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Induction of an Experimental PKU-Like Condition in Infant Rats During the First Two Weeks After Birth

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新生쥐의 生後 2週間에 있어서 Phenylketonuria 的 條件의 實驗的 誘導

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=국문초록=

Phenylketonuria (PKU)의 여러가지 特性을 硏究하기 爲하여 新生쥐에 實驗的으로 PKU 를 誘導시키는 方法을 實驗하였다.

新生쥐에 生後 2일부터 5일까지는 體重 kg 당 400mg 의 Phenylalanine 을, 6일부터 14일까지는 500mg 의 Phenylalnine 을 午前 6時부터 每 6時間마다 胃에서 注入시켰으며 生後 3일부터 14일까지는 體重 kg 당 0.00625~0.0125mg 의 amethopterin 을, 5일부터 14일까지는 體重 kg 당 50mg 의 P-chlorophenylalanine 을 午前 및 午後 9時 每日 2회 投與한후 Phenylalanine/tyrosine (P/T), 와 여러가지 外觀的인 症狀을 調査한 結果 Phenylalanine, amethopterin 및 P-chlorophenylalanine 을 同時에 投與한 경우는 P/T-比가 正常値以上으로 增加됨과 同時에 非正常的인 姿勢, 비틀거리는 걸음걸이와 같은 PKU 症狀이 나타났으나 Phenylanine 이나 沮害制 單獨投與時는 PKU 症狀이 나타나지 않았다.

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=ABSTRACT=

The objective of this study is to induce the primary characteristics of phenylketonuria in infant rats during the first 2 weeks after birth. The critical biochemical parameter in the development of phenylketonuria is the elevation of plasma phenylalanine while tyrosine is maintained at a relatively low level. A PKU-like condition was induced in infant rats during the first 2 weeks after birth using a modification of our previously published procedure for the development of a temporary (1 to 3 days) PKU-like condition. Phenylalanine was administered by stomach intubation every 6 hours (starting at 6 : 00 a.m.) at a dose level of 400mg per kg body weight (after birth-day 2 thru 5) and 500mg per kg body weight (day 6 thru 14). Amethopterin was given at 0.00625 or 0.0125mg per kg body weight (day 3 thru 14) and p-chlorophenylalanine at 50 mg per kg body weight (day 5 thru 14) at 9 : 00 a.m. and 9 : 00 p.m. At the times measured (6, 10 and 14 days) plasma phenylalanine/tyrosine (P/T) ratios were elevated from a normal value of the or less to values ranging from 6 to 15. During the second week after birth a staggering gait, abnormal stance and decreased social behavior were also observed. None of these PKU-like characteristics were apparent in the three control groups receiving (a) no phenylalanine or inhibitors, (b) phenylalanine alone, or (c) inhibitors alone. The establishment of these primary biochemical characteristics of phenylketonuria by stomach intubation of phenylalanine and a combination of low dose levels of enzyme inhibitors to infant rats provides an experimental system which should be valuable for extensive biochemical, histological and behavioral studies in phenylketonuria.

Introduction

Various investigators [1)-9)] have attempted to induce PKU experimentally in an animal system to facilitate studying the mechanism of brain damage in PKU. The minium criteria to me met to establish a model experimental PKU system appear to be; (a) The major biochemical abnormality of PKU, i.e., elevated plasma phenylalanine (PHE) concentrations compared to tyrosine (TYR) concentrations, must be maintained continually on a 24 hour cycle; (b) The treatment to create the PKU-like condition must be imposed in early life when neurological growth is taking place by hyperplasia or hyperplasia and hypertrophy if a permanent effect in later life is to be expected (this critical period is 6 to 12 months after birth for humans and 2 to 3 weeks after birth for the rat [10)-12)]; (c) The toxicity of the treatment must be minimized to allow a reasonable survival rate.

