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Full Name of Author — Nom complet de l'auteur

Marian Kim Brunet

Date of Birth — Date de naissance

July 10, 1954

Country of Birth — Lieu de naissance

CANADA

Permanent Address — Résidence fixe

1405 - 105 St.
EDMONTON, ALTA
T6J 5P3

Title of Thesis — Titre de la thèse

Vitamin B-6 Intake and Blood Levels in Breast- and Formula-Fed Infants

University — Université

ALBERTA

Degree for which thesis was presented — Grade pour lequel cette thèse fut présentée

MSc.

Year this degree conferred — Année d'obtention de ce grade

1984

Name of Supervisor — Nom du directeur de thèse

Dr. E.A. DONALD

Date

Apr. 24, 1984

Signature

M.K. Brunet

UNIVERSITY OF ALBERTA

VITAMIN B-6 INTAKE AND BLOOD LEVELS IN BREAST- AND FORMULA-FED INFANTS

by

M. KIM BRUNET

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
AND RESEARCH IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

IN
NUTRITION

FACULTY OF HOME ECONOMICS

EDMONTON, ALBERTA

Spring, 1984

THE UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR M. Kim Brunet

TITLE OF THESIS Vitamin B-6 Intake and Blood Levels in Breast- and Formula-Fed Infants

DEGREE FOR WHICH THESIS WAS PRESENTED M.Sc.

YEAR THIS DEGREE GRANTED 1984

(Signed) M K Brunet

PERMANENT ADDRESS:

1405 - 105 Street

Edmonton, Alberta

DATED April 24 19 84

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Vitamin B-6 Intake and blood levels in breast-and formula-fed infants" submitted by M. Kim Brunet, B.Sc., in partial fulfilment of the requirements for the degree of Master of Science in Nutrition.

Elizabeth A. Donald
Supervisor

Date

ABSTRACT

The purpose of this study was to document vitamin B-6 intakes and blood levels in breast- and formula-fed infants to determine if any differences existed. Twenty-seven mothers, ranging in age from 17 - 33 years, volunteered their healthy infants and themselves for participation. The infants were classified into 3 feeding groups; breast-fed (group I), proprietary formula-fed (group II) and non-proprietary formula-fed (group III). The mean age of group I and II infants was 12.4 ± 1.6 weeks and 12.1 ± 2.4 weeks, respectively. These means were not significantly different ($p<0.05$). Due to the small sample size in group III comparative statistical analyses were not done.

Dietary intakes for the breast-fed infants were determined by weighing the infant before and after feeding, the difference represented the weight of breast-milk ingested. At each feeding, samples of fore-milk were collected by the mothers. Breast milk samples were analyzed for protein and vitamin B-6. The B-6 vitamers were quantitated by paired-ion reverse phase high pressure liquid chromotography (HPLC) and total vitamin B-6 content was calculated. Proprietary formula-fed (group II) intakes were determined by having the mother measure and record the volume ingested. Beikost, if taken, was recorded by the mothers on food records. None of the infants consumed vitmain B-6 supplements. Fasting blood samples were collected from the mothers and infants in heparinized capillary tubes to which phosphatase inhibitor was pre-added. The B-6 vitamers were quantitated using the same method as described for breast-milk.

Vitamin B-6 intake (mg) expressed per gram of protein ranged from 0.004 - 0.031 mg per gram with a mean of 0.013 mg/g per day in breast-fed infants (group I). This was significantly lower ($p<0.01$) than the mean intake of 0.031 mg/g/day observed in infants fed a proprietary formula (group II). Intake for Group II ranged from 0.027 - 0.040 mg/g/day.

The mean whole blood pyridoxal phosphate (PLP) level in breast-fed infants was 11.47 ± 4.61 ng/ml which was lower than the mean of 16.11 ± 3.11 ng/ml observed in proprietary formula-fed infants. These means, however were not significantly different ($p<0.05$). Half of the infants in group I had PLP levels in a very low range indicating that their vitamin B-6 status was not optimal while all of the group II infants had blood PLP levels within the reported accepted range.

Correlation of total vitamin B-6 intake with whole blood PLP levels was not significant in group I ($r=0.387$) or Group II ($r=0.309$). Further analysis of the breast-fed infant's intake showed highly significant correlation between total vitamin B-6 intake, PL intake and whole blood PLP in mothers who were not consuming additional vitamin B-6 supplements.

The mean whole blood PLP in mothers who breast-fed their infants was 16.98 + 6.86 ng/ml which was not significantly different than the blood PLP of 8.91 + 4.65 ng/ml in mothers who fed a proprietary formula. Breast-feeding mothers had vitamin B-6 concentrations in milk which ranged from 44.24 - 359.92 mg/l. Based on blood PLP levels and breast milk concentrations half of the breast-feeding mothers had suboptimal viatmin B-6 status. Additionally over half of the non breast-feeding mothers had sub-optimal status as judged by whole blood PLP levels.

This study showed that over half of the breast-fed infants had sub-optimal vitamin B-6 status as did their mothers. It can be concluded that dietary sources of vitamin B-6 can be inadequate for lactation and this is reflected in suboptimal vitamin B-6 status in their infants. In contrast, all the infants in this study who were fed a proprietary formula had adequate dietary intakes of vitamin B-6 as evidenced by whole blood PLP levels that were in the reported normal range.

ACKNOWLEDGEMENTS

The author wishes to sincerely thank Dr. Elizabeth Donald, Professor of Foods and Nutrition, and Dr. E.E. McCoy, Professor of Pediatrics for their continuous guidance, support and encouragement throughout the project and preparation of this manuscript. The cooperation of the mothers and infants who participated in the study is very much appreciated.

Thanks to Doady Paddon of the Edmonton Local Board of Health and Drs. Boyd, Barr and Still for their invaluable assistance in recruiting subjects. Special thanks to Ken Strynadka for his long, arduous hours of dedication while developing the biochemical methodology and completing the analyses of the samples.

An invaluable contribution to the completion of this project has been made by the author's husband through his constant understanding and patience.

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INTRODUCTION

There is presently very limited data regarding the vitamin B-6 status and intakes of infants. Inadequate data are available to satisfactorily evaluate the vitamin B-6 requirements in this age group[17,42]. The present allowances for infants and children are an extrapolation of requirements based on experimentally obtained intake data of vitamin B-6 and protein in adults and expressed as a ratio of vitamin B-6 to protein.

There are a number of methods for biochemically assessing vitamin B-6 status. Of these varying methods, the measurement of pyridoxal 5' phosphate (PLP) has been reported by investigators to be both a sensitive and reliable indicator[47]. The dietary intake of vitamin B-6 is also commonly assessed in status studies with adults. However, there have been few reports regarding the actual dietary intake in infants and how this intake correlates with other parameters of vitamin B-6 status.

The present study was undertaken in a group of normal, healthy infants in Edmonton, Alberta and surrounding area with the following objectives:

1) to determine the usual intake of vitamin B-6 for formula and breast-fed infants;

2) to determine the pyridoxal 5' phosphate levels in whole blood from these subjects;

3) to correlate the dietary intake of vitamin B-6 with pyridoxal 5' phosphate levels in infants; and

4) to compare dietary vitamin B-6 intake and pyridoxal 5' phosphate levels in breast-fed vs. proprietary formula-fed infants.

REVIEW OF LITERATURE

The incidence of breast feeding in North America is on the upswing according to recent reviews [32,33,35,59]. In a recent survey conducted across Canada 58 - 83 percent of mothers were found to be breastfeeding upon discharge from hospital[36]. The higher levels were observed in the Western provinces. This is a substantial increase over 1965 - 1978 when only 25 percent of women were reported to be breastfeeding their infants at birth[35]. While this trend is impressive, the duration of breastfeeding continues to be relatively short in many instances. Myres[36] reported 28.1 percent of mothers stopped breastfeeding by the time the infant was 2 months old, 55.7 percent by 4 months and 74.8 percent by six months of age. Longer duration of breast feeding was associated with (1) residing in the Prairie provinces and/or in smaller towns (2) high income and better education (3) English speaking and (4) 30 - 34 years of age.

The Canadian Pediatric Society has recommended that "the best food source for the first 6 months of life is breast milk"[37]. In view of this recommendation and the increasing incidence of breast feeding, the assessment of the nutrient composition of human milk as it relates to infant needs is critical.

Vitamin B-6 is one nutrient for which there is insufficient data to permit satisfactory evaluation of the requirements for infants. Vitamin B-6 is an overall term for a vitamin which exists in multiple forms in

the body; pyridoxal (PL), pyridoxine (PN), pyridoxamine (PM), and their 5-phosphate esters. PLP and PMP are the predominant forms of vitamin B-6 in mammalian tissues; PM, PMP and PLP are the principal forms in whole blood[47]; and PL and PLP are the principal forms found in human breast milk[56]. Pyridoxal 5' phosphate is the biochemically active form of the vitamin and acts as a coenzyme in protein; carbohydrate and fat metabolism[42].

There is limited information on the relative biological activity of the vitamers. In rats however, they are equally active if given parenterally.[17] Lumeng[30] reported a study involving 6 human subjects whose plasma B-6 compounds were measured before and after 100 mg of pyridoxine hydrochloride (PN-HCl) daily for 1 - 3 weeks. Pyridoxal 5' phosphate and 4-pyridoxic acid (4PA), the metabolically inactive degradation product of vitamin B-6, were the most abundant forms found in the plasma before supplementation; after supplementation PLP, PL and 4PA increased 5-fold but little change was observed in the other vitamers. This suggests that PN, which is the most common form of the vitamin used for oral supplementation, is both rapidly metabolized and converted to PLP, PL and 4PA in plasma.

High pressure liquid chromatography (HPLC) has recently been considered as a promising analytical method for the separation and quantification of the 6 forms of vitamin B-6 as well as 4PA. Vanderslice et al.[55] have reported the separation and quantification of these vitamers using a dual HPLC system of 2 anion exchange columns and a

single buffer. An effective run time with the dual column system required approximately 100 minutes. These authors reported the sensitivity of detection to be 0.1 - 0.5 ng/ml for all the vitamers and the reproducibility to be ± 5 percent. Tryfiates et al.[54] have recently reported an HPLC system using ion-pairing chromatography. This method required only one analytical column and a typical run time of approximately 40 minutes. The sensitivity and reproducibility of the technique were not reported. The small sample size of 0.5 ml of whole blood or plasma required for analysis by HPLC techniques is very advantageous when dealing with human subjects.

Adult requirements for vitamin B-6 were derived by placing small numbers of individuals on vitamin B-6 deficient diets until biochemical evidence of deficiency was observed[12,28]. The increased urinary excretion of xanthurenic acid, following a tryptophan load, was a common test used for evidence of deficiency. The requirement was determined as the level of the vitamin required to normalize the xanthurenic acid excretion. The tryptophan load test has been reported to correlate well with other indices of vitamin B-6 nutrition[5], however, it may be difficult to interpret in persons with a concomitant disease process or in individuals using estrogen therapy. The increased xanthurenic acid excretion in these situations is probably due to stress induction of the pathway rather than vitamin B-6 deficiency. A number of other parameters have also been measured in blood and/or urine in various studies to

assess the requirement. Parameters include plasma PLP levels[6,24] aspartate amino transferase (ASP-AT) activity[6,13,46], alanine amino transferase[6,13,46] (ALA-AT) and 4PA excretion[6,13,24] in the urine. For various reasons many of these procedures have proven unreliable or unsatisfactory[45].

Pyridoxal 5' phosphate has been reported to be a sensitive indicator of the state of vitamin B-6 nutrition [47]. In whole blood, PLP is distributed approximately equally between plasma and erythrocytes thus measurement of either could serve equally well as a status indicator for the vitamin[4]. The levels of the B-6 vitamers in whole blood are listed in Table 1. Shane[47] reported that PLP levels did not appear to fluctuate over several months of study although the other compounds varied considerably. This suggests that PLP levels are a better indicator of vitamin B-6 nutriture than total blood levels of the vitamin.

Table 1. Vitamin B-6 Compounds in whole blood of adult males, and females [47].

Compound	Male (mean±SD)	Female (mean±SD)
	ng/ml	ng/ml
PLP	13.5 ± 2.9 (5)	12.1 ± 2.3 (23)
PNP	0	0
PMP	20.0 ± 5.5 (4)	9.3 ± 7.4 (21)
PL	1.3 ± 0.9 (4)	0.8 ± 0.2 (2)
PN	0	0
PM	24.7 ± 19.5 (3)	18.0 ± 3.9 (21)
4PA	31.9 ± 6.4 (4)	31.8 ± 10.1 (23)

Numbers in brackets indicate number of subjects used to derive the mean.

