한국영양학회지 20(5) : 318 - 329 , 1987
Korean J Nutr Vol 20(5) : 318 - 329, 1987

Effect of Long-term Pyridoxine Depletion on Lipid Composition in the Developing Rat Brain*

Jung Hee Kim, Haymie Choi, Sun Kyung Ji
Dept. of Food and Nutrtion, Seoul National University

장기간의 Pyridoxine 부족이 새끼쥐 뇌의 지방조성에 미치는 영향

김정희 · 최혜미 · 지선경
서울대학교 가정대학 식품영양학과

□ 국 문 초 록 □

이유한 Sprague Dawley 암컷 쥐에게 성장, 임신, 수유기간동안 pyridoxine이 풍분한 식이와 pyridoxine이 부족된 식이를 주어 사육했으며, 또는 다른 군은 성장, 임신기간은 pyridoxine이 부족된 식이를 공급받다가 생후 5, 10, 21일에 각각 pyridoxine이 풍부한 식이로 바꾸어 주어 회복정도를 살펴보았다.

출생 후 5, 10, 15, 21, 35, 50일에 체중을 재고 새끼를 희생시켜 뇌와 적혈구를 실험에 사용하였다. 적혈구에서는 alanine aminotransferase 활성을 측정하였고, 뇌에서는 cholesterol, proteolipid protein, cerebroside를 측정하였다.

Pyridoxine이 부족된 쥐는 실험기간동안 유의적으로 체중과 뇌무게가 감소했으며, erythrocyte alanine aminotransferase 활성의 증가정도가 훨씬 높은 것으로 보아 체내 비타민 B_6의 영양상태가 나쁨을 알 수 있었다. 한편, 뇌의 cerebroside의 함량은 유의적인 차이를 보였으며 5, 10, 21일에 supplementation 시킨 쥐의 이유후의 cerebroside의 생성속도의 증가로 보아 supplementation시킨 쥐의 myelination이 저해되었음을 알 수 있다. 한편 cholesterol, proteolipid protein도 부족식이에 의해 영향을 받았는데 생후 5일에 supplementation시킨 쥐는 정상수준으로 회복되나 10일 이후에 supplementation 시켰을 때는 정상수준에 미치지 못하였다.

INTRODUCTION

Mature brain is refractive to marked change in the diet inclusing malnutrition. However, neonatal and developing brains are known to be highly susceptible to the nutrition of the mother during the

* *This work was supoorted in part by a research grant from Korean Science and Engineering Foundation.*
접수일자 : 1987년 8월20일

period of active myelination.

Vitamin B_6 has been demonstrated to be an essential nutrient in the normal development of the central nervous system(CNS) [1~3]. Pyridoxal phosphate, the active coenzyme form of vitamin B_6, affects brain development by participating in the transport and metabolism of amino acids, in the synthesis of fatty acids, in the γ-aminobutyric acid(GABA) shunt pathway, etc. More specifically, in brain, vitamin B_6 is required for the activation of sphingolipid synthesis[4]. The carbon atoms of sphingosine are donated by serine and palmityl CoA or in the absence of palmityl CoA, by palmitic aldehyde. The addition of palmitic aldehyde to serine requires the activation of the methylene group of serine. This is accomplished by the formation of a schiff base metal complex of serine with pyridoxal phosphate and Mn^{++}. Since pyridoxine is required for the synthesis of sphingosine, a component of cerebrosides, it is expected that a reduction in dietary pyridoxine during myelination could result in a reduction of cerebroside levels in the brain.

Myelin is a highly specialized membrane characteristic of the vertebrate nerves. The myelin-forming cells of the CNS is oligodendroglia cells. Myelin is relatively abundant in all parts of the nervous system, and gives rise to the appearance of 'white matter' as a result of its high concentration(50~70% of dry weight) in fiber tracts in the brain. Myelin is comprised of 70~75% lipid and 25~30% protein(dry weight)[5].

Davison and Dobbing[6] reported that myelination is a vulnerable period in brain development and that nutritional deprivation occuring during that period will have a depressant action on myelination. Myelination of the rat brain begins at 10~15 days, after which time the rate abruptly declines to adult levels. Undernutrition in the rat prior to and during the time of rapid myelination result in a decreased synthesis of myelin. Cerebral development of pups from dams deficient in protein and energy was also usually improved if nutrition supplementation was begun before myelination[7]. However, if malnutrition persisted during myelination, irrepairable damage can result[8].

As a coenzyme role in the metabolism of amino acids is well established. Especially, transaminases are more sensitive than amino acid decarboxylase to the pyridoxine deffciency. Several investigators [9~11] have suggested that changes in percentage stimulation of erythrocyte alanine aminotransferase (E-ALAT) activity after in vitro additon of pyridoxal phosphate is a more reliable measure of the nutritional adequacy of vitamin B_6 than the measurement of the activity. Thus, % stimulation of E-ALAT is used as indicators of B_6 status in the body and can also be used as parameter as to determine vitamin B_6 requirement.

