Pharmacology Biochemistry & Behavior, Vol. 17, pp. 91–97, 1982. Printed in the U.S.A.

Seizure Susceptibility of the Pregnant Mouse[1]

ANDREW SMOLEN,[2] TONI NESS SMOLEN,[2,*] AND ALLAN C. COLLINS

*School of Pharmacy, *Department of Psychology*
Institute for Behavioral Genetics, and Alcohol Research Center, University of Colorado, Boulder, CO 80309

Received 24 January 1982

SMOLEN, A., T. N. SMOLEN AND A. C. COLLINS. *Seizure susceptibility of the pregnant mouse.* PHARMAC. BIOCHEM. BEHAV. 17(1) 91–97, 1982.—The effect of pregnancy on chemically-induced seizures in mice was studied. Latencies to myoclonic and clonic seizures induced by inhalation of flurothyl were significantly reduced at days 12 through 18 of gestation. Parturition resulted in a return of seizure susceptibility to control levels. The possibility that this effect might be mediated by decreased neurotransmitter levels subsequent to the decreased vitamin B_6 levels which are known to occur during pregnancy was suggested. A pregnancy-associated liver cytosolic aldehyde dehydrogenase (π-AlDH) utilized pyridoxal as a substrate, and the peak of π-AlDH activity was shown to coincide with the peak of seizure susceptibility. The activity of aldehyde oxidase, the major enzyme normally responsible for the metabolism of pyridoxal, was reduced in pregnant animals. The pyridoxal 5′-phosphate synthesizing enzymes, pyridoxal kinase and pyridoxamine phosphate oxidase, were marginally increased in activity during pregnancy. It was suggested that the increased activity of π-AlDH was indirectly responsible for the increased seizure susceptibility due to increased metabolism of pyridoxal.

Aldehyde dehydrogenase Aldehyde oxidase Pyridoxal kinase Pyridoxamine phosphate oxidase
Flurothyl Pregnancy Seizures Eclampsia

A CYTOSOLIC form of aldehyde dehydrogenase (AlDH), which has been termed π-AlDH is induced in the pregnant mouse [15,21]. The activity of this enzyme increases 2- to 3-fold during pregnancy, and its activity drops rapidly after parturition. π-AlDH has broad substrate specificity, is NAD^+ linked, and is induced specifically in liver cytosol. The factors which cause the induction of this pregnancy-associated enzyme are not known. The physiological substrate of π-AlDH is also not known, but it was discovered that pyridoxal, a form of vitamin B_6, could serve as a substrate. (Throughout this paper vitamin B_6 refers to any of the several forms of the vitamin, whereas pyridoxal 5′-phosphate refers to that vitamer exclusively.)

There is an increased requirement for vitamin B_6 in pregnant women [11] and experimental animals [3]. The blood (or plasma) concentration of pyridoxal 5′-phosphate (PLP), the coenzyme form of vitamin B_6, is very low during pregnancy as evidenced by direct measurement of PLP [3,11], or by measurement of urinary metabolites which are dependent upon PLP-linked enzyme reactions for further metabolism [8]. The deficiency of vitamin B_6 in toxemic pregnancies is apparently greater than in normal pregnancies [2,26], and vitamin B_6 supplementation has been advocated by several workers as a means of preventing or reducing some of the complications of pregnancy [2, 11, 26].

Pyridoxal 5′-phosphate serves as coenzyme for a number of enzymes involved in the intermediary metabolism of amino acids. The synthesis of the neurotransmitters norepinephrine, dopamine, serotonin, and γ-aminobutyric acid (GABA) are all dependent on PLP-linked reactions. Deficiency of PLP is known to be associated with increased susceptibility to experimentally induced seizures in mice [18] and spontaneous seizures in humans [14]. It seemed reasonable to predict that pregnancy would, therefore, create a predisposition to induced or spontaneous seizures; and that the risk of suffering a seizure would be greatest late in pregnancy. In this paper we report that pregnant mice are more susceptible to seizures than are virgin controls.