Depletion-repletion experimentation to determine the requirement of vitamin B-6 has not been done with infants. This is mainly due to the possible deleterious effects of depletion of the vitamin on the growing child and its consequent ethical concerns. Bessey[3] took advantage of the accidental vitamin B-6 depletion which occurred in a group of 9 young infants. The depletion in 5 of the 9 infants was due to the destruction (during the manufacturing process) of a very heat-sensitive form of vitamin B-6 used in a commercial formula. Two of the 9 infants experienced deficiency symptoms relating to low vitamin B-6 content in their mother's breast milk; while the other two suffered vitamin B-6 deficiency apparently due to an aberration in the metabolism of vitamin B-6 rather than to a simple dietary deficiency. Providing these infants with an alternate formula in which the vitamin B-6 content was intact, or providing supplements of PN-HCl, reversed the clinical symptoms. Provision of 0.26 mg/day stopped the convulsive seizures, but 1.0 - 1.2 mg/day were required to reduce the xanthurenic acid excretion to normal levels. In this limited sample, the amount of vitamin B-6 required to normalize xanthurenic acid excretion was larger than the current Canadian recommended nutrient intakes (RNI)[42] of 0.015 mg of vitamin B-6 per gram of protein or 0.2 mg - 0.3 mg vitamin B-6 per day. The amount

needed to prevent convulsions was 0.26 mg which is similar to the current recommendations.[42]

Snyderman[48], as reported in a review by McCoy[34], studied 2 infants with severe cerebral abnormalities. The infants, aged 2 months and 8 months, were placed on a vitamin B-6 deficient diet for 76 and 140 days, respectively. The younger infant developed listlessness after 72 days and convulsive seizures after 76 days of vitamin B-6 deprivation. After 140 days on the vitamin B-6 free diet, the 8 month old showed a severe microcytic, hypochromic anemia which was unresponsive to iron supplementation. Symptoms were relieved within 3 hours by the administration of 50 mg of PN-HCl. Snyderman then attempted to determine the vitamin B-6 requirement of these infants. This was to be accomplished by measuring the level of PN-HCl intake at which the urinary excretion of 4PA and PN would reach the original levels observed in the infants before the administration of the vitamin B-6 free diet. Snyderman was unable to reach the original level by gradually decreasing the PN-HCl intake over a 50 day period. The tissue stores were presumably saturated with the vitamin and restriction of pyridoxine over this time period was not sufficient to produce a deficiency which could be measured by these parameters.

The effect of the use of oral contraceptive agents (OCA's) on vitamin B-6 requirement of the adult female has been reviewed [2,40,53]. Conflicting results have been reported, seemingly independent

of the method used to measure the B-6 status. The effect of prior OCA use on the outcome of subsequent pregnancy and the quality of the breast milk have been dealt with infrequently. A recent paper[44] demonstrated that the use of OCA's for more than 30 months (long term) significantly decreased the levels of vitamin B-6 in maternal serum and milk at 3 days and 14 days post-partum compared to no use or short term use of 1 to 30 months. These data suggest that long term use of OCA's prior to conception reduces the reserves of vitamin B-6 thereby increasing the risk of suboptimal vitamin B-6 nutriture during pregnancy. The same authors reported in another paper[43], that mothers whose infants had unsatisfactory Apgar scores of less than 7 at 1 minute, had significantly lower intakes of vitamin B-6, and lower levels of the vitamin in both serum and breast milk than those mothers whose infants had satisfactory Apgar scores. Williams et al.[58] reported results which conflict with Roepke's work[43,44]. They found that long term OCA use was not associated with a decrease in total vitamin B-6 content of the breast milk. These authors, however, analyzed the breast milk at 1 month, 3 months and 6 months post partum compared to Roepke's work [43,44] where the milk was analyzed at 3 days and 14 days post partum. The differences in timing may account for the apparent differences observed in the vitamin B-6 levels of breast milk thus suggesting the OCA dependant changes may be only short term.

Current information regarding the vitamin B-6 content of human breast milk shows that this level is influenced by the vitamin B-6 intake[26,43,44,52,57], the stage of lactation[26,43,52], and the vitamin B-6 nutritional status of the mother[26,43,44].

Vitamin B-6 Intake

The content of vitamin B-6 in breast milk is dependant upon the level of vitamin B-6 in the diet. West and Kirksey[57] showed this in a group of 19 lactating women classified according to 3 different levels of vitamin B-6 intake: <2.5 mg/day, 2.5 - 5.0 mg/day and >5.0 mg/day. They found that the women consuming the lowest mean level of vitamin B-6 (<2.5 mg/day) also had the lowest mean level of vitamin B-6 in milk (129 ± 37 ug/l). Those consuming 2.5 - 5.0 mg and >5.0 mg/day had 239 ± 51 and 314 ± 52 ug vitamin B-6/l milk, respectively. Although these values were not significantly different they are 2 times greater than the group consuming <2.5 mg vitamin B-6/day. The low levels of vitamin B-6 in breast milk, with low intakes of the vitamin, demonstrates the inability of the mammary gland to concentrate vitamin B-6 when the intake of the vitamin is deficient.

In another study, Kirksey[26] observed that concentrations of the vitamin in breast milk rose to 4 times the original level 3-5 hours after a 20 mg oral dose of PN-HCl. Karlin[23] noted similar findings after

administering large oral doses (500 -1000 mg) of PN-HCl. A rise of 100 times the original level was observed in the breast milk 3 hours post-dose. Diurnal variations of vitamin B-6 in breast milk were only observed following the use of a vitamin B-6 supplement[57]. These fluctuations were associated with the lapsed time between the vitamin B-6 ingestion and its appearance in the milk. These results further emphasize the lack of regulatory mechanisms in the breast to maintain the concentration of vitamin B-6 within defined limits.

Stage of Lactation

The vitamin B-6 content of milk has been observed[43] to be markedly lower in colostrum at 3 days post partum (16 ug/l) and in transitional milk at 14 days post partum (57 ug/l) compared to values for mature milk obtained at 4 to 6 weeks lactation ($\geq$ 200 ug/l). The average level of vitamin B-6 in mature breast milk has not been agreed upon, and has been reported to range from 100 ug/l[23,31] to 200 ug/l[57] when no supplements were consumed. Values are generally higher in women who consume vitamin B-6 supplements than in those who do not take supplements[26,57].

The ratio of vitamin B-6 to protein in milk is also lower at 3 to 14 days lactation (1.0 ug/g protein) than at 4 weeks or later stages (>15.0 ug/g protein)[43]. This indicates that the mammary gland is

able to concentrate vitamin B-6 in later lactation compared with earlier stages and thus the level of the vitamin is unrelated to the protein content of breast milk.

Vitamin B-6 Nutritional Status

The vitamin B-6 nutritional status of the mother appears to significantly influence the vitamin B-6 content of human milk. Roepke and Kirksey[44] suggested this relationship when they determined that maternal nutritional status, assessed by the total vitamin B-6 activity in maternal serum, was positively correlated with the level of vitamin B-6 in milk at 14 days post partum. Animal studies have further supported this view. Felice et al.[15] showed that in rats, the mammary gland was the tissue most sensitive to a dietary vitamin B-6 deficit. The concentration of vitamin B-6 in breast milk was affected sooner than levels in liver and/or muscle tissue and was an earlier indicator of impending vitamin B-6 deficiency.

McCoy[34] has suggested the usual intake of vitamin B-6 and protein for a breast-fed infant of 3 kg would be in the range of 0.045 - 0.050 mg of vitamin B-6 (mainly as PL) and 6.7 - 7.5 g protein per day. The vitamin B-6 to protein ratio would then be 0.006 mg/g protein per day based on a breast milk content of 0.010 mg vitamin B-6/l. This compares to a formula-fed infant of the same weight who would receive 0.18 mg per day of vitamin B-6 (as PN) and 11.2 g protein for a ratio of 0.016 mg/g protein intake. The RNI for Canadians[42] recommends an intake of

0.015 mg vitamin B-6/g protein based on observed intakes of formula-fed infants. Breast-fed infants would only meet this requirement if their mothers consumed 2.5 - 5.0 mg of vitamin B-6 per day. Roepke[43] reported a milk content of 237 ug/l in women who consumed 2.5 - 5.0 mg of the vitamin per day. At this level of vitamin B-6 in breast milk the calculated intake for a breast-fed infant would be 0.10 - 0.12 mg. An estimated protein intake of 6.7 - 7.5 g protein would result in a ratio of 0.015 mg vitamin B-6/g protein.

To date, few researchers have reported data correlating dietary intakes of vitamin B-6 in infants with biochemical parameters. The present study was undertaken to answer the following questions: (1) What is the usual intake of vitamin B-6 for a group of formula- and breast-fed infants? (2) What is the average whole blood pyridoxal 5' phosphate levels in these infants? (3) How does the vitamin B-6 intake correlate with the level of pyridoxal 5' phosphate in whole blood? and (4) How do the dietary vitamin B-6 intakes and pyridoxal 5' phosphate levels compare in breast-fed vs. proprietary formula-fed infants?

EXPERIMENTAL PROCEDURE

I. SUBJECTS

Women who had recently given birth to a healthy infant and who lived in or near Edmonton, Alberta volunteered to participate in the survey. Subjects were recruited in several ways. Information sheets (Appendix 1) describing the study were available at City of Edmonton Health Units and City of Edmonton sponsored prenatal classes for interested mothers to pick up. Other subjects were recruited with the co-operation of a group of obstetricians who admit patients to the University of Alberta Hospitals and still others were recruited through personal contacts. Interested mothers registered for the survey by telephone and were later recontacted, also by telephone, by the registered dietitian to confirm their interest in participating, to outline the study protocol and to arrange an appointment for a home visit. Any subjects with acute or chronic illness were excluded. Infants accepted for the study were considered to be "normal and healthy", thus they met the following criteria: (1) they were born to mothers who had an uncomplicated pregnancy and delivery and (2) each had had an uncomplicated neonatal period. Infants were also free from acute or chronic illness and were not routinely taking medication other than vitamin preparations.

The protocol and use of human subjects in this study was reviewed and approved by the University of Alberta Hospital Ethics Committee. Each woman acknowledged her understanding of the project and agreed to participate in the study by signing the consent form (Appendix 2a,b).

II. DIETARY INTAKE

(a) Breast Milk

Each mother who was breast feeding her infant was supplied with an electronic scale. The model used was a Sartorius 3862-MP6 top loading electronic scale with a capacity of 16,000 gm x 0.1 gm. This unit was equipped with a Sartorius data terminal #71401-1011 animal weighing program. This program enabled the scale to take a maximum of 20 individual weights of the infant over a period of approximately 10 seconds and to display only the mean of these weights. This equipment eliminated problems in recording weights when the infant was active. Instruction on how to use the scale was provided to the mother by demonstration as well as written instruction (see Appendix 3).

The infant was weighed prior to (pre) and after each feeding (post) for 3 consecutive 24-hour periods. The 24-hour period commenced at different hours for each mother but, began at the time of the infant's first feeding after the mother had received the equipment. The mother

was instructed not to change the infant's clothing or diapers from the time of the pre-feeding until after the post-feeding weighing, thus weight change should be soley due to the weight of milk consumed. The mother recorded both pre- and post-feeding weights on the breast milk record sheet (Appendix 4a). The weight of breast milk consumed by the infant was taken to be the post-weight minus the pre-weight. The volume of breast milk was calculated assuming 1 ml as equivalent to 1.031 gm.

In order to quantitate the vitamin B-6 and protein content of the breast milk the mother was instructed to express 5 - 10 ml of fore-milk, manually or by breast pump, at each feeding. Opaque polypropylene containers were supplied to the mother for this purpose.

Breast milk was collected in subdued light if possible, and put into the household freezer immediately. The breast milk was transported to the laboratory in an insulated container to keep it in the frozen state. It was then stored at -40°C until analyzed.

The vitamin B-6 and protein intake of the infant was obtained for each feeding by calculating the volume of milk taken multiplied by the analyzed vitamin B-6 or protein value at that feeding. The total daily vitamin B-6 and protein intake were then calculated for each 24-hour period. Mean daily intake was calculated by averaging intakes from the three 24-hour periods.

(b) Proprietary (P) and Non-Proprietary (Non-P) Formula

Each mother who fed her infant with P or non-P formula recorded the volume of intake for 3 consecutive 24-hour periods. The beginning of the 24-hour period began at different hours for each subject but all mothers commenced with the first feeding after all instructions were given. Mothers were instructed to measure the volume of formula given to the infant in a graduated Imperial or metric measuring cup to the nearest ounce or 30 ml. This volume was recorded on the formula record sheet (Appendix 4b). Once the infant had finished feeding, the mother was instructed to measure any formula not consumed to the nearest 1 tsp. or 5 ml and to record this on the same formula record sheet. Mothers also recorded the brand name of the formula used on this record. Volume of formula ingested was calculated as the difference between the amount of formula fed and any formula not consumed. To arrive at a 24-hour total, the volumes of formula consumed at each feeding were added together.

Vitamin B-6 and protein content of the formulae were determined by using manufacturer's published values. The vitamin B-6 content was verified in our laboratory by the method described in Section IV, and the published values were found to be accurate. The vitamin B-6 and protein intake per 24-hour period was calculated by multiplying total volume by the published values. Mean daily intake was calculated from averaging intakes from three 24-hour periods.

(c) Breast Milk And Proprietary or Non-Proprietary Formula

Infants who were breast-fed and who received supplements of P or non-P formula had intakes determined by combining techniques described in (a) and (b) above.