In this study, brain lipid and E-ALAT were compared during different stages of postnatal development in progeny of rats fed a low level and adequate level of vitamin B_6 during growth, gestation and lactation. And another objective of this study was to determine whether altered brain lipid compositions produced by a vitamin B_6 deficiency of the mother, can be prevented or reversed, when vitamin B_6 supplementation of the mother is initiated before or after the critical period of brain development of the progeny.

MATERIALS AND METHODS

1) Animal and Diet

Two hundred weanling female Sprague Dawley rats weighting 50~60g supplied by Animal Breeding Laboratory of Seoul National University,

Table 1. Composition of experimental diet(g/100g diet)

	Pyridoxine -deficient	Pyridoxine -suffieient
Vitamin-free easein	20.0	20.0
Corn starch	58.5	58.5
Corn oil	5.0	5.0
Beef tallow	5.0	5.0
Salt mixture[1]	4.0	4.0
Cellulose	2.0[8]	5.0
Vitamin mixture $-B_6$[2]	2.08	—
$+B_6$[3]	0.12	2.2
DL-Methionine	0.3	0.3

1) Composition of salt mixture. g/kg of mixture: $CaHPO_4$ 500g, NaCl 74g, K_2SO_4 52g, Potassium Citrate Monohydrate 220g, MgO 24g, Manganous Carbonate(43-48 % Mn) 3.5g, Ferric Citrate(16-17 % Fe) 6.0g, Zinc Carbonate 1.6g, Cupri Carbonate(53-55 % Cu) 0.3g, KIO_3 0.01g, Chromium Potassium Sulfate 0.55g, $Na_2SeO_3 \cdot 5H_2O$ 0.01g, Suerose, finely powedered 118.0g.

2) Nutritional Biochemicals, ICN Life Science Group, Cleveland, Ohio, Vitamin mixture ($-B_6$): vitamin A conc. (200,000 units per gm) 4.5g, vitamin D conc. (400,000 units per gm). 0.25g, *α-to-chloride* 75.0g, menadione 2.25g, p-Aminobenzoic acid 5.0g, Niacin 4.5g Riboflavin 1.0g, Thiamine Hydrochloride 1.0g, Calcium Pantothenate 3.0g, Biotin 0.02g, Folic acid 0.09g, vitamin B_{12} 0.00135g and starch to 1kg.

3) Vitamin mixture ($+B_6$) contained, in addition to the above vitamins 1g of pyridoxine hydrochloride per kg of vit. mixture.

were divided into two groups, control and deficient. When female rats grew to approximately 180~200g in weight, they were mated with males of the same strain(2 : 1). Pregnancy assumed to have begun when sperms were found in a vaginal smear (x200) in the morning of test day. After postpartum, deficient groups were divided into three groups according to rehabilitation period of 5(D_5), 10(D_{10}), and 21(D_{21}) days postpartum, and one deficient(D) group continued the same diet throughout the experimental period of 50 days.

Rats were housed in a plexiglas cage. Temperature and humidity were kept 20± 1℃, 55± 1%, respectively. Light and darkness were also controlled.

Two kinds of experimental diet were used. The compositions are given in Table 1. Pyridoxine-sufficient diet(control diet) contains pyridoxine · HCl 22mg/kg diet and pyridoxine-deficient diet contains pyridoxine · HCl 1.2mg/kg diet.

2) Sampling

On the first day of lactation, pups from each dam were counted, weighed, and randomly reduced to 8. At the age of 5, 10, 15, 21, 35 and 50 days, body and brain weights were measured. Pups were sacrificed by cervical dislocation, and brains were removed and stored at-20℃. Bloods were collected in a syringe treated with heparin by cardiac puncture. They were placed immediately on ice without delay and centrifuged. The plasma and buffy coat were discarded. Cells were washed by mixing with twice the volume of physiological saline solution followed by centrifuging and discarding the supernatant. The procedure was repeated. Erythrocytes were pipeted 200μl or 500μl in the packed red cell layer and frozen until assayed for erythrocyte alanine aminotransferase (E-ALAT) activity. Hemolysates were made immediately before the measurement of E-ALAT activity.

