTUM	0.9 ± 0.3	0.8 ± 0.4	NS
14 DAYS POSTPARTUM	5.3 ± 1.1	2.2 ± 0.6	P<0.01
MEAN ± SE FOR 8 TO 46 SUBJECTS			

only 26 µg for long-term OC users or approximately one-half that of nonusers. The ratio of vitamin B-6/protein in milk also was significantly lower for OC users compared to nonusers. These findings raise pertinent questions about the adequacy of vitamin B-6 especially relative to the protein content in milk of long-term OC users.

Roepke and Kirksey (1979) observed that levels of vitamin B-6 in maternal serum at delivery and in milk at 3 and 14 days postpartum were significantly lower for mothers whose infants had unsatisfactory Apgar scores (<7) at 1 min after birth compared to values for mothers whose infants had satisfactory ratings. These data suggested that vitamin B-6 nutritional status was poorer for mothers whose infants had low Apgar scores than for mothers whose infants received acceptable ratings. The mean intake of vitamin B-6 (4.2 mg/day) of women whose infants had low Apgar scores was considerably greater than the RDA of 2.6 mg/day. Our findings suggest that intakes of vitamin B-6 greater than 4 mg/day may be needed on a regular basis by some women during pregnancy.

SUMMARY

Because of the widespread occurrence of vitamin B-6 in foods, a human deficiency of this vitamin has been considered unusual. However, it is not known the extent to which marginal