Since the recent attempts[1)9)] to develop a model experimental PKK system do not satisfactorily meet all three of these condi tions, the present study has been designed to develop an experimental PKU-like rat model which will meet these criteria. It is known that in the newborn rat liver PHE hydroxylase activity is low for 12 to 48 hours after birth[13)14)]. Previous work from this laboratory[15)] has shown that the stomach intubation of an L-PHE milk solution to newborn rats produced a temporary biochemical condition of PKU for the first 2 to 3 days after birth

when the PHE hydroxylase activity is low. However, by this treatment the PKU-like condition cannot be mainained for more than a few days after birth since the hydroxylase activity is not inhibited permanently. This temporary experimental PKU-like model has been used to assess the effects of the PKU-like condition on various aspects of intermediary metabolism[16)17)].

In the present study techniques and procedures reported previously[15)] to induce a tempoary experimental PKU-like condition were modified to induce continually on a 24 hour cycle the requisite biochemical characteristic of PKU in rats for a period of 2 weeks after birth when neurological growth is primarily by cell division. Specifically, stomach intubation of excess PHE was performed every 6 hours and amethopterin (AMP) and p-chlorophenylalanine (PCP), two inhibitors of the PHE hydroxylase system (Fig. 1), every 12 hours. AMP inhibits dihydrobiopterin reductase[18)] and PCP inhibits PHE hydroxylase[8)], both of which function in the PHE hydroxylase enzyme system. Inhibition of the conversion of PHE to TYR could be accomplished with high dose leveals of either inhibitor alone, but toxicity becomes a problem. Therefore, treatment with low doses of two inhibitors blocking the PHE hydroxylase system at two different sites provided a better chance of producing a significant reduction in the conversion of PHE to TYR while minimizing the toxicity of the inhibitors. Also, the low dose of AMP should minimize the inhibitory effect of AMP on the hydroxylation of tyrosine and tryptophan, the initial rate limiting steps in the biosynthesis of the neurotransmitters, dopamine and serotonin, respectively[19)].

If successful, this experimental PKU model will allow biochemical and histological studies during the most critical time of brain development in rats which corresponds to the first 6 to 12 months of life in human infants[10)12)]. Furthermore, success in these studies will make it possible to test whether any neurological changes caused by the PKU-like condition during this early stage in life has a permanent effect on the mental capability of the rat after the treatment is removed. Such studies will provide the critical test of the validity of the experimental PKU model system.

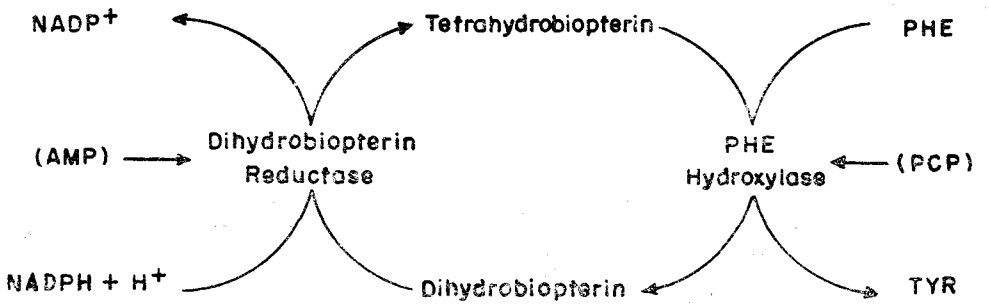

Fig. 1. Reaction sequence of PHE hydroxylase enzyme system.

Material and Methods

Treatment of Rats

Pregnant albino rats of the Holtzman strain were bred in our animal colony. The pregnant females were housed individually in stainless steel cages (7″×9″×7″) with wire mesh bottoms and fronts. One day before the expected delivery day, the rats were transferred to maternity cages (7″×9″×6″) with wire mesh tops and solid stainless steel sides and bottoms. Wood shavings were placed on the bottom of the cage for nesting. Purina laboratory rat chow and water were given ad lib. at all times.