3) Experimental Methods

The activity of E-ALAT before and after addition of PLP was measured by the colorimetric method of Tonhazy et al[12] and Bayoumi et al[13] as modified by Woodring and Storvick[14]. Vitamin B_6 status was classified as "adequate" (≤25%) or "poor" (>25 % E-ALAT stimulation)

Total protein was measured colorimetrically by the method Lowry et al[15]. Proteolipid protein was determined from the chloroform : methanol extract by the modification of Lowry procedure[16]. Chole-

Table 2. Effect of long-term pyridoxine depleted diet on body weight of offsprings (g)

Group days	C	D	D_5	D_{10}	D_{21}
1	7.69± 0.25[a](13)[b]	6.67± 0.74 (59)**	6.67± 0.74 (59)**	6.67± 0.74 (59)**	6.67± 0.74 (59)**
5	14.20± 0.58 (72)	13.23± 1.10 (286)**	13.23± 1.10 (286)**	13.23± 1.10 (286)**	13.23± 1.10 (286)**
10	27.97± 1.26 (54)	25.05± 2.07 (158)**++	27.48± 1.97 (54)**	25.05± 2.07 (158)**++	25.05± 2.07 (158)**++
15	446.38± 2.52 (36)	36.53± 6.94 (72)** ++	44.29± 4.52 (36)*	43.17± 1.85 (36)**	36.53± 6.94 (72) **++
21	70.32± 3.85 (27)	51.15± 11.92 (47)** ++	67.94± 6.82 (27)	67.64± 3.79 (27) *	51.15± 11.72 (47)**++
35	136.67± 12.43 (18)	115.23± 9.20 (16)**++	133.49± 11.67 (18)	136.32± 15.55 (18)	126.20± 19.87 (15)** ++
50	190.33± 18.36 (9)	169.96± 9.78 (7)*+	185.29± 14.93 (9)	181.82± 20.92 (8)	177.23± 19.39 (7)*+

a : Mean± S.D.
b : Number of animals used for calculation.
* : $p < 0.05$, significantly different from control group.
**: $p < 0.01$, significantly different from control group.
+ : $p < 0.05$, significantly different from D_5 group.
++: $p < 0.01$, significantly different from D_5 group.
: $p < 0.01$, significantly different from D_{10} group.

C : Control.
D : Deficient throughout.
D_5 : Deficient group supplemented at 5 days postpartum.
D_{10} : Deficient group supplemented at 10 days postpartum.
D_{21} : Deficient group supplemented at 21 days postpartum.

sterol was determined by the fluorometric method of McDougal and Farmer[17)18)].

Galactocerebroside was determined by the orcinol-sulfuric acid reaction as modified by Hess and Lewin[19)].

4) Statistical analysis

The data were analyzed statistically by the student t-test[20)].

RESULTS

1) Body Weight and Physical Appearance

At almost all ages, body weights of offsprings of deficient group were significantly lower than those of the control and deficient groups supplemented at 5(D_5) and 10(D_{10}) days(Table 2). At weaning, body weight of deficient group showed 75% of the control. At 15, 21 and 35 days of age, body weights of deficient supplemented at 5(D_5) and 10(D_5) days were higher than those of the deficient group(D) but no differences appeared between deficient supplemented at 21(D_{21}) days and the deficient group (D). These results explain that the later supplementation, the less chance of recovery.

From 10 to 21 days of age, some of unsupplemented dams were nervous and offsprings were cannibalized by their dams. And unsupplemented dams became slender and hair of their pups became darker in color, rarer in number, and had scaly skin than the control. Between 15 and 21 days, some signs of pyridoxine deficiency such as tremors, rapid running movement, and convulsion were observed. After these signs appeared, some of the pups did not survive their lactation period. Several investigatiors[1)21)22)] have also found that progeny of rats fed a vitamin B_6 free diet or low levels of the vitamin during gestation and lactation showed

Table 3. Effect of long-term pyridoxine depleted diet on brain weight of offsprings(g)

Grdoup days	C	D	D_5	D_{10}	D_{21}
1	0.298±0.021[a](13)[b]	0.256±0.032(46)**	0.256±0.032(46)**	0.256±0.032(46)**	0.256±0.032(46)**
5	0.568±0.030 (18)	0.537±0.045(69)**	0.537±0.045(69)**	0.537±0.045 (69)**	0.537±0.045(69)**
10	1.024±0.068 (18)	0.996±0.059(50)*	1.011±0.053(50)	0.996±0.059 (50)*	0.996±0.059(50) *
15	1.385±0.072 (9)	1.290±0.093 (18)**	1.284±0.127 (9)	1.132±0.051 (9)	1.290±0.093 (18) **
21	1.541±0.113 (9)	1.422±0.106(16) *++	1.549±0.061 (9)	1.489±0.037 (9)	1.422±0.106(16) *++
35	1.811±0.103 (9)	1.641±0.075 (8)*++	1.740±0.071 (9)	1.767±0.108 (9)	1.680±0.099 (8)*
50	1.860±0.118 (9)	1.794±0.058 (7)	1.860±0.122 (9)	1.833±0.105 (9)	1.849±0.082 (7)

a : Mean±S.D.
b : Number of animals used for calculation.
* : $p < 0.05$, significantly different from control group.
** : $p <$
+ ..
‡ ..