(d) Beikost

Some infants received beikost (foods other than breast milk or formula). The beikost intake was recorded for 3 consecutive 24-hour periods which coincided with the same periods of time of recording breast milk or formula. At the time of the initial visit, those mothers whose infants were consuming beikost were instructed on how to record this intake. The beikost to be fed was measured to the nearest 1 tsp. or 5 ml and any not consumed was then re-measured. Mothers recorded the amount actually taken on the food intake record sheet (Appendix 4c). The brand names of products used in the recipe of home prepared foods were also recorded. When the infant assisted in his/her feeding the actual food eaten was estimated less accurately as more food was usually spilled.

Food records were reviewed by a registered dietitian with the mother shortly after the recording period to ensure as much accuracy as possible in recording.

III. SAMPLE COLLECTION AND STORAGE

(a) Blood Collection

Blood samples were collected from mother and infant between 0730 and 1100 hours. The mother had completed a minimum 8 hour, overnight fast. The infants had fasted at least 3 hours at time of sampling. Vitamin supplements were withheld until after the blood was collected.

Finger or heel pricks (infants) and finger pricks (mothers) were done using an Autolet® or surgical lancet. Blood was collected from the finger or heel using heparinized Natelson Pipettes. A 0.4 - 0.5 ml sample of capillary blood was put into a dark colored heparin-coated tube which had Na_2HPO_4 (0.3 ml) pre-added to inhibit degradation of PLP. Samples were capped, immediately put on ice in an insulated container, and transported to the laboratory within 2 hours of collection and stored at -40°C.

(b) Breast Milk Collection

i) Collection of Milk: Mothers were given polypropylene, 125 ml. opaque specimen containers in which to collect breast milk samples. Five to 10 ml samples of fore-milk were expressed manually or with a breast pump, each time the infant fed during the study period. These samples were collected in subdued light (if possible); labelled for time and date, and frozen immediately in the household freezer. The dietitian picked up the completed samples and transported them in an insulated container to the laboratory. They were stored at -40°C until analyzed.

ii) Fore- and hind-milk: Additional human milk samples were collected in order to determine any variation in vitamin B-6 concentration during a single nursing. Samples were provided by 3 lactating women who did not wish to participate in the intake/blood phase of the study. Each lactating woman was asked to collect 18 samples. One sample from the morning (0800 - 1200 hours); one from the afternoon (1201 - 1800 hours) and one from the evening (1801 - 2400 hours) were to be collected by each mother for 3 consecutive days. Each sample consisted of 2 parts; (a) a fore-milk sample and (b) a hind-milk sample. Once the infant began nursing and the "let down" reflex was initiated, a fore-milk sample was expressed from the opposite breast. A hind-milk sample was taken from the same breast after the infant finished feeding. Each sample was labelled for date, time and whether it was fore- or hind-milk. Quantity of sample, storage and transport to the laboratory was identical to that described in (i) above.

(c) Nutrition Survey Questionnaire

The nutrition survey questionnaire (Appendix 5) was administered by the registered dietitian during the initial home visit.

IV. BIOCHEMICAL ANALYSES

(a) Vitamin B-6

Whole blood and breast milk samples were prepared for analysis by the same method. Samples were titrated with 0.5 ml perchloric of whole blood or breast milk to remove protein and protein-bound B-6 forms. The sample was centrifuged at 10,000 x g for 10 min then filtered through a 0.45 millipore filter. An aliquot ranging from 0.1 ml to 0.5 ml of the sample was then injected into the sample port leading to the chromatographic columns. Each sample was then analyzed by reverse phase ion-paired chromatography using a modification of procedure described by Tryfiates[54]. The method to be described was developed over a 2 year period in the Department of Pediatrics, University of Alberta, Edmonton, Alberta. The Heritage Foundation for Medical Research provided a grant for purchase of the equipment.

Samples were first run through a pre-column of CO:PELL ODS (Whatman) and then through the PRP-1 5mm x 25cm analytical column. Two solvents were used. The first was a mixture of 1.6% propanol at pH 2.3, 0.004 M heptanesulfonic acid and 0.004 M octanesulfonic acid, the second was 5.5% propanol at pH 2.3. A Perkin-Elmer LS-5000 spectrophotofluorometer with a 0.20 ml flow cell was used to identify the individual vitamers as they were eluted. Excitation wavelengths were set at 380 nm and emission wavelength at 400 nm. An Oriel multifunctional signal processor using a Hewlett-Packard model 3390A integrator printed and quantitated each peak of the chromatogram. Standards were prepared

daily to program the integrator before each days' samples were run. The total run time for each sample was approximately 50 minutes. The entire system was kept in a room with yellow flourescent lights to prevent photodegradation of the B-6 vitamers.

The sensitivity of this method was found to be 1.0 - 2.0 ng/ml and the reproducibility was ± 10 percent. A sample size of 0.4 - 0.5 ml of whole blood or breast milk was required in order to do the analysis.

(b) Protein

The total protein content of breast milk was done in duplicate according to the method described by Lowry et al[29].

V. STATISTICAL ANALYSES

All data were analyzed using a Hewlett-Packard 97 programmable calculator. The Student's t-test program was used to determine significant differences between 2 means. Correlation coefficients were obtained between certain variables to measure their consistency. When the correlation coefficient was significant a linear regression program was used between variables to predict values of one variable in terms of the other. All statistical analyses were compared to standard tables[60] at two significant levels ($P<0.05$ and $P<0.01$). All average values were reported as the mean ± standard error of the mean.

RESULTS AND DISCUSSION

I. SUBJECTS

(a) Mothers

Data was collected between September 1982 and September 1983. Twenty-seven women ranging in age from 17 to 33 years volunteered to participate in the study. The mean age of mothers who breast-fed their infants (feeding group I) was 30.6 $\pm$ 0.5 years compared to 26.4 $\pm$ 2.1 years for proprietary formula feeding mothers (feeding group II) and 29.6 $\pm$ 1.4 years for mothers feeding a non-proprietary formula (feeding group III) (Table 2). Mothers who fed their infants a proprietary formula were significantly younger ($p \leq 0.05$) than those mothers who breast-fed their infants. This is consistent with the data reported by Myres[36] in which longer duration of breast-feeding was associated with women 30 - 34 years of age. Comparative statistical analyses were not done on feeding group III due to the small sample size. Fourteen of the women were first time mothers, 13 had more than one child.

(b) Infants

Twenty-eight full term infants, including one set of twins, were studied and their general characteristics are summarized in Table 3. Group I had 9 males and 7 females while group II had 7 males and 2 females. All subjects in group III were females. The mean age of the breast-fed infants (feeding group I) was 12.4 $\pm$ 1.6 weeks and 12.1 $\pm$ 2.4 weeks for the proprietary formula-fed infants' (feeding group II), while

Table 2. Description of Mothers[1]

Feeding Group	Subject	Age (years)	Para[2]	Gravida[3]
I. Breast-feeding (n=16)	CW	27	1	1
	MK	28	1	1
	TE	29	4	4
	LG	30	2	2
	MM	33	1	1
	MT	28	1	1
	PB	33	2	2
	MC	32	2	2
	KT	30	1	1
	JDE	32	2	2
	LL	32	1	1
	SED	31	2	2
	JP	31	3	3
	FW	32	3	3
	LM	30	1	1
	LW	31	2	2
Mean Values ± SEM[4]		30.6[a]±1.5		
II. Proprietary Formula-Feeding (n=8)	MB	17	1	1
	RW	33	2	3
	RB	30	1	1
	MS	25	1	1
	LD	29	2	2
	JN	29	1	2
	MH	30	1	1
	LW	18	1	1
Mean Value ± SEM		26.4[b]±2.1		
III. Non-proprietary Formula-Feeding[5] (n=3)	JDE	32	2	2
	LB	30	1	1
	MG	27	4	3
Mean Value ± SEM		29.6±1.4		

1. Significantly different means are followed by different superscripts ($p<0.05$).
2. Number of pregnancies.
3. Number of live births.
4. Standard error of the mean.
5. Comparative statistical analyses not done due to small sample number.

Table 3. Description of Infants[1]

Feeding Group	Subject	Sex	Age (weeks)
I. Breast-Fed (n=16)	KW	M	10
	DK	M	6
	EE	M	16
	SG	M	6
	BM	F	7
	MT	M	7
	EB	F	8
	HC	F	13
	GT	M	17
	KDE	F	18
	NL	M	23
	EED	F	26
	LD	F	6
	MW	M	8
	RE	M	8
	MEW	F	19
Mean Values ± SEM[2]			12.4[a]±1.6
II. Proprietary Formula-Fed (n=9)	CB	M	6
	LW	M	6
	TW	M	6
	MB	M	7
	LS	F	9
	SD	M	12
	CN	F	20
	AH	M	25
	MIW	M	18
Mean Value ± SEM			12.1[a]±2.4
III. Non-Proprietary Formula-Fed[3] (n=3)	KDE	F	38
	SB	F	45
	SAG	F	40
Mean Value ± SEM			41.0±2.1

1. Significantly different means ($p \leq 0.05$) are followed by different superscripts.
2. Standard error of the mean.
3. Comparative statistical analyses not completed due to small sample number.

the 3 infants who were fed a non-proprietary formula (feeding group III) had a mean age of 41.0 $\pm$ 2.1 weeks. There was no significant difference between the age of groups I an II. Comparative statistical analysis were not completed for subjects in group III due to the small sample number.

The growth patterns of the infants are summarized in Table 4. There was no significant difference between expected weight change and actual weight change in feeding groups I and II. Differences in actual weight change were also not significant between breast-fed infants and those fed proprietary formula. Expected change in length was significantly higher ($p<0.05$) than actual change in feeding groups I and II, however, there was no significant difference in actual change in length. Measurement of length, in this study, was subject to error due to inter-observer error, the use of different measuring apparatus and the co-operation of the infant. Measurements made at birth were taken by various hospital personnel and reported to the researcher by the mother. Length measurements at the time of the study were taken by the research during the intital home visit. The combination of these factors may have resulted in significant error and could account for an apparent difference rather than a real difference in growth rates. When the length data was plotted on growth curves[17] all the infants, except one (KDE - group III), had achieved lengths of greater than the 10th percentile for age, thus indicating that actual length attained was well within population norms.

Table 4. Expected change in weight and length of infants from birth to present age compared to actual change[1].

Treatment	Subject	Weight (kg) Expected[2] Change	Weight (kg) Actual Change	Length (cm) Expected[2] Change	Length (cm) Actual Change
I. Breast-fed (n=16)	RE	2.02	1.95	8.06	2.60
	MW	3.87	2.40	15.31	14.60
	DK	1.45	1.70	6.17	6.70
	EB	2.02	0.66	NA[4]	NA[4]
	HC	2.71	3.50	11.51	7.70
	EED	4.60	4.82	15.30	9.50
	KDE	3.54	2.18	14.30	8.00
	SG	1.45	1.34	6.17	7.00
	NL	4.72	4.83	18.44	12.40
	GT	3.82	3.22	15.00	7.30
	BM	0.96	2.58	6.79	8.90
	MT	1.74	1.53	6.97	5.10
	EE	3.66	3.22	14.41	9.20
	LP	0.90	2.20	6.40	5.91
	MW	2.02	1.00	8.06	3.85
	KW	2.46	1.44	9.75	5.40
Mean ± SEM[3]		2.62[a] ±0.31	2.41[a] ±0.31	10.87[b] ±1.10	7.21 ±0.89[c]
II. Proprietary Formula (n=9)	LS	1.41	1.30	8.46	8.50
	SD	2.91	3.61	11.45	10.10
	CN	3.84	3.96	15.89	7.70
	AH	4.97	5.02	19.38	15.00
	MB	1.73	1.89	7.12	6.33
	MLW	1.45	1.38	6.17	3.81
	MTW	1.45	1.06	6.17	3.81
	MIW	4.30	4.71	16.91	15.88
	CB	1.45	0.90	6.17	0.70
Mean ± SEM		2.61[a] ±1.43	2.65[a] ±1.66	10.86[b] ±1.75	7.98[c] ±1.69
III. Non-proprietary Formula[5] (n=3)	SB	6.34	5.00	25.61	21.97
	KDE	5.79	4.46	23.34	13.00
	SAG	5.94	7.08	23.99	25.24
Mean ±		6.02 ±0.16	5.51 ±0.80	24.31 ±0.67	20.07 ±3.66

1. Significantly different means ($p<=0.05$) are followed by different superscripts.
2. Expected change was calculated according to data from Pomerance[39].
3. Standard error of the mean.
4. Information not available.
5. Comparative statistical analysis not done due to small sample number.

None of the infants routinely consumed medication although some infants occasionally were given colic medications eg. Gripe Water® and Ovol®.

II. BREAST MILK

(a) Vitamin B-6 content of breast milk

Twenty mothers supplied a total of 275 breast milk samples for analysis. Table 5 summarizes the percentage of vitamin B-6 vitamers present in the milk. The principle form of vitamin B-6 found was PL at 55.2 ± 3.8% of the total. Pyridoxal 5' phosphate and PN were the next most abundant at 22.2 ± 3.2% and 16.5 ± 2.5%, respectively. Pyridoxamine and PMP contributed only a minor portion of the total vitamin B-6, 3.0 ± 0.7% and 3.1 ± 0.6%, respectively. These data are generally consistent with Vanderslice et al.[56] who also found that the principle forms of vitamin B-6 present in human milk were PL and PLP. However, these authors[56] reported that they detected PN in only 2 of their 13 subjects and found only trace amounts in the others. In contrast, the present study found PN contributed 1/6 of the total vitamin B-6 present or 16.5%. Vanderslice et al.[56] also reported no evidence of PM or PMP compared with a total of 6.1% of the vitamin B-6 present in the milk analyzed in this study. These differences are probably reflective of the greater sample size used in this study (275 samples) as compared to Vanderslice et al.[56] who based their results on a very small number of samples (n=13).