A summary of the treatment of the control and experimental rats is given in Table 1. The Exptl-PKU-like rats in Experiment Nos. 1 and 2 were administered orally by stomach intubation an L-PHE: Milk mixture every 6 hours, starting at 6 : 00 a.m. at a dose level of 400mg/kg body weight (BW) from day 2 to day 5, and a dose level of 500mg/kg BW from day 6 to day 14. The PHE:Milk mixture was prepared by dissolving 40mg/ml of K-PHE in 0.1N HCl and diluting to 20mg PHE/ml with equal volumes of rat milk and raw

Table 1. Dosage levels of L-phenylalanine, amethopterin and/or p-chlorophenylalanine administered to control and experimental rats

Group Description*	L-phenylalanine		Amethopterin		p-chlorophenylalanine
	400mg/kg	500mg/kg	0.00625mg/kg	0.0125mg/kg	50mg/kg
Experiment No. 1					
I. Normal-Control	−	−	−	−	−
II. Inhibitor-Control	−	−	−	+	+
III. Exptl-PKU-Like	+	+	−	+	+
Experiment No. 2					
I. Normal-Control	−	−	−	−	−
II. PHE-Control	+	+	−	−	−
III. Inhibitor-Control	−	−	−	+	+
IVa. Exptl-PKU-Like	+	+	−	−	+
IVb. Exptl-PKU-Like	+	+	+	−	+
IVc. Exptl-PKU-Like	+	+	−	+	+
Experiment No. 3					
I. Normal-Control	−	−	−	−	−
II. PHE-Control	+	+	−	−	−
III. Inhibitor-Control	−	−	+	−	+
IV. Exptl-PKU-Like	+	+	+	−	+

*L-Phenylalanine was admininistered by stomach intubation every 6 hours starting at 6 : 00 AM at dose levels of 400mg/kg BW (after bitrh-day 2 thru 5) and 500mg/kg BW (day 6 thru 14) along with amethopterin at dose levels of 0.00625mg/kg BW or 0.0125 mg/kg BW (day 3 thru 14) and p-chlorophenylalanine at 50mg/kg BW (day 5 thru 14) at 9 : 00 AM and 9 : 00 PM. For rats not receiving PHE, sham intubations of HCl: Milk were given in Experiment Nos. 1 and 2. In Experiment No. 3 rats of Group III were administered sham intubations of HCl:Milk and rats of Group I and II HCl:H_2O and PHE:H_2O, respectively. In all experiments rats not receiving inhibitors were given sham intubations of H_2O. Between treatments all pups were allowed to suckle with their mothers.

cow milk. Between treatment pups were placed with their mothers to suckle. Procedures for the stomach intubation of newborn pups as developed by Miller et al.[20] and milking of lactating rats as described by Nelson et al[21]. were modified in this laboratory for these studies[15] The inhibitors used were amethopterin (AMP-methotrexate of Lederle Laboratory)and DL-p-chlorophenylalanine (PCP-Sigma Chemical Co). The inhibitors were given twice daily at 9 : 00 a.m. and 9 : 00 p.m. with the intubation of AMP starting on day 3 and PCP on day 5 after birth. The level of AMP was 0.00625 or 0.0125mg/kg BW while PCP was 50mg/kg BW. Sham intubation of HCl: Milk replaced the PHE:Milk and water replaced the inhibitor solutions for all control groups.

In Experiment No. 3 several changes in the intubation procedure were made in an attempt to control the body weight gains of animals in the control groups, since it was found in the first two experiments that the intubation of the added milk to the rats of groups I and II produced body weight gains in excess of normal pups without treatment during lactation. Therefore, in Experiment No. 3 the same procedures were used as in the first two experiments except all intubation mixtures given to rats in Groups I and II were made with water replacing the rat and cow milk. In addition the number of pups in Experiment No. 3 was increased to permit statistical analyses of the data[22].