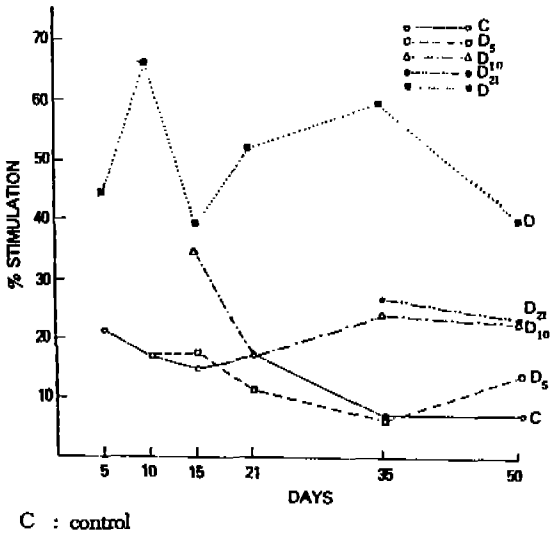
symptoms of pyridoxine deficiency between 10 to 18 days postnatally. This period coincided with the time of rapid myelination in the rat[1)21)22)].

2) Brain Weight

From birth, the brain weights of unsupplemented pups were significantly lower than the control(Table 3). But no significant differences were found between the control and deficient group supplemented at 5(D_5) or 10(D_{10}) days. Brain growth rate was higher in deficient pups than the control. Thus pyridoxine deficiency had a relatively more profound effect on the body weight than the brain weight. Therfore, at the end of experimental period, there are no significant differences in brain weight among the groups.

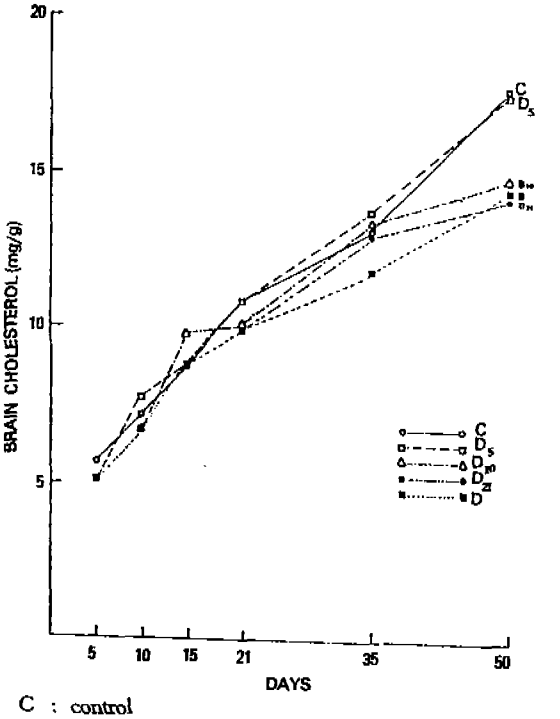
3) Percentage Stimulation of Erythrocyto Alanine Aminotransferase(E-ALAT)

Fig. 1 shows percentage stimulation of E-ALAT activity. When the conservative criterion for the vitamin B_6 inadequacy suggested by Sauberlich et al. (E-ALAT stimulation$>$25%) is used, deficient groups at all ages showed inadequacy in vitamin B_6 status. There was signifficant difference in E-ALAT activity between the control and deficient group. Especially, at 10 days of age, vitamin B_6 status in the deficients(D) group is the poorest. D_5 as well as control group showed an adequacy in vitamin B_6 D_{10} and D_{21} were also rehabilitated to adequacy ($\leq$25%). But their values were not reached to that of the control group. These results indicate that a good maternal vitamin B_6 status is very important to the offsprings, especially if breast fed. A good maternal vitamin B_6 status results from high dietary intake of vitamin B_6.



C : control
D_5 : deficient group supplemented at 5 days postpartum
D_{10}: deficient group supplemented at 10 days postpartum
D_{21}: deficient group supplemented at 21 days pospartum
D : deficient throughout

Fig. 1. Effect of long-term pyridoxine depleted diet on percentage stimulation of E-ALAT activity of offsprings.



C : control
D_5 : deficient group supplemented at 5 days postpartum
D_{10}: deficient group supplemented at 10 days postpartum
D_{21}: deficient group supplemented at 21 days postpartum
D : deficient throughout

Fig. 2. Effect of long-term pyridoxine depleted diet on brain cholesterol of offsprings.