Table 5. Percentage of Vitamin B-6 vitamers present in breast milk

Subject n=20	Number of Samples Analyzed	Percent of total Vitamin B-6 present PLP	PL	PN	PM	PMP
MM	17	18.1	45.2	29.9	2.0	4.7
MT	16	18.8	45.4	30.9	1.8	3.0
TE	16	17.3	45.3	27.9	3.5	6.0
JP	16	15.1	50.4	32.1	2.4	0.0
FW	13	28.2	31.6	22.7	13.3	4.3
CW	16	13.6	73.1	11.1	0.8	1.3
CT	14	36.0	45.0	11.9	3.0	3.8
MC	15	37.0	44.2	11.5	5.4	1.9
MK	22	14.5	80.7	2.0	0.2	2.7
JDE	14	46.5	48.3	3.9	0.7	0.5
LG	11	30.9	36.9	25.2	2.4	4.5
LL	18	20.9	78.6	0.1	0.0	0.4
PB	18	15.8	75.3	4.9	1.0	3.0
SED	12	20.0	58.6	10.5	3.2	7.7
MM	8	24.8	51.1	20.4	1.8	1.9
UG	5	3.7	85.9	9.9	0.0	0.4
JE	9	9.3	62.5	10.7	8.3	9.1
MN	16	9.5	46.5	37.1	5.3	1.5
PM	10	4.0	70.9	17.9	2.5	4.7
ZG	9	59.1	27.9	9.0	2.6	0.8
MEAN ± SEM[1]		22.2 ±3.2	55.2 ±3.8	16.5 ±2.5	3.0 ±0.7	3.1 ±0.6

1. Standard error of the means.

The average amount of vitamin B-6 found in the breast milk of mothers, who did not consume vitamin B-6 supplements, was 96.58 ± 36.62 ug/l with a range of 44.24-170.22 ug/l (Table 5). The average level of vitamin B-6 found in breast milk has not yet been agreed upon. Some investigators[11,23] have reported the mean level to be in the range of 100 ug/l while others[21,43,57] have reported values of 200 ug/l. Roepke et al [43] reported a vitamin B-6 concentration of 130 ug/l to be associated with an average vitamin B-6 intake of <2.0 mg per day. The vitamin B-6 intake of the mothers in this study, other than recording the amount of vitamin B-6 supplements ingested, was not quantitated. Based on the observation by Roepke et al.[43] however, one could speculate that the unsupplemented mothers in this study would have consumed a mean of <2.0 mg of vitamin B-6 per day since the mean breast milk concentration was <130 ug/l. Bessey[3] has suggested that infants receiving milk containing less than 100 ug of vitamin B-6 per liter may develop convulsions. Seven out of 13 unsupplemented mothers in this study had a vitamin B-6 concentration in their breast milk of less than 100 ug/l (Table 6) but none of the infants were reported to have suffered convulsions. The method of analysis of vitamin B-6 in 1953 would not have been as precise as the methods currently in practice. Bessey's[3] method therefore may have overestimated the concentration of vitamin B-6 necessary to prevent convulsions. West and Kirksey[57] observed milk levels of <100 ug/l of vitamin B-6 to be associated with intakes currently thought to be below optimal. One could speculate that breast milk vitamin B-6 concentrations of <100 ug/l may be considered suboptimal but not to

Table 6. Total vitamin B-6 and protein content in breast milk of vitamin B-6 supplemented and unsupplemented mothers[1]

Subject	Number of Samples Analyzed	Level of Supplement	Mean Vitamin B-6 Content	Mean Protein Content	Ratio of B-6 to Protein
		mg/day	ug/l	g/100ml	mg/g
Unsupplemented n=13					
ZG	9		141.96	0.94	0.015
CT	16		112.24	0.99	0.011
MC	15		73.77	1.03	0.007
MK	22		170.22	1.16	0.015
JDE	14		85.14	1.24	0.007
LG	11		62.18	1.11	0.006
SED	12		77.06	0.89	0.009
MM	17		64.82	1.58	0.004
MT	16		92.09	1.08	0.008
TE	16		128.94	1.14	0.011
JP	16		44.24	1.12	0.004
MGM	8		126.77	1.05	0.012
PM	10		76.08	0.84	0.009
MEAN ± SEM[2]			96.58 ±36.62 [a]	1.09 ±0.05 [c]	0.009 ±.001 [d]
Supplemented n=7					
MN	16	2.0	108.57	1.38	0.008
JE	9	5.0	142.79	1.01	0.014
LL	18	2.0	239.77	1.07	0.022
PB	18	1.0	112.16	1.35	0.008
FW	13	1.0	208.54	0.87	0.024
CW	16	4.0	359.92	1.10	0.033
UG	5	50.0	282.20	0.96	0.029
MEAN ± SEM			207.71 ±93.91 [b]	1.11 ±0.07 [c]	0.020 ±.004 [e]

1. Significantly different means (≤ 0.05) are followed by a different superscript.
2. Standard error of the mean.

such a degree as to elicit clinical symptoms.

Mothers who consumed vitamin B-6 supplements took an average of 9.3 mg of the vitamin per day, with intakes ranging from 1.0-50.0 mg. Excluding (UG), the mother who regularly consumed a very large vitamin B-6 supplement of 50 mg per day, the mean intake changes to 2.5 mg per day. The average level of vitamin B-6 found in the breast milk of these mothers was 207.71 $\pm$ 93.91 ug/l with a range of 112.16 - 359.92 ug/l. This value is significantly larger than the vitamin B-6 concentration found in unsupplemented mothers ($p<0.05$). Analysis of the major vitamers present in the breast milk (Table 7) indicates that this difference is largely due to a significantly ($p<0.05$) higher level of PL found in the breast milk of vitamin B-6 supplemented mothers. This larger amount of PL observed in breast milk following vitamin B-6 supplementation (as PN) may be explained by the observations of Anderson et al.[1]. These authors reported that ingested PN is taken up by red blood cells and converted to PLP and then to PL followed by a gradual release of the PL into plasma. This may also hold true in breast tissue where the PN may be converted to PLP and then to PL where it is released into breast milk, larger amounts of PL being found in those individuals who ingest larger amounts of PN. West and Kirksey[57] reported an average value of 239$\pm$51 ug/l of vitamin B-6 in breast milk of women who consumed 2.5 to 5.0 mg of vitamin B-6 per day. Their findings are similar to that found in the vitamin B-6 supplemented group in the present study where although the vitamin B-6 content of foods was not quantitated, the mean vitamin B-6 supplement ingested was 2.5

Table 7. Major vitamers present in breast milk of vitamin B-6 supplemented and unsupplemented mothers[1]

Subject	Number of Samples Analyzed	PLP	PL	PN
		ug/l	ug/l	ug/l
Unsupplemented n=13				
ZG	9	83.92	39.61	12.79
CT	16	40.46	50.64	13.43
MC	15	27.32	32.57	8.47
MK	22	24.63	137.32	3.34
JDE	14	39.57	41.12	3.37
LG	11	19.20	22.97	15.73
SED	12	15.44	45.19	8.07
MM	17	11.73	29.31	19.40
MT	16	17.33	41.80	28.48
TE	16	22.28	58.45	35.95
JP	16	6.67	22.32	14.22
MGM	8	31.48	64.74	25.84
PM	10	3.08	53.96	13.62
MEAN ± SEM[2]		^{a} 26.39 ±5.73	^{b} 49.23 ±8.17	^{d} 15.59 ±2.68
Supplemented n=7				
MN	16	10.30	50.52	40.31
JE	9	13.34	89.22	13.04
LL	18	50.12	188.46	0.23
PB	18	17.77	84.49	5.45
FW	13	58.74	65.90	47.29
CW	16	49.12	263.19	39.88
UG	5	10.56	242.45	28.00
MEAN ± SEM		^{a} 29.99 ±8.14	^{c} 140.60 ±33.46	^{d} 24.89 ± 7.06

1. Significantly different means ($p \leq 0.05$) are followed by different superscripts.
2. Standard error of the mean.

mg/day. These mothers had a corresponding mean vitamin B-6 concentration in breast milk of 207.71 ± 93.91 ng/ml.

(b) Protein content of breast milk

The mean protein content of breast milk was found to be 1.10±0.04 g per 100 ml. This value is consistent with the work of others [18,50,57]. There was no significant difference between the protein content in breast milk of vitamin B-6 supplemented and unsupplemented and mothers. There was, however, a significantly larger ($p<0.05$) amount of vitamin B-6 per gram of protein in the breast milk of mothers who consumed supplements (Table 6). This further supports suggestions[23,57] that the vitamin B-6 content of breast milk is related to the intake of the vitamin and not to the protein content of the milk.

(c) Vitamin B-6 content of fore- and hind-milk

Three mothers, ranging in age from 27-32 years supplied 22 fore- and 22 hind-breast milk samples. Table 8 shows that although fore-milk had a higher level of vitamin B-6 this level was not significantly different from the level of vitamin B-6 found in hind-milk. Comparison between individual subjects also indicated no significant difference between fore- and hind-milk samples. The average protein content of fore-milk was 1.01± 0.03 g/100 ml and hind-milk was 1.07±0.04 g/100 ml. These values were not significantly different.

Table 8. Variation in the total vitamin B-6 content of fore- and hind-breast milk in 3 women[1].

Subject	Fore-milk	Hind-milk	Difference (Fore-milk minus Hind-milk)
	ng/ml	ng/ml	ng/ml
A	150.42	117.04	33.38
	144.28	88.12	56.16
	112.48	238.60	-126.12
	132.74	103.63	29.11
	105.62	113.10	-7.48
	128.42	98.62	29.80
	154.44	165.55	-11.1
	85.76	85.52	0.24
B	191.00	140.84	50.16
	246.66	194.90	51.76
	312.1	282.34	29.76
	380.88	261.08	119.80
	280.44	329.98	-49.50
C	129.50	150.02	-20.52
	123.70	79.52	44.18
	150.13	138.64	11.49
	104.34	81.42	22.92
	159.60	79.53	80.07
	181.50	140.32	41.18
	117.99	110.81	7.18
	172.30	151.52	20.78
	146.11	130.72	15.39
MEAN ± SEM[2]	168.66^{a} ±15.71	149.17^{a} ±15.03	

1. Significantly different means are followed by different superscripts.
2. Standard error of the mean.

(d) Diurnal changes in vitamin B-6 content of breast milk

Diurnal changes in the vitamin B-6 concentration of breast milk were studied in vitamin B-6 supplemented and unsupplemented mothers grouped according to 6 different times of feeding on 3 consecutive days (Figure 1). Little diurnal variation was observed in mothers not consuming vitamin B-6 supplements. Average minimal and maximal values ranged from 87 ug/l to 106 ug/l at different feeding times. Mothers who consumed vitamin B-6 supplements showed greater variation but these changes were not statistically significant. Minimal and maximal mean values in this group ranged from 173 ug/l to 213 ug/l. The peak vitamin content of the milk of supplemented mothers occurred between 1600-1800 hours. West and Kirksey[54] observed marked diurnal change in individual subjects taking vitamin B-6 supplements. This variation was reported to be associated with the time elapsed between vitamin ingestion and its appearance in the milk. West and Kirksey's[57] supplemented mothers consumed the vitamin B-6 supplement at approximately 10 A.M. The pattern observed and time of peak vitamin content observed in this study was similar to West and Kirksey[57] suggesting vitamin supplement ingestion also occurred at approximately the same time.