Sample Preparation and Analysis

Control and experimental rats were sacrificed by decapitation one hour after the 12 noon tubing of sham or PHE: Milk at 6, 10 and 14 days of age. The blood was collected in a 3×150mm heparinized Trident Micro Blood Collection tube. The tube was sealed by heat and centrifuged at 3000 RPM for 20minutes at 0°C. The hematocrit was measured and recorded for each animal. Plasma was deproteinized by the addition of 5 volumes of 3% sulfosalicylic acid and the protein free supernatant stored at −70°C.

Microanalyses for plasma PHE and TYR levels of the protein free supernatant were performed with a Technicon Amino Acid Analyzer (Model TSM) employing a 0.4×25 cm column packed with type C-3 chromobeads ion exchange resin (No. T15-0360). PHE and TYR were separated by eluting with a 0.3N lithium citrate buffer of pH 3.80 for 30 minutes at a flow rate of 0.45ml per minute. The temperature of the column was maintained at 63°C. Concentrations of plasma PHE and TYR were calculated against an amino acid standard using a Technicon integrator calculator. Standard runs of plasma samples with PHE and TYR added gave recoveries of 100±3%. Additionally, PCP is eluted considerably after the PHE and TYR peaks and does not interfere with their analyses. Absolute concentrations of plasma PHE and TYR in mg percent were obtained and P/T ratios calculated to serve as an index of PHE hydroxylase activity in the liver as described by Longenecker et al[15].

Results and discussion

Previous work from this laboratory has shown that the treatment of adult rats with increasing doses of enzyme inhibitor of treatment of infant rats with increasing dose of L-PHE[15] suppressed temporarily PHE hydroxylase activity and resulted in a substaintial elevation of the P/T ratios. This

Table 2. The average plasma PHE and TYR concentrations and P/T ratios of control and experimental PKU-like rats Experiment No. 1

Group Description*	Age (days)	Sex	No. of Pups	Body Wt** Gain (g)	Plasma Conc.** (mg/dl)		P/T Ratios**
					PHE	TYR	
I Normal-Control	6	M	2	8.8±3.1	2.1±0.3	6.8±1.5	0.3±0.0
II Inhibitor-Control	6	M	1	6.3±0.0	3.9±0.0	9.5±0.0	0.4±0.0
III Exptl-PKU-Like	6	M	2	8.1±1.8	138.9±0.2	15.0±0.5	9.3±0.3
I Normal-Control	6	F	3	8.0±1.2	1.9±0.2	5.1±0.4	0.4±0.1
II Inhibitor-Control	6	F	2	6.6±3.4	3.1±0.5	5.7±1.9	0.1±0.1
III Exptl-PKU-Like	6	F	3	7.8±6.3	115.3±24.2	14.1±3.4	9.3±3.3
I Normal-Control	10	M	3	23.7±1.0	2.2±0.5	8.0±0.7	0.3±0.1
II Inhibitor-Control	10	M	2	12.5±0.2	7.8±1.8	7.2±0.1	1.1±0.3
III Exptl-PKU-Like	10	M	2	8.5±2.1	148.2±38.5	17.1±5.0	8.7±0.3
I Normal-Control	10	F	2	18.5±0.9	1.6±0.0	7.6±1.8	0.2±0.0
II Inhibitor-Control	10	F	2	14.5±0.1	7.8±2.8	10.6±1.3	0.8±0.4
III Exptl-PKU-Like	10	F	3	10.1±2.2	127.5±0.7	19.2±0.2	6.7±0.2
I Normal-Control	14	M	3	35.6±0.9	2.0±0.7	5.3±0.8	0.3±0.1
II Inhibitor-Control	14	M	3	20.6±4.0	5.6±1.2	4.5±0.2	1.2±0.3
III Exptl-PKU-Like	14	M	5	14.3±2.2	130.8±14.0	11.6±0.7	11.4±1.5
I Normal-Control	14	F	5	38.5±1.8	1.7±0.1	4.8±0.9	0.4±0.1
II Inhibitor-Control	14	F	5	18.2±2.6	7.4±1.0	4.8±0.7	1.7±0.3
III Exptl-PKU-Kike	14	F	8	11.9±1.2	125.4±7.7	8.3±0.5	15.5±1.5