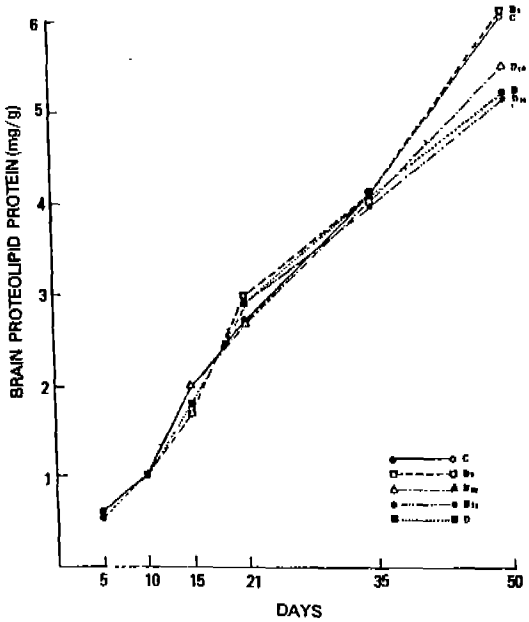
4) Cholesterol Content

Cholesterol content(Fig. 2) increased steadily throughout the 50 day period in all 5 groups. Cholesterol contents of deficient group were significantly lower than those of the control at 5, 21, 35, and 50 days. There were significant differences between the control and D_{10} at 21 days. At 50 days of age, cholesterol contents of D_{10} and D_{21} were significantly lower than those of the control and D_5.

5) Proteolipid Protein and Cerebroside

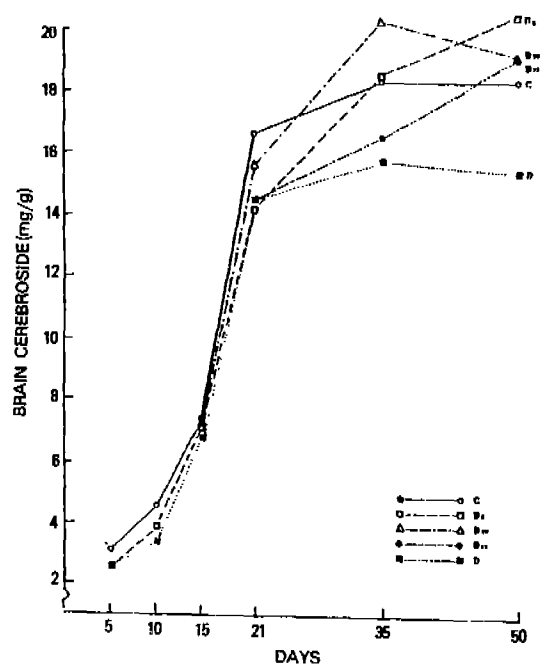
Proteolipid protein is considered as one of the myelin marker during the development. Proteolipid protein content(Fig. 3) also increased steadily throughout the 50 day period in all 5 groups. At 5, 15, and 50 days of age, the deficient group(D) was significantly lower than the control. At 50 days of age, the deficient group(D) was significantly lower than the control and D_5.

Cerebroside content (Fig. 4) was significantly different between the control and deficient group(D) at 10, 21, 35, and 50 days. The normal rate of increase in cerebroside levels in brain during the postnatal development was inhibited in pups from unsupplemented dams, and delayed in pups from deficient supplemented dams(D_5, D_{10} and D_{21}) in postweaning period. In developing brain, significant increase in cerebrosides during 15 to 21 days were observed. After weaning increase rate of cerebroside



C : control
D_5 : deficient group supplemented at 5 days postpartum
D_{10}: deficient group supplemented at 10 days postpartum
D_{21}: deficient group supplemented at 21 days pospartum
D : deficient throughout

Fig. 3. Effect of long-term pyridoxine depleted diet on brain proteolipid protein of offsprings.



C : control
D_5 : deficient group supplemented at 5 days postpartum
D_{10} : deficient group supplemented at 10 days postpartum
D_{21} : deficient group supplemented at 21 days postpartum
D : deficient throughout

Fig. 4. Effect of long-term pyridoxine depleted diet on brain cerebroside of offsprings.

was slightly depressed. Each of the lipid fractions (cholesterol, proteolipid) in brain increased between 10 and 21 days of the lactation. But the increment in the cerebroside content was the greatest (about fourfold) among the lipid fractions analyzed.

DISCUSSION

The National Research Council[23] recommended that 7mg of vitamin per kg diet was needed for adequate growth of rat. The level of pyridoxine, approximately 90μg/day meets this recommendation. Driskell et al[2] reported that vitamin B_6 requirement as determined by behavioral patterns and biochemical assessment such as DNA, RNA, and E-ALAT, appeared to be approximately 45μg daily for weanling and sexually mature male rats. According to Kirksey et al[24], the needs for both young and older pregnant rats possibly exceeded 19.2mg pyridoxine/kg diet for vitamin B_6 saturation for maternal liver, fetus and fetal brain. Several investigators[2,10,25] have suggested that the pyridoxine requirement can be ascertained by determining for the level required to produce a plateau in E-ALAT enzyme response. In this study, pyridoxine · HCl 22mg/kg diet was used as pyridoxine supplemented diet, pyridoxine · HCl 1.2mg/kg diet as pyridoxine deficient diet. And these diets were fed throughout the growth as well as during the gestation and the lactation.