III. DIETARY INTAKE OF INFANTS

Fourteen breast-fed (group I), 9 proprietary formula-fed (group II) and 3 non-proprietary formula-fed infants had intake data calculated. Two of the group I infants consumed either proprietary formula supplements or beikost as a major proportion of their vitamin B-6 intake and therefore

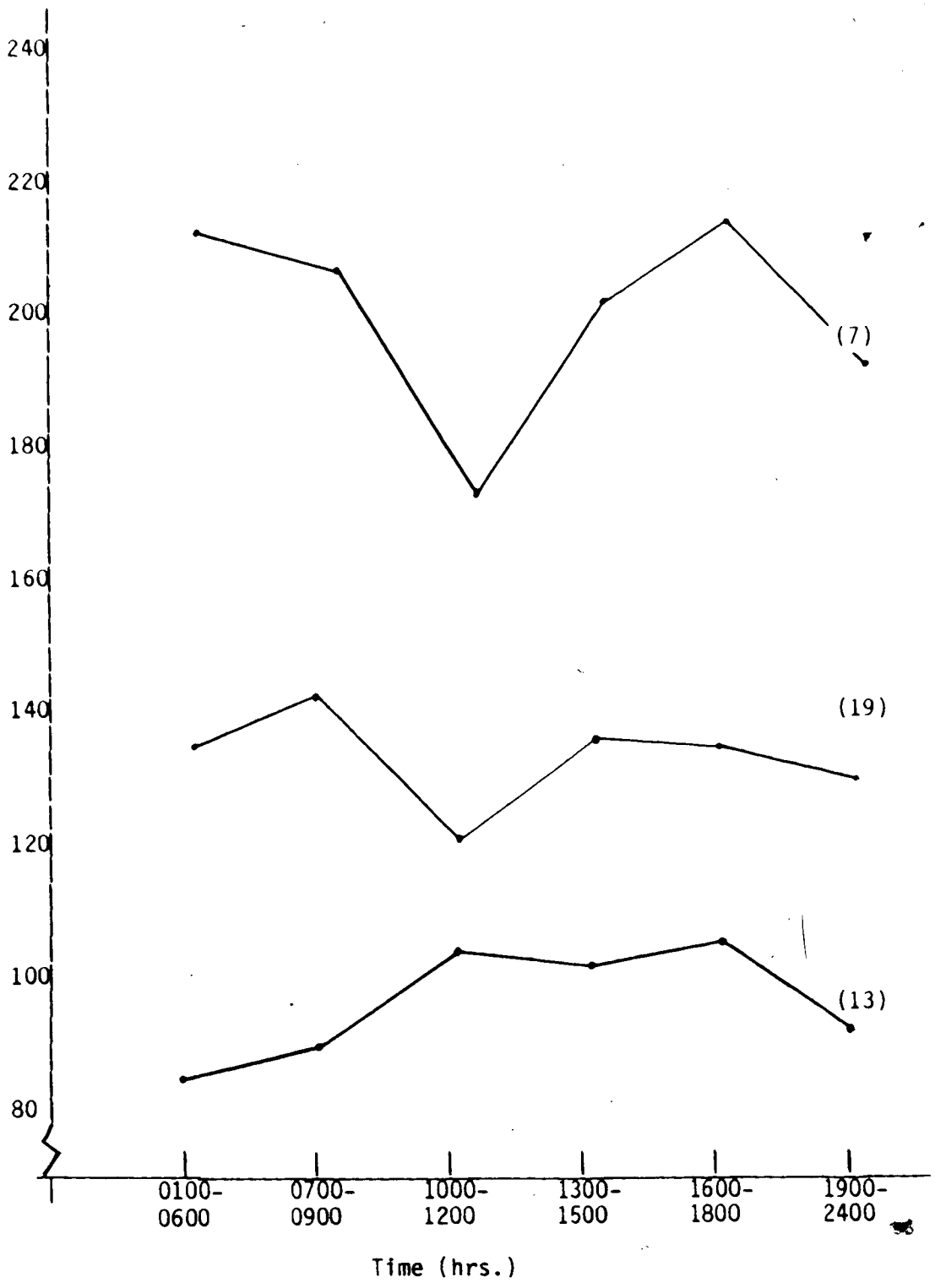

Figure 1. Mean diurnal patterns of vitamin B-6 content in mother's milk.
Mothers who did not consume vitamin B-6 supplements;
Mean values of all mothers, supplemented and unsupplemented;
Mothers who consumed vitamin B-6 supplements.

Numbers in brackets indicate number of subjects used.

were excluded from the breast-fed group. Two group II infants received beikost which contributed $\geq$ 15 percent of the vitamin B-6 intake and were excluded from group II. All 3 infants in group III consumed cow's milk and beikost, however, due to the small sample comparative statistical analyses were not completed.

(a) Volume of intake of breast milk and proprietary formula

Infants, who obtained $\geq$ 90 percent of their vitamin B-6 intake from breast milk, consumed 634±47 mls per day with a range of 384-914 mls. Infants fed proprietary formula had a mean intake of 810±57 mls per day when $\geq$ 90 percent of vitamin B-6 intake was consumed as formula. Intake ranged from 608-1061 mls. The mean intakes for these two groups were statistically different ($p \leq 0.05$). The difference may be partly due to the use of different methodology for determination of intake. Butte et al.[7] suggested that the test-weighing procedures, in which infants are weighed before and after a feeding, would underestimate breast milk intake by 3 percent when compared to measuring intake by volume. They postulated that this difference was due to insensible water loss during a feeding. Additionally, mothers may encourage formula-fed infants to "finish the bottle" thereby increasing the volume of intake. The volume consumed by breast-fed infants in this study is in agreement with the work of others[8,38]. The volume of intake by formula-fed infants in this study is similar to the data presented by Fornon[16] for formula-fed infants.

(b) Protein intake

The mean protein intake of infants who were breast-fed (group I) was 7.1±0.7 g per day and in the formula-fed (group II) the mean was 13.0±1.0 g per day (Table 9). These means were significantly different ($p \leq 0.01$). The reported intake of protein in breast-fed infants of comparable age ranges from 6.1 g per day[7] to 8.1 g per day[38]. This is consistent with the mean protein intake of breast-fed infants in this study. Protein intake in formula-fed infants ranges from 10.2-13.6 g per day as calculated from data by Fomon[16]. The protein intake of formula-fed infants in this study is consistent with this.

(c) Vitamin B-6 intake

Vitamin B-6 intake in breast-fed infants (group I) was 0.088±.004 mg per day and this level of intake was significantly lower ($p \leq 0.01$) than the calculated mean intake of 0.408±0.010 mg per day by formula-fed infants (group II). This difference is also reflected in a significantly lower ($p \leq 0.01$) ratio of vitamin B-6 to protein of 0.013±0.002 mg per gram in group I compared to a ratio of 0.031±0.002 mg per gram for infants in group II.

The suggested adequate ratio of vitamin B-6 to protein for infants is 0.015 mg per gram[42]. Eight of the 12 infants in group I did not meet this level of intake in contrast to group II where all infants did.

The 3 infants in group III had mean intakes of vitamin B-6 and protein which were higher than those observed in groups I and II. This is not unexpected due to the age difference (Table 3). The mean ratio of

Table 9 Effect of breast-feeding vs proprietary formula-feeding on Vitamin B-6 intake and ratio of vitamin B-6 (mg) to protein (g) of infants[1]

Subject	Vitamin B-6 Intake	Protein Intake	Ratio Vitamin B-6 to Protein
I. Breastfed[2] (n=12)	mg	g	mg/g
HC	0.045	5.8	0.008
KDE	0.051	7.6	0.007
NL	0.212	9.3	0.023
GT	0.051	4.6	0.011
DK	0.124	8.1	0.015
EB	0.042	4.9	0.009
BM	0.049	12.4	0.004
MT	0.042	4.9	0.009
EE	0.092	8.0	0.011
LP	0.034	8.8	0.004
MW	0.091	3.7	0.025
KW	0.213	6.9	0.031
MEAN±SEM[3]	0.088 ±0.004 [a]	7.1 ±0.7 [c]	0.013 ±0.002 [e]
II. Proprietary Formula-fed[4] (n=7)			
LS	0.292	10.8	0.027
SD	0.523	13.1	0.040
MB	0.240	12.2	0.020
MLW	0.342	12.8	0.027
MTW	0.347	13.0	0.027
MIW	0.709	18.8	0.038
CB	0.405	10.1	0.040
MEAN±SEM	0.408 ±0.010 [b]	13.0 ±1.1 [d]	0.031 ±0.002 [f]
III. Non-proprietary formula-fed[5] (n=3)			
KDE	0.594	45.7	0.013
SB	1.23	40.6	0.030
SG	1.70	60.8	0.027
MEAN±SEM	1.17±0.32	49.0±6.0	0.023±0.005

1. Significantly different means ($p \leq 0.01$) are followed by different superscripts.
2. Infants received > 90% of vitamin B-6 intake from breast milk.
3. Standard error of the mean.
4. Infants received ≥ 90% of the vitamin B-6 intake from proprietary formula.
5. Comparative statistical analyses not completed due to small sample size.

vitamin B-6 per gram of protein was 0.023 ± 0.005 mg/g which is within the suggested range.[42]

IV. B-6 CONTENT OF WHOLE BLOOD

(a) Mothers

Whole blood vitamin B-6 determinations were available for 25 mothers. Table 10 indicates that PLP and PL were the major forms present at 35.0±5.2 percent and 26.2±4.9 percent of the total, respectively. Pyridoxine was found to constitute 13.0±3.4 percent, PM 13.9±5.4 percent and PMP 11.8±2.0 percent of the total B-6. These results do not agree with those reported by Shane[47] who suggested that PLP, PM, and PMP were the major forms present in whole blood. Shane[47] found that 30 percent of the total B-6 was PLP which gives reasonably good agreement with our data. However, the total amount of PL and PN found by Shane[47] was only 2 percent compared with 39 percent in this study. Pyridoxamine and PMP totalled 26 percent of the vitamer content while Shane[47] reported these vitamins present at 68 percent of the total. He[47] also suggested that B-6 vitamers, other than PLP, fluctuate considerably over time and this may, in part, account for the differences in results between the 2 studies. Additionally, the methodology used by Shane[47] for the determination of the B-6 vitamers was developed in 1968. The sensitivity and specificity of this method has been greatly improved upon since then so that the method used in this study would be more reflective of the actual vitamin B-6 levels present. The number of subjects used for the determination of mean vitamer levels was variable in

Table 10. Percentage of Vitamin B-6 vitamers present in whole blood of mothers

Subject	Percent of total Vitamin B-6 present				
	PLP	PL	PN	PM	PMP
(n=25)					
MM	0.0	16.3	53.8	29.9	0.0
MT	0.0	0.0	41.4	23.7	35.0
RB	0.0	30.4	28.8	17.0	23.8
TE	57.6	0.0	0.0	28.4	14.0
RW	24.4	26.0	25.2	24.4	0.0
LW	42.7	10.3	28.7	7.5	10.8
JP	1.4	0.2	0.4	97.7	0.2
FW	89.0	0.0	0.0	11.0	0.0
CW	17.2	81.7	0.0	0.0	1.1
KT	62.0	12.0	5.8	6.0	14.0
MC	35.0	0.0	47.5	0.0	17.5
JN	71.5	19.9	0.0	4.7	3.9
MK	18.3	56.4	0.0	0.0	25.3
MH	57.0	19.4	0.0	4.5	19.1
JDE	9.0	61.6	3.7	17.6	7.9
LG	27.3	50.2	0.0	5.8	16.7
PB	40.8	33.3	0.0	20.3	5.6
SED	27.1	19.8	8.0	26.3	18.8
MS	40.4	17.7	5.3	11.7	24.9
LD	2.8	81.7	7.3	3.1	5.0
LM	86.3	9.9	1.2	0.0	2.5
LW	45.8	7.4	34.2	1.0	11.5
LB	53.6	24.8	21.5	0.0	0.0
JDE	48.4	25.5	0.0	2.1	24.0
SAG	27.9	50.5	12.2	5.8	13.5
MEAN	35.0	26.2	13.0	13.9	11.8
± SEM[1]	±5.2	±4.9	±3.4	±5.4	±2.0

1. Standard error of the mean.

Shane's[47] report i.e. only 2 subjects were used to determine the mean PL concentration, which could further explain the differences observed between these two studies.

Mothers who breast-fed their infants had a higher blood PLP level of 16.98±6.86 ng/ml compared to 8.91±4.65 mg/ml in mothers who fed their infants proprietary formula, however, these differences were not statistically different (Table 11). The higher values observed in breast-feeding mothers is probably reflective of the fact that 1/3 of the breast-feeding mothers consumed vitamin B-6 supplements whereas none of the feeding group II mothers consumed any. One mother in feeding group I (LM) routinely consumed a large dose of vitamin B-6 (9mg/day) and her whole blood PLP was 4 times higher than any of the other reported values. Exclusion of this subject from the analysis lowered the group mean to 10.53±2.51 ng/ml which is similar to the mean for group II.

Group III mothers, those who fed their infant non-proprietary formula, had a mean blood PLP level of 10.24 ± 2.59 ng/ml which is similar to the mothers in the other 2 groups. Comparative statistical analyses were not done with this group due to the small sample number.