* Treatment as given in Table 1.
** Mean±Scandard Error of Mean.

research has shown that the in vivo P/T ratio is a valid index to monitor the relative PHE hydroxylase activity in both infant and adult rats[15]. Therefore, in the present studies the P/T ratio technique was used to follow the primary biochemical condition of PKU, i.e., maintenance of high plasma PHE relative to TYR, and to determine the effectiveness of the various treatments of proceducing an experimental PKU-like condition in newborn rats for 2 weeks after birth.

The effects produced by the administration of the combination of L-PHE and PHE hydroxylase inhibitors on the plasma concentration of PHE and TYR as well as their corresponding P/T ratios are summarized in Tables 2 thru 4. For Experiment No. 1 the average plasma PHE and TYR levels for 6−, 10− and 14-day-old rats and their average P/T ratios are given in Table 2 For the two control groups, i.e., Group I (Normal-Control) rats receiving no PHE or inhibitors, and Group II (Inhibitor-Control) rats receiving 0.0125 mg/kg BW of AMP and 50 mg/kg BW of PCP but no L-PHE, plasma PHE and TYR levels and the corresponding average P/T ratios are relat vely low for all age groups. It should be noted that in newborn

rats, plasma TYR levels generally fall between 4 to 8 mg percent while corresponding PHE values range between 1 to 3 mg percent. These results have been found consistently in all our studies with newborn rats. Furthermore, frequent checks of these results have been made since the values are in contrast to the equal levels (3 to 5 mg percent) of TYR and PHE found in adult rats and human infants. All checks have proved positive. In contrast, plasma PHE concentrations are elevated 50 to 100 times their normal levels and remain substantially high for the rats in Group III which were given the same amount of inhibitors as Group II along with L-PHE intubation. Correspondingly, plasma TYR levels are elevated far less (2 to 4 times their base values) and remain relatively low compared to the plasma PHE concentrations. The P/T ratios increase with age to a high value of 15.5 for the 14-days-old rats of Group III (Exptl-PKU-Like) compared to the relatively low and constant P/T ratios found for the control groups. There are no sex differences in the development of the PKU-like condition. These results show that intubation of L-PHE along with low doses of AMP and PCP is capable of producing a biochemical PKU-like condition in infant rats during the first 2 weeks after birth, whereas treatment with inhibitors alone does not induce the condition.

The average plasma PHE and TYR levels and the corresponding P/T ratios for Experiment No. 2 are given in Table 3 The Normal-Control rats (Group I) which were administered sham solutions have mean values of normal plasma PHE concentrations ranging from 1.0 to 1.3 mg/dl and TYR levels from 4.2 to 5.1 mg/dl resulting in average P/T ratios of 0.2 to 0.3 for all age groups. PHE-Control rats (Group II) administered PHE but no inhibitors have mean values of plasma PHE concentrations ranging from 23 to 34 mg/dl and TYR levels from 24 to 30 mg/dl. Mean P/T ratios are 1.0, 1.1 and 1.2 for the 6−, 10− and 14-day-old rats, respectively These data indicate that intubation of excess PHE alone causes an elevation of plasma levels of both PHE and TYR when no inhibitor is present to block the hydroxylase system. It is apparent that the presence of the excess PHE saturates the PHE hydroxylase enzyme system with substrate an forces a rapid synthesis of TYR to occur. Thus the primary biochemical parameter of PKU, i.e., elevated levels of plasma PHE with low levels of plasma TYR, is not produced by this treatment, but an elevation of both plasma PHE and TYR levels occurs. The Inhibitor-Control rats (Group III) administered inhibitors without PHE have mean values of plasma PHE concentrations ranging from 1.5 to 7.6 mg/dl and TYR levels from 3.7 to 4.7 mg/dl. These same rats have average P/T ratios of 0.5, 1.9 and 1.6 at 6, 10 and 14 days after birth respectively. As was the case in Experiment No. 1, treatment with inhibitors alone does not produce the PKU-like condition.