In this study, it was demonstrated that long-term

maternal pyridoxine depletion affects body weight of offsprings. Especially before weaning, the difference in body weight between the control and deficient group increased. This result means that when maternal pyridoxine depletion period was longer during the most critical period of brain development, the effect on the growth of offsprings become more severe. Growth is more affected by protein, by food deprivation, or by pyridoxine-free diet[26] than long-term pyridoxine depleted diet. Many investigators have also reported the similar results of effect of pyridoxine deficient diet on the body weight of offspring[1~3,21,24].

This study also demonstrated a significant decrease in brain weight. However, total protein content in brain was not significantly different among the groups. Thus, the decrease in brain weight was not a reflection of a decrease in total protein content. Stephens et al[22] suggested the possibility that myelination might be affected in the pyridoxine deficient rats at preweaning period, since the myelin lipids contribute greatly to the total brain weight.

The adverse effects observed postnatally in body and brain weights in pups of dams fed low levels of pyridoxine for long term. This discrepancy in brain and body weights mean that food intake of dams may be depressed or may result in a reduction in the amount of available milk, or may be too weak to suckle adequatley to obtain sufficient nourishment. In the present study, food intake was not correctly measured. But generally, food intake of deficient dams was slightly decreased. Roberts and Williams[27] showed that vitamin B_6 deficient diet resulted in decreased vitamin B_6 content in milk rather than in decreased milk production.

Several investigators[28,29] reported that rehabilitation during lactation did not fully reverse the growth retardation of pups resulting from food and protein restriction imposed on dams during gestation. However, in this study the effect of vitamin B_6 deficiency in dams on growth of their pups were almost reversed when pyridoxine supplementation was given. But the earlier supplementation given, the better growth resulted.

Some investigators[1,21] found that vitamin B_6 concentration by microbiological assay in brain was significantly reduced in pups of dams fed low levels of pyridoxine. Pang and Kirksey[1] found that the concentration of vitamin B_6 increased during postnatal development of brain except in pups from deficient dams fed 1.2mg pyridoxine/kg diet. Thomas et al[21] reported that vitamin content in whole brain cerebrum and cerebellum did not increase during 5 to 10 days. William and Coniglio[30] found little increase in pyridoxine content during 7 to 21 days in brains of pups suckled by pyridoxine deficient dams.

However, in the present study, vitamin B_6 content in brain was not directly measured. Percentage stimulation of E-ALAT was used to assess the status of vitamin B_6 in tissue. Some investigators[31] reported that at early stage of pyridoxine deficiency E-GOT (Erythrocyte GOT) is a more sensitive indicator of vitamin B_6 status than E-GPT (E-ALAT). But recently several investigators[9~11,14,24] have suggested that changes in percent stimulation of E-ALAT (EGPT) activity after in vitro addition of pyridoxal phosphate is a more reliable measure of the nutritional adequacy of the coenzyme form of pyridoxine and would not be directly affected by variation in enzyme activity rates occurring with endogenous hormonal or chemical fluctuations. They suggested that apoenzyme as well as coenzyme was reduced by the low level of vitamin B_6 intake. And they also suggested % stimulation was significantly, influenced by age as well as diet. Kirk-

sey et al[24] found that the greatest percentage stimulation of E-ALAT was found in young animals receiving the lowest level of dietary pyridoxine (1.2 mg/kg diet). This is in an agreeement with our experiment. Values for % stimulation of E-ALAT tended to be higher for the younger animal. This result indicates that the younger the animal, the higher requirement of vitamin B_6.

The deposition of the various lipids in the brain during 5 to 50 days of age was altered in whole brain by the low levels of maternal intake of pyridoxine. Especially, the effects of pyridoxine depleted diet on cerebroside, is characteristic[21]. Sphingolipids have also been shown to be reduced in pups of dams fed pyridoxine-free diet and low levels of pyridoxine in several studies[1)21)32]. Kurtz et al[32] found that sphingolipids were reduced 30% to 58% in brains of progeny from dams fed pyridoxine-free diet from parturition. Williams and Coniglio[30] also found low levels of cerebrosides in pups from dams fed pyridoxine-free diet from parturition. Pang and Kirksey[1] found that cerebroside content was significantly lower in the brains of 12 and 21 day-old progeny from dams fed 1.2mg pyridoxine/kg diet compared with the control rats fed 19.2mg/kg diet. Brain ganglioside values of pups from deficient dams were also reduced[32].

Results of our experiment showed that all lipid fractions in brain increased from 10 to 20 days but the greatest increase were found in the cerebroside content. This is in an agreement with Davison and Dobbing's postulation[6] that myelination occurs rapidly between 10 and 21 days postpartum in the rat. Thus, it is confirmed that cerebroside is the most specific marker in myelination.

Ceison and Waisman[33] reported no significant differences in the ganglioside content in brain but found the myelin lipids lower in protein-calorie restricted pups. These authors suggested that normal concentration of ganglioside found in the undernourished rats may indicate that the synthesis of synaptic neuronal membranes, which contain greater concentrations of gangliosides, was less affected by the stress of malnourishment than the synthesis of myelin.