The absolute amount of the vitamers present in whole blood have not been frequently reported. Shane[47] reported that 12.1±2.3 ng/ml of PLP was present in whole blood in a group of 23 non-pregnant women. Descriptive information, ie. amount of vitamin B-6 supplementation and dietary intake data, were not available. In this study, the mean amount of PLP present in the whole blood of mothers when feeding groups I and II were combined was 14.42 ± 4.90 ng/ml, and this value decreased to 9.99 ± 2.22

Table 11. Whole blood pyridoxal 5' phosphate (PLP) and whole blood total vitamin B-6 content in mothers who breast-fed, those who fed proprietary formula and those who fed non-proprietary formula[1]

Subjects	Whole blood PLP	Whole blood Total Vitamin B-6
I. Breast-fed (n=15)	ng/ml	ng/ml
LM	107.31	124.32
LW	25.59	55.87
LG	18.61	68.27
MC	5.23	14.96
JDE	8.00	88.69
KT	19.15	31.00
MK	17.41	94.95
PB	27.78	68.08
SED	5.48	20.23
MM	ND	6.49
MT	ND	4.52
TE	2.98	5.17
JP	4.45	--[2]
FW	3.25	3.65
CW	9.53	55.40
MEAN ± SEM[3]	16.98 ±6.86[a]	45.83 ±10.51[b]
II. Proprietary Formula (n=7)		
MH	34.62	60.71
JN	14.40	20.14
MS	6.26	15.50
LD	0.93	33.28
RB	ND	10.07
RW	2.82	11.57
LW	3.35	7.85
MEAN ± SEM	8.91 ±4.65[a]	22.73 ±7.10[b]
III. Non-proprietary Formula[4] (n=3)		
JDE	15.42	31.84
SB	7.49	13.95
SAG	7.82	43.68
MEAN ± SEM	10.24 ± 2.59	29.82 ± 8.64

1. Significantly different means are followed by different superscripts.
2. Not possible to integrate due to large amount present; not used in analysis.
3. Standard error of the mean.
4. Statistical analysis not completed due to small sample numbers.

ng/ml if subject LM was excluded. These mean values are similar to Shane's[47] values. It is important to note, that in this study, there was considerable variation between subjects and that blood PLP values ranged from an undetectable amount to 34.62 ng/ml (excluding subject LM). Nine of the 22 mothers had PLP levels less than 5 ng/ml indicating suboptimal B-6 status. No correlation was found between the lactating mother's whole blood PLP levels and the total vitamin B-6 in breast milk. This may be explained by the observation of Kirksey[26] that the level of the vitamin present in breast milk changes rapidly (within hours) depending upon the amount of the vitamin in the diet. The concentration of blood PLP, however, has been reported to be relatively constant over time[27,47]. It is thought to decrease and increase relative to the dietary intake and to take 3-4 weeks to reach new steady state levels if intake is changed[27]. These differences in sensitivity of the tissues to vitamin B-6 intake may explain why correlation of mean vitamin B-6 content of breast milk with whole blood PLP is difficult. Based on whole blood PLP levels and the total vitamin B-6 content in breast milk approximately half of the lactating mothers studied had sub-optimal vitamin B-6 status.

(b) Infants

Results of whole blood vitamin B-6 vitamers were available for twenty-seven infants. Three of these infants were in feeding group III and results for this group were not statistically analyzed due to the small sample number. An additional 5 infants were excluded from the stat-

istical analysis because they consumed less than 90 percent of their vitamin B-6 intake from breast milk or proprietary formula and therefore did not fit into either group I or II. Results from the remaining 19 infants are summarized in Table 12. All the infants had been consuming breast milk (group I) or proprietary formula (group II) for at least 4 consecutive weeks before they were studied. The major vitamers present in both breast- and proprietary formula-fed infants were PLP and PL. These vitamers contributed 52.7 percent of the total vitamin B-6 present in group I and 79.6 percent in group II infants. There were no significant differences ($p \leq 0.05$) between the percentage of PLP and PL present in breast-fed infants when compared to formula-fed infants. It is interesting to note however, that the mean whole blood PL concentration in formula-fed infants is 2 times that observed in breast-fed infants. This difference may be related to the fact that formula-fed infants consume all of their vitamin B-6 as PN. Anderson et al[1] has suggested that PN is converted to PLP in the red blood cell then to PL for gradual release into plasma. A large amount of PN ingested could then result in a larger concentration of PL in whole blood. The breast-fed infants however, consume their vitamin B-6 as a mixture of the vitamers (Table 5) so the resultant concentration of the vitamers in whole blood may be more evenly distributed. There was no significant difference when the percentage of PLP and PL found in the infants was compared to the percentage found in the mother's whole blood in this study.

Group I was observed to have a significantly larger ($p \leq 0.05$) percentage of PM present when compared to group II. The explanation for this

Table 12. Percentage of Vitamin B-6 vitamers present in whole blood of infants[1,2]

Subject	Percent of total Vitamin B-6 present				
	PLP	PL	PN	PM	PMP
I. Breast-fed (n=12)					
GT	0.0	22.0	12.0	40.0	25.5
HC	18.7	41.8	18.0	17.8	3.7
DK	61.2	11.0	0.0	19.5	8.3
KDE	37.0	37.3	11.4	12.2	2.1
EB	57.7	5.0	9.4	10.7	17.1
BM	25.1	17.6	36.1	11.4	9.8
MT	18.1	14.0	36.1	19.6	12.3
EE	27.6	27.4	28.2	9.3	7.4
LP	39.9	14.3	35.2	10.5	0.0
MW	41.8	0.0	39.3	11.2	7.7
KW	49.8	19.7	15.9	8.5	6.1
MEW	41.5	3.0	12.1	14.5	29.0
	a	b	c	d	f
MEAN ± SEM[3]	34.9 ±5.1	17.8 ±3.7	21.1 ±3.8	15.4 ±2.5	10.8 ±2.6
II. Propietary Formula-Fed (n=7)					
LS	3.1	94.9	0.0	0.5	1.4
SD	14.4	71.6	1.7	1.8	10.4
MB	57.3	15.7	17.2	2.3	7.5
MTW	58.1	11.4	24.2	0.0	6.2
MLW	58.4	15.2	26.4	0.0	0.0
MIW	87.3	11.8	0.0	0.9	0.0
CB	22.8	35.1	21.2	6.9	14.0
	a	b	c	e	f
MEAN ± SEM	43.1 ±11.4	36.5 ±12.7	13.0 ±4.5	1.8 ±0.9	5.6 ±2.1

1. Significantly different means ($p \leq 0.05$) are followed by different superscripts.
2. Infants receiving >90% of vitamin B-6 intake from breast milk or proprietary formula.
3. Standard error of the mean.

is not readily apparent but may be related to the differences in B-6 vitamers ingested between these 2 groups, as previously suggested. Presently there is no other published research data with which to compare these results. Comparison with the mother's data (Table 10) indicated that no significant differences ($p<0.05$) were found between the percentage of vitamers present in breast-fed infants and the percentage found in the mothers. The formula-fed infants had significantly less ($p<0.05$) PM present than found in the mothers but no other differences were determined.

The absolute values for PLP and whole blood vitamin B-6 are summarized in Table 13. Breast-fed infants (group I) had PLP concentrations ranging from an undetectable amount to 60.00 ng/ml with a mean of 11.47±4.61 ng/ml. This concentration is lower than that observed in those infants who were formula-fed (group II) who had a mean of 16.11±3.11 ng/ml with a range of 9.86 to 30.76 ng/ml. The difference between groups was not statistically different. Subject DK, who was breast-fed, had a whole blood PLP concentration which was 4 times higher than the next highest reported value in Group I. The mother of the infant had not consumed additional vitamin B-6 supplements, thus the reason for this high value is not readily apparent. If this subject were excluded from the calculation of the mean for feeding Group I the mean would decrease to 7.06±1.45 ng/ml. This lower mean would then be significantly smaller ($p<0.05$) than the mean PLP concentration for infants who were formula-fed. Comparative data for whole blood PLP concentrations are lacking for infants. Ejderhamn and Hamfelt[14] have reported an average PLP level of 30.23

Table 13. Effect of breast-feeding vs proprietary formula-feeding on whole blood pyridoxal 5' phosphate (PLP) and whole blood total vitamin B-6 of infants[1].

Subjects	Whole blood PLP	Whole blood Total Vitamin B-6
I. Breast-fed[2] (n=12)	ng/ml	ng/ml
GT	TR[3]	22.63
HC	4.96	26.54
DK	60.00	98.01
KDE	14.11	38.03
EB	11.63	20.15
BM	4.48	17.83
MT	2.59	14.34
EE	4.39	15.88
LP	6.60	16.53
MW	3.63	8.68
KW	13.34	26.83
MEW	11.89	28.67
MEAN ± SEM[4]	11.47^{a} ±4.61	27.84^{b} ±6.77
II. Proprietary Formula-Fed[5] (n=7)		
LS	9.86	--[6]
SD	10.03	69.69
MB	24.53	42.79
MTW	13.95	23.99
MLW	13.40	22.95
MIW	30.76	35.22
CB	10.21	44.83
MEAN ± SEM	16.11^{a} ±3.11	39.91^{b} ±7.03

1. Significantly different means ($p<0.05$) are followed by different superscripts.
2. Received >90% of vitamin B-6 intake from breast milk.
3. Trace, nor able to be quantitated therefore calculated as zero.
4. Standard error of the mean.
5. Received >90% of vitamin B-6 intake from formula.
6. Integration not possible therefore not included in analysis.

ng/ml in a group of seven 6 week-old breast-fed infants. The reported range was 12.3 ng/ml-69.2 ng/ml. The higher values were found in those infants whose mothers had consumed vitamin B-6 supplements during pregnancy and lactation. The mean whole blood PLP levels observed in this study are considerably lower than those reported by Ejderhamn and Hamfelt[14]. The infants in this study were older with an average age of 12 weeks compared with Ejderhamn and Hamfelt's study[14] where the infants were only 6 weeks of age. This difference in age may, in part, account for the differences observed in the levels of whole blood PLP. Additionally, Ejderhamn and Hamfelt[14] do not report vitamin B-6 intakes of the infants in their study which perhaps would have further explained the differences.

Comparison of the whole blood PLP levels found in infants with those found in adults shows that by the age of 12 weeks the level in the infant is similar to that found in the adult. It is important to note, however, that half of the infants in group I had PLP levels of less than 5.0 ng/ml indicating that their vitamin B-6 status may not be optimal.

Group III infants (non-proprietary formula-fed) had a mean blood PLP level of 16.47$\pm$ 8.49 ng/ml ranging from a trace amount to 28.31 ng/ml. No further statistical analyses were completed due to the small sample number.

Whole blood vitamin B-6 was not significantly different between those infants who were breast-fed (group I) and those who were formula-fed (group II). The mean concentration for group I was 27.84$\pm$6.77 ng/ml and 39.91$\pm$7.03 ng/ml for group II (Table 13).

Eight infants whose mothers did not consume vitamin B-6 supplements had a mean PLP level of 12.14±7.81 ng/ml in whole blood compared with 10.12±2.2 ng/ml in the infants of four mothers who used a supplement. Although these means are similar further statistical analyses were not completed due to the small number of supplemented mothers. The similarity of the means may be due to the low level of vitamin B-6 content in the supplements taken by these mothers (mean=1.75 mg/day). Additionally, no information is available concerning other dietary sources of vitamin B-6 ingested by the mothers in either group. If total vitamin B-6 intakes had been determined the level of intake might have been more reflective of the infant's blood levels.

Pyridoxal phosphate levels were also compared to determine whether there was any sex difference (Table 14) when infants were grouped together regardless of method of feeding. Shane[47] has reported slightly lower blood PLP values for adult females (12.1±2.3 ng/ml) compared with males (13.5±2.9 ng/ml). The mean PLP level in male infants was 16.96±4.65 ng/ml whereas females had a mean level of 11.99±2.44 ng/ml. There was no significant difference between these means. The lower level observed in female infants may be partially due to the small number of female infants (4) fed proprietary or non-proprietary formula compared with male infants (7) and hence the differences may be more reflective of the type of feeding rather than any difference due to sex.

Table 14. Effect of the sex of infants on blood pyridoxal 5' phosphate (PLP) and whole blood total vitamin B-6 irrespective of feeding group[1].

Subjects	PLP	Total Vitamin B-6
Males (n=15)	ng/ml	ng/ml
GT	TR[3]	22.63
DK	60.00	98.01
SG	16.04	30.15
AH	ND[4]	30.48
MT	2.59	14.34
MB	24.53	42.79
SD	10.03	69.69
EE	4.39	15.88
MTW	13.95	23.99
MLW	13.40	22.95
MIW	30.76	35.22
CB	10.21	44.83
MW	3.63	8.68
KW	13.34	26.83
RE	51.46	107.63
MEAN ± SEM[2]	16.96 ± 4.65^{a}	39.61 ± 7.66^{b}
Females (n=12)		
HC	4.96	26.54
EB	11.63	20.15
KDE	14.11	38.03
CN	23.31	69.89
EED	7.67	28.49
LS	9.86	NA[5]
KDE	28.31	100.55
BM	4.48	17.83
LP	6.60	16.53
MEW	11.89	28.67
SB	TR[3]	19.62
SAG	21.10	45.68
MEAN ± SEM	11.99 ± 2.44^{a}	37.45 ± 7.86^{b}

1. Significantly different means ($p<0.05$) are followed by different superscripts.
2. Standard error of the mean.
3. Trace amount present therefore calculated as zero.
4. Not detected therefore calculated as zero.
5. Large amount present. Not able to be integrated, therefore not included in the calculation.