Infant rats receiving by intubation one or both of the enzyme inhibitors along with PHE as a means of inducing an experimental PKU-like condition were studied in three groups. Rats of Group IVa receiving 50mg/kg BW of PCP along with the PHE: Milk intubation have average plasma PHE concentrations of 63, 91 and 66mg/dl and plasma TYR levels of 21, 28 and 11 mg/dl at 6, 10 and 14 days after birth, respectively. Corre-

Table 3. The average plasma PHE and TYR concentrations and P/T ratios of 6—, 10—, and 14-day-old control and experimental PKU-like rats Experiment No. 2

Group	Description*	Age (days)	No. of Pups	Body Wt.** Gain (g)	Plasma Conc.** (mg/dl) PHE	Plasma Conc.** (mg/dl) TYR	P/T Ratios**
I	Normal-Control	6	4	6.9±0.5	1.3±0.3	5.1±1.1	0.3±0.0
II	PHE-Control	6	3	6.9±0.5	23.4±1.9	26.3±5.6	1.0±0.2
III	Inhibitor-Control	6	3	5.3±1.3	1.5±0.1	3.7±1.0	0.5±0.2
IVa	Exptl-PKU-Like	6	2	7.2±0.2	62.5±1.3	21.1±5.6	3.1±0.8
IVb	Exptl-PKU-Like	6	3	7.1±0.2	78.4±13.8	19.4±0.6	4.1±0.1
IVc	Exptl-PKU-Like	6	2	7.2±0.4	69.4± 9.7	24.5±1.4	2.8±0.2
I	Normal-Control	10	3	19.0±1.2	1.0±0.2	4.7±0.8	0.2±0.0
II	PHE-Control	10	2	17.2±0.4	24.8±5.6	24.0±2.3	1.1±0.4
III	Inhibitor-Control	10	2	10.7±0.2	7.6±0.7	4.2±0.6	1.9±0.1
IVa	Exptl-PKU-Like	10	4	10.9±0.8	91.1±4.3	28.0±5.2	3.6±0.7
IVb	Exptl-PKU-Like	10	2	8.9±0.9	141.3±45.3	18.6±1.3	7.5±1.8
IVc	Exptl-PKU-Like	10	4	8.6±1.1	122.8±13.5	10.4±1.4	11.4±0.7
I	Normal-Control	14	3	34.8±2.3	1.0±0.1	4.2±0.4	0.3±0.0
II	PHE-Control	14	2	37.1±0.5	34.0±14.4	29.9±11.6	1.2±0.1
III	Inhibitor-Control	14	4	17.5±1.1	7.2±0.8	4.7±0.7	1.6±0.1
IVa	Exptl-PKU-Like	14	3	14.5±2.0	65.9±8.2	11.0±1.6	6.1±0.4
IVb	Exptl-PKU-Like	14	2	12.9±1.2	127.9±2.8	10.1±1.9	13.0±2.8
IVc	Exptl-PKU-Like	14	2	12.5±4.5	122.9±2.8	7.1±1.0	16.5±1.8

* Treatment as given in Tabl e 1. Male rats used in this experiment.
** Mean±Standard Error of Means.