The deposition of various lipids was altered by long-term pyridoxine depleted diet of dams. The normal rate of increase in cerebroside levels in brain during postnatal development was inhibited in pups from unsupplemented deficient dams (D), and delayed in pups from supplemented deficient dams (D_5, D_{10}, D_{21}) in postweaning period. Especially, cholesterol and proteolipid protein contents were also significantly influenced by longterm pyridoxine depleted diet. A number of investigator[34~36] have employed proteolipid protein and cerebroside content as indices of myelination and myelin integrity. If this is valid, the pyridoxine deficient pups might be expected to have reduced levels of myelin. Especially, when the degree of myelination was determined at 15 days. Electron micrographs showed marked decrease of myelination in lower level of pyridoxine diet group[37].

The various parameters of brain development assessed in pups from D_5 were not significantly different from those in pups of control dams. However, some differences in brain development of pups were evident when supplementation of dams was delayed to 10 days or at weaning. Especially, cholesterol and proteolipid protein of D_{10} and D_{21} were similar to values of unsupplemented deficient group (D). These results indicate that brain development, particulary myelination, was affected by a deficiency of vitamin B_6 prior to and including the period of rapid myelination. Thus, the present results are in an agreement with the postulation of Bass[38].

They postulated that this lipid abnormality and others are a consequence of decreased myelin formation resulting from damage of glia cell precursors which fail to undergo differentiation. Differentiations of both neurons and oligodendroglia cells are responsible for the marked changes in lipid content that accompany early growth of the central nervous system.

SUMMARY

Weanling female Sprague Dawley rate were fed 1.2mg pyridoxine · HCl/kg diet(depleted diet) and 22mg pyridoxine · HCl/kg diet(control diet). The control and one depleted group were fed their diets throughout growth, gestation, and lactation. Other three depleted groups were fed the depleted diet throught growth and gestation, and then pyridoxine was supplemented by feeding control diet at 5, 10, and 21 days postpartum. The brains were analyzed for proteolipid protein, cholesterol, and cerebroside. Percentage stimulation of erythrocyte alanine aminotransferase activity was also determined.

Body and brain weight were significantly lower at all ages in depleted group than the control and depleted group showed inadequacy of B_6 at all ages. Proteolipid protein and cholesterol were significantly lower in the depleted group at 10, 21, 35 and 50 days. The postnatal development of cerebroside in brain was delayed in depleted groups supplemented at 5, 10, and 21 days. When supplementation was initiated at 5 days postpartum, contents of cholesterol and proteolipid protein were reversed. But some differences in brain development of pups were evident when supplementation of dams was delayed to 10 days or 21 days.

REFERENCES

1) Pang RL, Kirksey A. *Early postnatal changes in brain composition in progeny of rats fed different levels of dietary pyridoxine. J Nutr 104 : 111~117, 1974*

2) Driskell JA, Stricklan LA, Poon CH, Foshee DP. *The vitamin B_6 requirement of the male rats as determined by behavioral patterns, brain pyridoxal phosphate and nucleic acid composition and erythrocyte alanine aminotransferase activity. J Nutr 103 : 670~689, 1973*

3) Moon WY, Kirksey A. *Cellular growth during prenatal and early postnatal period in progeny of pyridoxine deficient rats. J Nutr 103 : 123~133, 1973*

4) Brady RO, Fomica JU, Koval GJ. *The enzymatic synthesis of sphingosine. II. Further studies on the mechanism of reaction. J Biol Chem 233 : 1072~1075, 1958*

5) Wiggins RC. *Myelin development and nutritional insufficiency. Brain Res Rev 4 : 151~175, 1982*

6) Davison AN, Dobbing J. *Myelination as a vulnerable period in brain development. Br Med Bull 22 : 40~44, 1966*

7) Winick M. *Cellular growth during early malnutrition. Pediatrics 47 : 969~978, 1971*

8) Dobbing J, Sands J. *Vulnerability of developing brain . IX. The effect of nutritional growth retardation on the timing of the brain growth spurt. Biol Neonate 19 : 363~378, 1971*

9) Cinnamon AD, Beaton JR. *Biochemical assessment of vitamin B_6 status in man. Am J Clin Nutr 23 : 696~702, 1970*

10) Driskell JA, Wiley JH, Kirksey A. *Alanine ami-*

notransferase activity in liver and erythrocyte of pregnant and nonpregnant rats fed different levles of pyridoxine. J Nutr 101 : 85~91, 1971.