V. CORRELATION OF THE INFANT'S DIETARY INTAKE WITH WHOLE BLOOD PYRIDOXAL 5' PHOSPHATE (PLP)

As previously stated, the breast-fed infants (group I) had lower PLP levels in whole blood (11.47 $\pm$ 4.61 ng/ml) when compared with proprietary formula-fed infants (group II) (16.11 $\pm$ 3.11 ng/ml). The mean dietary intake of 0.088 $\pm$ 0.004 mg/day of vitamin B-6 was observed to be significantly ($p<0.01$) lower in group I infants compared to the mean intake of group II infants of 0.408 $\pm$ 0.010 mg/day. Analysis to determine if a significant correlation could be found between the level of vitamin B-6 intake and the whole blood PLP levels in both groups of these infants found a correlation coefficient of $r=0.387$ for breast-fed infants and $r=0.309$ for formula-fed infants. These correlations were not significant. When the data from breast-fed infants was further analyzed, a significant correlation ($r=0.820$; $p<0.05$) was found between the infants total vitamin B-6 intake and blood PLP levels if their mothers did not consume vitamin B-6 supplements. Additional analysis to determine if any correlation existed between the infant's blood PLP levels and the level of the individual vitamers in the mother's breast milk was done. The only significant correlation found was between the vitamin B-6 unsupplemented mother's PL content of breast milk and their infant's blood PLP level ($r=0.910$; $p<0.01$). When the vitamin B-6 supplemented mothers were added to the analysis the correlation coefficient decreased to 0.420, a level which was not significant at $p<0.05$. Further analysis to assess the correlation of PL intake (mg) with blood PLP levels (ng/ml) for breast-fed

infants (Table 15) also showed a highly significant value ($r=0.946$; $p<0.01$). Similarly, when supplemented mothers were added to the analysis the correlation coefficient was no longer significant ($r=0.463$). The decrease in correlation when vitamin B-6 supplemented mothers were added to the analysis may be due to the significantly higher level of PL found in the breast milk of these mothers. The higher content of PL in the breast milk did not appear to be directly reflected in higher blood PLP levels in the infant.

Model II linear regression[49] (Figure 2) was calculated to determine the expected increase in blood PLP (ng/ml) per mg of increase in vitamin B-6 intake. Using the mean value of 12.14 ng/ml of whole blood PLP (the mean blood PLP level in breast fed infants of unsupplemented mothers), the regression equation predicts that an intake of 0.061 mg. of vitamin B-6 would have been ingested. This predicted value is the same as the mean total vitamin B-6 intake in this group. (Table 15) The vitamin B-6 to protein ratio can be calculated. Based on the mean protein intake of 7.1 ± 0.7 g/day (Table 9) observed in breast-fed infants, the ratio would be 0.009 mg vitamin B-6 per gram of protein. Based on this prediction, 1/2 of the unsupplemented breast-fed infants consumed an intake of less than 0.009 mg vitamin B-6 per gram of protein. However, actual whole blood PLP levels in this group of infants, showed that 3/4 of the infants had levels lower than 12.14 ng/ml. This mathematical relationship must be interpreted cautiously since it cannot account for differences in absorption and utilization of the vitamin between subjects and because the data on which it was based are very limited in numbers.

Table 15. Total vitamin B-6 intake, pyridoxal (PL) intake and blood pyridoxal 5' phosphate (PLP) level in breast-fed infants

Type of Feeding	Subject	Total Vitamin B-6 Intake	Ratio of Vitamin B-6 to protein	PL Intake	PL Intake as a percentage of total	Blood PLP levels
		mg	mg/g	mg	%	ng/ml
Breast-fed Unsupplemented Mother n=8	HC	0.045	0.008	0.020	44.0	4.96
	KDE	0.051	0.007	0.026	50.9	14.11
	GT	0.051	0.011	0.023	45.0	Tr
	DK	0.124	0.015	0.095	76.6	60.00
	BM	0.050	0.004	0.024	48.0	4.48
	MT	0.042	0.009	0.020	47.6	2.59
	EE	0.092	0.011	0.040	43.5	4.39
	LP	0.034	0.004	0.017	50.0	6.60
	Mean ± SEM[1]	0.061±0.011	0.009±0.001	0.033±0.009	50.7±3.82	12.14± 6.99
Breast-fed Supplemented Mother n=3	EB	0.042	0.009	0.032	76.2	11.63
	MW	0.091	0.025	0.030	33.0	3.63
	KW	0.213	0.031	0.173	81.2	13.34
	Mean ± SEM	0.115±0.051	0.022±0.006	0.078±0.047	63.5±15.3	9.53±2.99

1. Standard error of the mean

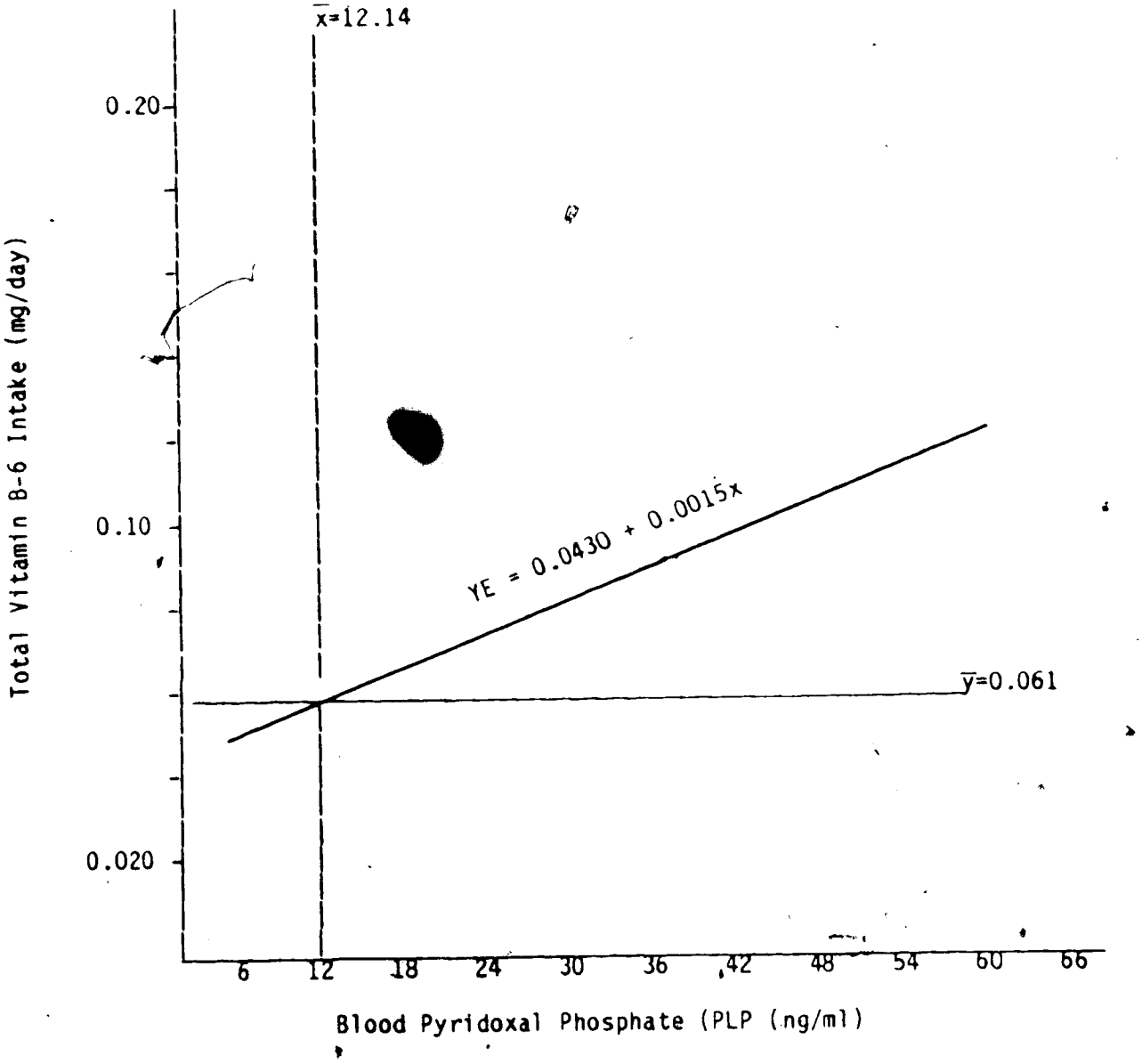

Figure 2. The relationship of the dietary pyridoxal (PL) intake and whole blood pyridoxal 5' phosphate (PLP) levels in breast-fed infants whose mothers did not consume vitamin B-6 supplements.

The reason for the lack of correlation between the intake of vitamin B-6 by proprietary formula-fed infants and their blood PLP levels is not readily apparent. It may reflect a difference in absorption of the vitamin from formula or perhaps the major form in which the vitamin is present in proprietary formulas (as PN) has a varying, individual effect, on the infant's ability to utilize and convert the PN to PLP.

VI. ORAL CONTRACEPTIVE USE (OCA)

The mean levels of the vitamin as measured by whole blood PLP for mothers and their infants grouped according to no use (A), short-term use (B), or long-term use (C) of OCA's prior to conception are similar (Table 16). Statistical analyses were not completed due to the small sample numbers in group B and C. The means however, do not appear to indicate a trend toward lower values with increased use of OCA's. This is contrary to the report by Roepke et al.[44] in which long-term use of OCA was associated with lower levels of vitamin B-6 in serum of women at 5 months gestation, at delivery, in cord serum and in milk at 14 days post-partum compared to women who did not use OCA. Williams[58], however, reported that there was no effect of OCA use on breast milk vitamin B-6 content at 1 month, 3 months and 6 months post-partum. This suggests that OCA-dependent changes are short-term and the data in the present study appear to agree with that suggestion.

Table 16. Effect of oral contraceptive use prior to conception on the whole blood pyridoxal 5' phosphate (PLP) levels in mothers and infants[1]

	Subject	PLP of Mother ng/ml	PLP of infant ng/ml
A. No use[2] n=15	MC	5.23	4.96
	JN	14.40	23.31
	MK	17.41	60.00
	MH	34.62	ND
	JDE	8.00	14.11
	LG	18.61	16.04
	LD	.93	10.03
	MM	ND	4.48
	MT	ND	2.59
	RW	2.82	13.95
	RW	-	13.40
	LW	3.35	30.76
	FN	3.25	3.63
	CW	9.53	13.34
	CB	-	10.21
	Mean ± SEM[5]	9.09±2.76	14.72±3.85
B. Short-term Use[3] n=4	PB	27.78	11.63
	SED	5.48	7.67
	MS	6.26	9.86
	TE	2.98	4.39
	Mean ± SEM	10.63±5.76	8.39±1.56
C. Long-Term Use[4] n=3	JP	4.45	6.60
	KT	19.15	Tr
	RB	ND	24.53
	Mean ± SEM	7.87±5.79	10.38±7.33

1. Statistical analyses not done due to small sample number in group B and C.
2. The mother had not used oral contraceptive agents within 1 year prior to conception.
3. The mother had used oral contraceptives within 1 year prior to conception and had used them for <30 months consecutively.
4. The mother had used oral contraceptives within 1 year prior to conception and had used them for >30 months consecutively.
5. Standard error of mean.

GENERAL DISCUSSION AND CONCLUSIONS

All the infants studied were normal and healthy as judged by the criteria previously stated (pg 15). No significant differences were determined between the growth rates of the breast-fed infants (group I) vs formula-fed infants (group II) even though gains in weight and length were slightly larger in group II. This finding is in agreement with Fomon[14] when he reported that formula-fed infants in general gained more weight and length than breast-fed infants. The difference in the present study may have been significantly different if a larger sample size had been used. The dietary intake of the formula-fed infants was significantly greater ($p<0.01$) for total volume, protein and total vitamin B-6 compared to the breast-fed infants. The observed larger gains in weight and length in proprietary formula-fed infants are consistent with the greater dietary intake. This finding could be interpreted to conclude that the breast-fed infants were underfed compared to the formula-fed infants. The mean weights and lengths of both the breast-fed and formula-fed infants in this study were on the 50th percentile when compared to the data of Hamill et al[19]. This would indicate that the intakes of both the breast-fed and the formula-fed infants in this study were adequate for growth.

The mean vitamin B-6 intake in the breast-fed infants was 0.088 mg per day or 0.013 mg of vitamin B-6 per gram of protein ingested. This mean value is less than the suggested intake of 0.015 mg vitamin B-6 per gram of protein[42] for infants. These suggested intakes however, are

not based upon actual requirement studies with infants but are instead, based on theoretically calculated adequate intakes for formula-fed infants. In the present study, there was a lack of correlation between the total dietary intake of vitamin B-6 and the level of PLP in the blood of breast-fed infants unless the infants of mothers who consumed vitamin B-6 supplements were excluded from the analysis. A correlation coefficient of 0.802 was found between the infants' blood PLP levels and the total vitamin B-6 intake if the mother did not consume vitamin B-6 supplements. A significant correlation was also determined between the infant's PL intake and the whole blood PLP level ($r=0.946$; $p<0.01$). These correlations indicate that as total vitamin B-6 or PL intake increases, so does the whole blood PLP concentration. Linear regression analysis (Figure 2) predicted that a ratio of 0.009 mg of vitamin B-6 per gram of protein was required to achieve a blood PLP level of 12.14 ng/ml. This suggested that the vitamin B-6 requirement may be lower than the current suggested level[42] of 0.015 mg per gram of protein ingested. This suggestion does appear to hold true for infants of unsupplemented mothers. The relationship, however, cannot be extrapolated to all breast-fed infants, since the mean vitamin B-6 to protein ratio for all infants in group I was found to be 0.013 mg/g and the mean blood PLP level was only 11.47 ± 4.61 ng/ml. In this small number of infants the only conclusion which can be made is that the intake of vitamin B-6 and the whole blood PLP levels were lower in infants who were breast-fed when compared to infants who were fed via a proprietary formula. The mean ratio of vitamin B-6 to protein intake in formula-fed infants (group II) was 0.031 mg/g and

the average whole blood PLP was 16.11 ± 3.11 ng/ml. These data however, give rise to some interesting speculation. Firstly, the formula-fed infants had a total vitamin B-6 intake which was 58 percent greater than the breast-fed infants intake. The whole blood PLP levels, however, were only 29 percent greater in the formula-fed infants. This may imply better utilization of the ingested vitamin B-6 from breast milk compared to proprietary formula. Secondly, 6 out of 11 infants who were breast-fed (group I) had blood PLP levels in a low range (≤ 5 ng/ml) while none of the infants who were fed proprietary formula (group II) had levels considered to be suboptimal. This observation could lead one to speculate that, in some women, dietary sources of vitamin B-6 may be inadequate for lactation and this was reflected in low blood PLP levels in the infants.