sponding P/T ratios are 3.1, 3.6 and 6.1 Although somewhat elevated, these values for this group are not comparable to those of human PKU subjects. Rats in Group IVb were tubed the PHE/Milk along with a combination of AMP and PCP. The AMP dose level was 0.00625 mg/kg BW and PCP 50mg/kg BW. The average plasma PHE concentrations are 78, 141 and 128mg/dl and the respective plasma TYR levels, 19, 19 and 10mg/dl at 6, 10 and 14 days after birth, respectively. As found in Experiment No. 1, the plasma PHE levels are elevated 50 to 100 times and plasma TYR 2 to 4 times respective control values. Therefore, the respective average P/T ratios of 4.1, 7.5 and 13.0 produced by this treatment are of a magnitude similar to values found in human PKU subjects. Rats in Group IVc received the same treatment as those in Group IVb except the dose level of AMP was increased from 0.00625 mg/kg BW to 0,0125 mg/kg BW. Levels of plasma PHE, TYR and P/T ratio are similar to those found for Group IVb. Therefore, doubling the dose level of AMP of offers no advantage over the lower dose level of 0.00625 mg/kg BW used in Group IVb in producing the PKU-like condition. The mortality rate in Experiment Nos. 1 and 2 ranged from approximately 15% for Group I

(Normal-Control) to a high value of approximately 25% for Group IVc (Exptl PKU-Like). These findings clearly show that the major biochemical conditions of PKU can be induced successfully over a 2 week period by treating infant rats with an oral dose of PHE along with AMP and PCP, two inhibitors of the PHE hydroxylase system.

However, in the third and final study the optimal procedure established in Experiment Nos. 1 and 2was used and the number of pups per group increased to permit statistical analyses of the data. Also, in Experiment No. 3 control rats were given sham intubations using water instead of rat milk and cow milk as given in Experiment Nos. 1 and 2. This change was made since rats of these control groups in the first two experiments were over nourished by the added milk and had body weight gains in excess of growth curves of normal pups during lactation. The average plasma PHE and TYR concentrations and P/T ratios for the control and experi mental rats in Experiment No. 3 are given in Table 4. Statistical analyses of the data using a pair-wise multiple comparison of means[22] of plasma levels of PHE, TYR and P/T ratios indicate that the values for the rats in Group IV (Exptl-PKU-Like) at 10 and 14 days of age are significantly different ($P<0.001$) from those obtained for the control groups (Group I, II and III). It should be noted that the plasma PHE levels for Group IV (Exptl-PKU-Like) are 50 to 100 times greater than the control values (Group I, Normal-Control), and even thought the plasma TYR leveals are significantly higher for the Exptl-PKU-Like rats than the Normal Control rats, they are only elevated 2 to 4 fold. As previously found in the preliminary

Table 4. The average plasma PHE and TYR concentration and P/T ratios of 6—, 10—, and 14-day old control and experimental PKU-like rats Experiment No. 3

Group	Description*	Age (days)	No. of Pups	Body Wt.** Gain (g)	Plasma Conc.** (mg/dl) PHE	Plasma Conc.** (mg/dl) TYR	P/T Ratios**
I	Normal-Control	6	8	6.7±0.5	1.1±0.2[1]	4.9±0.6[1]	0.2±0.01[1]
II	PHE-Control	6	10	5.6±0.6	29.3±2.9[2]	16.4±1.9[3]	1.9±0.2[2]
III	Inhibitor-Control	6	8	6.3±0.6	1.7±0.3[3]	4.0±0.7[1]	0.5±0.1[3]
IV	Exptl-PKU-Like	6	8	5.8±0.5	70.5±7.5[4]	15.6±1.7[3]	4.7±0.5[4]
I	Normal-Control	10	7	15.9±0.6	1.1±0.2[1]	4.4±0.7[1]	0.3±0.1[1]
II	PHE-Control	10	5	14.8±1.2	39.6±5.4[2]	25.4±6.2[3]	1.8±0.3[3]
III	Inhibitor-Control	10	9	10.8±0.8	6.3±0.9[3]	5.5±0.8[1]	1.4±0.3[3]
IV	Exptl-PKU-Like	10	9	8.9±0.9	87.4±7.7[4]	12.4±0.9[3]	7.2±0.7[2]
I	Normal-Control	14	9	28.8±1.9	1.5±0.2[1]	3.4±0.5[1]	0.4±0.1[1]
II	PHE-Control	14	10	27.0±1.5	17.2±2.9[2]	13.2±2.1[2]	1.5±0.3[3]
III	Inhibitor-Control	14	9	18.8±1.4	6.5±0.7[3]	4.4±0.7[1]	1.7±0.3[3]
IV	Exptl-PKU-Like	14	11	13.6±1.1	99.5±8.2[4]	8.1±0.7[3]	12.8±1.0[2]

* Treatment as given in Table 1. Male rats used in this experiment.