11) Sauberlich HE, Canham JE, Baker EM, Raica N, Herman YF. *Biochemical assessment of nutritional status of vitamin B_6 in the human. Am J Clin Nutr 25 : 629~641, 1972*

12) Tonhazy NE. *Colortmetric assay.* In : *Methods of enzymatic analysis. Academic Press, New York and London. Vol 2 : 764, 1974*

13) Bayoumi RA, Rosalki SB. *Evaluation of methods of coenzyme activation on erythrocyte enzymes for detection of deficiency of vitamin B_1, B_2 and B_6. Clin Chem 22(3) : 327, 1976*

14) Woording MJ, Storvick CA. *Effect of pyridoxine supplementation on glutamic-pyruvic transaminase and in vitro stimulation in erythrocyte of normal women. Am J Clin Nutr 23 : 1385, 1970*

15) Lowry OH, Roserough NJ, Farr AL, Randall RJ. *Protein mesurement with the Folin-Phenol reagent. J Biol Chem 193 : 265~275, 1951*

16) Lees MB, Paxman S. *Modification of the Lowry procedure for the analysis of proteolipid protein. Anal Biochem 47 : 184~192, 1972*

17) McDougal DB, Jr, Farmer HS. *A fluorometric method of total serum cholesterol. J Lab Clin Med 50 : 485~488, 1957*

18) Albers RW, Lowry OH. *Fluorometric determination of 0.1 to 10 micrograms of cholesterol. Anal Chem 27 : 1829~1932, 1955*

19) Hess HH, Lewin E. *Microassay of biochemical structural components in nervous tissues. J Neurochem 12 : 205~211, 1965*

20) Chao LL. *Statistics. In : Methods and analysis. 2nd ed MaGraw Hill, 268~274, 1974*

21) Thomas MR, Kirksey A. *Postnatal patterns of brain lipids in progeny of vitamin B_6 deficient rats before and after pyridoxine supplementation. J Nutr 106 : 1404~1414, 1976*

22) Stephens MC, Havlicek V, Dakshinamurti K. *Pyridoxine deficient and development of the central nervous system in the rat. J Neurochem 18 : 2407~2416, 1971*

23) National Research Council : *Nutrient requirements of laboratory animals. National Academy of Sciences. Washington, DC. 56~93, 1972*

24) Kirksey A, Pang RL, Lin WJ. *Effects of different levels of pyridoxine fed during pregnancy superimposed upon growth in the rat. J Nutr 105 : 607~615, 1975*

25) Beaton GH, Cheney MC. *Vitamin B_6 requirement of the male albino rat. J Nutr 87 : 125~132, 1965*

26) Choi H, Kang SA. *Effect of pyridoxine deficiency on neurotransmitters in the developing rat brain. Korean J Nutr 17 : 199~209, 1984*

27) Roberts BW, Williams R. *Lactation and human milk : Nutritional considerations In : Nutrition in pregnacny and lactation. 3rd ed Time Mirror /Mosby College Publishing. : 244~249, 1985*

28) Blackwell B, Blackwell PO, Yu T, Weng W, Chow BF, *Further studies on growth and feed utilization in progeny of underfed mother' rats. J Nutr 97 : 79~84, 1969*

29) Culley WJ, Lieneberger RO. *Effect of undernutrition on the size and composition of rat brain. J Nutr 96 : 375~381, 1968*

30) William B, Coniglio JC. *The effects of pyridoxine deficieney and calorie restricition on lipids in the developing rat brain. J Neurochem 18 : 267~276, 1971*

31) Thomas MR, Kirksey A. *Postnatal patterns of fatty acids in brain of progeny from vitamin B_6 deficient rats before and after pyridoxine supplementation. J Nutr 106 : 1415~1420, 1976*

32) Kurtz DJ, Levy H, Kanfer JN. *Cerebral lipids*

and amino acids in the vitamin B_6-deficient suckling rat. J Nutr 102 : 291~298, 1972

33) Ceison RL, Waisman HA. *Effects of nutritional status on rat brain maturation as measured by lipid composition. J Nutr 100 : 315~298, 1972*

34) Campagnoni AT, Campagnoni CW, Dutton GR, Cohen J. *A regional study of developing rat brain : The accurmulation and distribution of proteolipid proteins. J Neurobiol 7 : 313~324, 1976*

35) Nakhasi HL, Toews AD, Horrocks LA. *Effects of a postnatal protein deficiency on the content and composition of myelin from brains of weanling rats. Brain Res 83 : 176~179, 1975*

36) Bass NH, Hese HH. *A comparison of cerebrosides, proteolipid protein, and cholesterol as indices of myelin in the architecture of rat cerebrum. J Neurochem 16 : 731~750, 1969*

37) Morre DM, Kirksey A, Das GD. *Effects of vitamin B_6 deficiency on the developing central nervous system of the rat myelination. J Nutr 108 : 1260~1265, 1978*

38) Bass NH. *Influence of neonatal undernutrition on the development of rat cerebral cortex : A microchemical study. In : Chemistry and Brain Development. Paoletti, R and Davison, AN eds. Plenum Press. 413~424, 1972*