The mothers studied were free from acute and/or chronic illness and were not routinely taking medications other than vitamin supplements. Their mean vitamin B-6 status, as judged by whole blood PLP level, was in a low normal range with means for group I mothers of 10.53 ± 2.51 ng/ml (exlcuding subject LM) and 8.91 ± 4.65 ng/ml for group II mothers. The total whole blood vitamin B-6 concentration was 39.79 ± 9.30 (excluding LM) in group I mothers and 22.73 ± 7.10 in group II mothers. Seven of 15 mothers who breast-fed their infants had low blood PLP levels (≤ 5 ng/ml) and an approximately equal proportion (4/7) of mothers who fed their infant proprietary formula had low levels. It would have been valuable, in retrospect, to have obtained dietary intake information on the mothers in order to assess the quality of their diets in relation to their blood

PLP levels. The lack of correlation found between lactating mothers whole blood PLP levels and the total vitamin B-6 in their breast milk was explained by the relative differences in these tissues to depletion (page 45).

The breast milk obtained from 20 lactating mothers indicated suboptimal vitamin B-6 status (< 100 ng vitamin B-6 per liter) in over half of the mothers who did not consume vitamin B-6 supplements whereas none of the vitamin B-6 supplemented mothers had low levels. These low levels in 50 percent of the breast-feeding mothers has strong implications to the nutritional quality of her infant's intake. Intake data would have been valuable to determine if the low concentrations of vitamin B-6 in breast milk were also associated with suboptimal intakes. The mothers who consumed even low dose vitamin B-6 supplements (1 mg/day) had acceptable concentrations of vitamin B-6 in breast milk. This may implicate the mothers with low vitamin B-6 breast milk concentrations with having concurrently low intakes. It is always advisable to counsel mothers to improve overall dietary intake in order to increase the nutritional quality of the diet. The low concentrations of vitamin B-6 may also indicate that low levels of other nutrients may be present as vitamin B-6 is widely distributed among foods such as meat, poultry, milk, whole grains, fruits/vegetables. This study however, indicates that unless an effort is made to incorporate foods rich in vitamin B-6 into the diet a dietary supplement of vitamin B-6 is needed.

The vitamin B-6 content of fore-milk (168.66 ± 15.71 ng/ml) was higher than that observed in hind-milk, (149.17 ± 15.03 ng/ml) however, this difference was not significant. One might expect that the vitamin B-6 content would be higher in fore-milk since vitamin B-6 is a water-soluble vitamin and the water content of breast-milk is highest in fore-milk whereas, hind-milk is reported to be richer in fat[50]. The lack of a significant difference between fore-milk and hind-milk should be interpreted cautiously. The sample size (n=44) was relatively small and, therefore, a difference may have been elucidated if n had been increased. In fact, when the provability of detecting a difference, if one existed, was calculated [10] this probability was found to be only 16 percent.

From the present study, there appears to be little effect of oral contraceptive use (OCA) before conception on the vitamin B-6 status, as judged by blood PLP levels, of mothers and their infants at 12 weeks postpartum. A decrease in vitamin B-6 status of long-term OCA users (> 30 mos. consecutive use) has been reported by others[44] at the birth of the infant, in cord blood and in breast milk at 14 days post-partum. Statistical analyses were not completed on the data in this study due to insufficient sample numbers.

SUMMARY

1. The infants studied were normal, healthy and achieved growth patterns within population norms. These infants were born to mothers who were free from acute/chronic disease and were not regularly consuming medications.
2. The mean whole blood pyridoxal 5' phosphate (PLP) in the mothers was 9.99 $\pm$ 3.58 ng/ml. There was no significant difference between the blood PLP levels in mothers who breast-fed their infants and those that fed proprietary formula. Half of the mothers had blood PLP levels considered to be suboptimal ($\leq$ 5.0 ng/ml).
3. Lactating mothers who took vitamin B-6 supplements had significantly higher levels of pyridoxal (PL) in breast milk when compared to those mothers who did not consume supplements. Over half of the non-supplemented mothers had vitamin B-6 concentrations in breast milk that are considered to be suboptimal while none of the mothers who consumed supplements had low levels.
4. The mean vitamin B-6 intake for the breast-fed infants was 0.013 mg of vitamin B-6 per gram of protein ingested, with a range of 0.004 - 0.025 mg per gram. The proprietary formula-fed infants had a significantly higher intake with a mean of 0.031 mg of vitamin B-6 per gram of protein. The intake in this group ranged from 0.020 - 0.040 mg per gram.

5. The mean whole blood pyridoxal 5' phosphate (PLP) levels in breast-fed infants (group I) was 11.47 $\pm$ 4.61 ng/ml which was lower, but not significantly lower, than the mean blood PLP level of 16.11 $\pm$ 3.11 ng/ml observed in the proprietary formula-fed infants (group II). Half of the infants in group I had PLP levels in a very low range (< 5 ng/ml) indicating that their vitamin B-6 status was not optimal. All of the group II infants had blood PLP levels within the reported acceptable range.
6. No significant correlation was observed between the total vitamin B-6 intake and blood PLP levels in formula-fed infants. Analysis of the intakes of breast-fed infants revealed significant correlations between the infants total vitamin B-6 intake and pyridoxal (PL) intake ($r=0.802$ and $r=0.949$, respectively) and their blood PLP levels if the mothers did not consume vitamin B-6 supplements.

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Appendix 1. Nutrition Survey Information Sheet

NUTRITION SURVEY

Investigators (a pediatrician and a dietitian) at the University of Alberta are undertaking a nutrition survey of normal, healthy infants during 1982. The purpose of this survey is to determine the dietary vitamin B-6 intake and how much of this vitamin is retained in the body of the baby. This information will contribute to the very limited knowledge about vitamin B-6 requirement of infants.

We wish to obtain volunteers in the City of Edmonton, Alberta. We are seeking mothers who:

1. Had a normal pregnancy, a normal vaginal delivery and whose babies were placed in the regular nursery after birth.

2. Are willing to participate when their babies are between the ages of 1 month to 1 year.

3. If breastfeeding their infants, are willing to weigh their baby before and after feeding for 1-3 days. Scales will be provided by us. For this same time period are also willing to manually express breast milk samples at each feeding.

4. If formula-feeding their infants, are willing to record the volume of formula taken for 1-3 days.

5. Are willing to have one blood test taken from her baby and one from herself.

Your involvement in this study can take place at your home over 2-3 days.

If you wish to take part in this survey please register by calling the phone number below indicating your interest in the nutrition survey. Your participation will be greatly appreciated.

Appendix 2a. NUTRITION SURVEY: CONSENT FORM (A)

_____ _____

Name of Mother Date

_____

Name of Baby

CONSENT TO PARTICIPATE IN VITAMIN B-6 STUDY

We understand that the purpose of this study is to determine vitamin B-6 intakes of healthy babies. We understand that I will need to collect, for _____ consecutive 24-hour periods, samples of my breast milk, which will be analyzed for vitamin B-6 content. One blood sample will be obtained from me for determination of vitamin B-6 level in my blood. In order to determine the amount of vitamin B-6 our baby is receiving, it will be necessary to record the amount of breast milk our baby received by weighing him/her before and after feeding on the days that breast milk samples were obtained. If our child eats or drinks anything other than breast milk, I will also record this for each 24-hour period. We also understand that to determine the amount of vitamin B-6 in our baby's blood, one small blood sample will be obtained from finger or toe prick. We understand the purpose of this study and I agree to participate. We agree also that our baby will participate in the study.

_____ _____

Witness Mother

Appendix 2b. NUTRITION SURVEY: CONSENT FORM (B)

_____ _____

Name of Mother Date

_____

Name of Baby

CONSENT TO PARTICIPATE IN VITAMIN B-6 STUDY

We understand that the purpose of this study is to determine vitamin B-6 intakes of healthy babies. We understand that I will need to record for _____ consecutive 24-hour periods, the amount of formula our baby drinks. If our child eats or drinks anything other than formula, I will also record this for each 24-hour period. We also understand that to determine the amount of vitamin B-6 in our baby's blood, one small blood sample will be obtained from finger or toe prick. One blood sample will be taken from me to determine the amount of vitamin B-6 in my blood. We understand the purpose of these studies and agree to participate. We also agree that our baby will participate in the study.

_____ _____

Witness Mother

Appendix 3. Instruction for use of scale

INSTRUCTIONS

1. To turn on scale: use power switch labelled 9 .

   - the scale will read 88888 then automatically return to 0.0g.

2. To calibrate scale: turn calibration lever labelled 2 to the down position. Scale display will flash 3 times and then read 15000.0 og. Turn calibration lever back to original position.

3. Put scale top on balance pan labelled 1 .

4. Press red "T" button labelled 6 . Machine should return to 0.0g.

5. Press "A" button on the side panel.

6. Press "A" button again once display p[illegible] reads 20.

7. Put baby on scale top. DO NOT HOLD BABY WHILE WEIGHING.

8. Press "A" again .... Record final number displayed on Breast Milk Record Sheet. This is the weight before feeding.

9. Feed baby.

10. Put baby on scale top. Press "A" button .... record final number displayed on Breast Milk Record Sheet. This is the weight after feeding.

Appendix 4a. Breast Milk Record Sheet

NAME: ______________________ DATE: ______________________

BREAST MILK RECORD SHEET

Please record on this sheet the time of the feeding and the weight of the baby before and after the feeding.

Time ____________ Weight before feeding ________ Weight after feeding ________	Time ____________ Weight before feeding ________ Weight after feeding ________
Time ____________ Weight before feeding ________ Weight after feeding ________	Time ____________ Weight before feeding ________ Weight after feeding ________
Time ____________ Weight before feeding ________ Weight after feeding ________	Time ____________ Weight before feeding ________ Weight after feeding ________
Time ____________ Weight before feeding ________ Weight after feeding ________	Time ____________ Weight before feeding ________ Weight after feeding ________
Time ____________ Weight before feeding ________ Weight after feeding ________	Time ____________ Weight before feeding ________ Weight after feeding ________
Time ____________ Weight before feeding ________ Weight after feeding ________	Time ____________ Weight before feeding ________ Weight after feeding ________

Appendix 4b. Formula Record

NAME: _____

FORMULA RECORD SHEET

Please record on this sheet all the formula your child drinks today.

DATE: _____ BE SURE TO SPECIFY AMOUNTS.

Time _____

Amount _____

Formula Type _____

Time _____

Amount _____

Time _____

Amount _____

Time _____

Amount _____

Time _____ Time _____

Amount _____ Amount _____

Time _____ Time _____

Amount _____ Amount _____

Time _____ Time _____

Amount _____ Amount _____

Time _____ Time _____

Amount _____ Amount _____

Appendix 4c. Food Intake Record Sheet

NAME: _____

FOOD INTAKE RECORD SHEET

Please record on this sheet everything your child eats or drinks today. DATE _____. Specify amounts and types of foods as well as all beverages (IE. 1/2 cup orange juice, 2 tablespoons beans, 1/2 cup vegetable soup, 2 soda crackers).

BREAKFAST

Mid-Morning Snack

NOON MEAL

Afternoon Snack

EVENING MEAL

Evening Snack

Appendix 5. Nutrition Survey Questionnaire

NAME: MOTHER/CHILD

ADDRESS:
PHONE NUMBER:

CARD NUMBER

I.D. NUMBER

DATE

CHILD'S BIRTHDATE

SEX: 1=Female; 2=Male

WEIGHT at birth in gms.....

LENGTH at birth in cms.....

Any health problems, malformations etc.?
1=No; 2=Yes, describe.....
.....

PRESENT WEIGHT in gms.....

PRESENT LENGTH in cms.....

NUTRITION COLUMN: 1=Breast only; 2=Formula only, describe.....
.....; 3=Cow's milk only; 4=1+ solids; 5=2+ solids; 6=3+ solids;
7=Other, describe.....

MEDICATION COLUMN: 1=None; 2=Vitamins, describe.....
3=Iron, describe.....; 4=Others, describe.....

MOTHER'S BIRTHDATE

PARA GRAVIDA

The course of this last pregnancy was: 1=Normal; 2=Maternal complication;
3=Other, describe.....

ORAL CONTRACEPTIVES: 1=Never used; 2=Has used without interruption before this pregnancy but not now; 3=Has used in the past but not now; 4=Is using them now; 5=Other, describe .....
.....

Month/Year first used

Total number of months used

MEDICATION: 1=Multivitamins and/or iron, describe.....
2=Other, describe.....
3=None

PRE-EXISTING ACUTE/CHRONIC ILLNESS? Describe.....
1=Yes 2=No