** Mean±Standard Error of Means. Means not followed by same symbol are significantly different ($P<0.001$). Pair-wise multiple range comparisons in the statistical method used (22).

experiments, intubation of PHE alone (Group II, PHE-Control) elevated plasma PHE and TYR concentrations equally, while treatment with the inhibitors alone had no important effect on the plasma PHE or TYR values. Thus, the P/T ratios for the Exptl-PKU-Like rats are significantly higher than the values for the three control groups. At 6 days after birth, similar differences are apparent but the magnitude is somewhat less. These results reveal that the enzyme inhibition and thus the development of the biochemical conditions of PKU become greater the longer the treatment period.

Hematocrit values were not significantly different among groups. Also. as found in Experiment Nos. 1 and 2, the mortality rates ranged from a low value of 14% for Group I to a high value of 33% for Group IV. Observation of the rats during the 2 week period revealed the experimental PKU-like rats experience a staggering gait or inability to walk, frequent falling, inability to maintain balance and muscular hypertoxicity with a degree of muscular rigidity. Similar abnormalities are often seen in PKU patients[23)31)] Also, it was quite obvious that rats of Group IV had a lack of social behavior such as passive protection, active aggresion, competition and playing with littermates and playing or active interaction with their mother. None of these findings were observed with any of the 3 control groups.

The hepatic enzyme, PHE hydroxylase is a primary determinant of the capacity to metabolize PHE in vivo. Treatment of rats with low dose levels of AMP and PCP will produce maximum enzyme inhibition with minimum toxic effects from the inhibitors. This particular combination of inhibitors was employed because AMP reduces PHE hydroxylase activity by blocking the dihydrobiopterin reductase[18)], while PCP acts as a competitive inhibitor of PHE to inhibit PHE hydroxylase[8)]. The interrelationship between the hydroxylase and reductase enzymatic reactions is shown in Fig. 1. Tetrahydrobiopterin is also a required cofactor for the hydroxylation of tyrosine[32)] and tryptophan[33)] leading to the biosynthesis of the neurotransmitters, dopamine and serotonin, respectively[19)] The low dose levels of AMP employed in the model system should minimize the inhibitory effects of AMP on the neurotransmitter pathways. Therefore, in our experimental PKU model the dominant inhibition should be the combined effect of AMP and PCP on the PHE hydroxylase system.

Several investigators[34)39)] have reported a new variant of PKU with progressive neurological illness which is not reponsive to PHE restriction. The metabolic defect has been proposed to be the absence of enzyme activity of dihydrobiopterin reductase. This enzyme is not only important in mediating the conversion of PHE to TYR, but is also directly involved in the synthesis of neurotransmitters. It has been suggested that the lack of neurotransmitters may be a possible cause of the neurological deficit in PKU.

The present study demonstrates that stomach intubation of newborn rats with low dose levels of AMP and PCP, two inhibitors of PHE hydroxylase enzyme system, along with L-PHE during the first 2 weeks postnatally produces a PKU-like condition. This period of the life cycle of the rat was chosen since it corresponds to early infancy in man. It is likely and brain damage produced by PKU would be the result of the severe

imbalance of plasma PHE to TYR levels in early life when rapid cellular development of brain is occurring. The treatment employed here is an excellent means to produce a relatively long-term PKU-like condition in an animal which will make it possible for further biochemical and microscopic studies to elucidate more completely the nature and causes of brain damage in PKU.

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