The cause of the vitamin B_6 deficiency which accompanies pregnancy is not known. Certainly some PLP is shunted from the mother to the fetus as indicated by higher PLP concentration in cord blood than maternal blood at delivery in humans [1], however, it seems unlikely that this mechanism could account for the magnitude of the vitamin B_6 deficiency generally observed during pregnancy. It was reported that PLP deficiency in pregnant rats could not be overcome even with supplementation of their diets with excessive amounts of pyridoxine [19]. If shunting was responsible for the deficiency of PLP, then dietary supplementation would be expected to bring the levels up to normal. This lack of efficacy indicated some abberation in the synthesis or metabolism of PLP. The major metabolite of vitamin B_6 is

[1]Supported in part by grants AA-00029, AA-003527, DA-07043, and GM-07305.
[2]Current address: Department of Pharmacology, Medical Science Building, I, Room 6322, University of Michigan, Ann Arbor, MI 48109. Address reprint requests to Dr. A. Smolen in Ann Arbor.

ABBREVIATIONS USED IN THE TEXT

AlDH	Aldehyde dehydrogenase
AO	Aldehyde oxidase
ATP	Adenosine triphosphate
EDTA	Ethylenediaminetetraacetic acid, sodium salt
FAD	Flavin adenine dinucleotide
PLP	Pyridoxal 5'-phosphate
NMN	N^1-methylnicotinamide

4-pyridoxic acid, which is formed from pyridoxal in an FAD-linked oxidation catalyzed by aldehyde oxidase (AO) [22]. However, rats genetically lacking AO can still produce 4-pyridoxic acid from an NAD^+-linked oxidation involving AlDH, which is normally a minor pathway of pyridoxal metabolism [23]. Our studies suggest that synthesis of PLP is not changed markedly during pregnancy, but that induction of π-AlDH may result in increased metabolism of pyridoxal. The resultant reduction in PLP concentration is thought to predispose pregnant mice to experimentally-induced seizures.

METHOD

Animals

The animals used in this study were female Heterogeneous Stock (HS) mice [12], 60–100 days old. Mice were maintained on a 12 hr light/dark cycle (0700–1900) and were allowed free access to food (Wayne Sterilizable Lab Blox containing 8.13 ppm pyridoxine) and water.

For all of the experiments in this paper controls were virgin females of approximately the same age as experimentals. Day 1 of pregnancy was ascertained by observance of vaginal plug. Plugged females were separated from the males and housed in groups until the day they were used for the experiment. Separate groups of animals were tested for seizures at various times throughout gestation and lactation. After testing, animals which were not visibly pregnant (days 7, 9 and 12 after mating) were sacrificed, and the uterus was examined for the presence of fetuses. After parturition, females were allowed to nurse four pups each until the day they were used for an experiment. To test the effect of lactation on seizure susceptibility, the pups were removed from some mothers on the morning they delivered. This non-lactating group was seizure tested on the twelfth postpartum day and was compared to lactating animals. Animals were used only once. Mice which had been seizure tested were not used for subsequent biochemical determinations.

Seizure Testing Procedure

Seizures were induced by inhalation of bis[2,2,2-trifluoroethyl] ether (flurothyl, Indoklon®) which was obtained from Air Reduction Corporation. The procedure used has been detailed previously [6]. Briefly, the apparatus consists of a modified 435-ml ointment jar. The screw cap lid has a rubber septum mounted in the center through which the flurothyl is injected onto a 1-cm square of Whatman No. 1 filter paper which is suspended 15 mm below the septum and held in a Plexiglas support. Two glass tubes (one connected to a water aspirator) extending through the lid into the chamber can be closed off with pinchcock clamps to keep the chamber air tight. The tubes are cemented in place with epoxy. After the end of the test period, the clamps are released, the aspirator turned on, and the chamber flushed with air to remove the excess flurothyl.

The animal to be tested was placed in the chamber and 5 μl of flurothyl was injected into the chamber. This dose was chosen since it produces good separation of myoclonus and clonus in several inbred strains and selected lines of mice [6] (T.N.S., unpublished). The latencies to the first myoclonic and clonic seizure were recorded to the nearest second. All testing was done between 1300 and 1600 hrs because of the circadian rhythmicity of clonic seizure threshold [28]. The mouse remained in the chamber for 5 min or until onset of a clonic seizure. Animals which did not have a clonic seizure were given a score of 300 (sec). Under the conditions of time and dose of flurothyl used in this study, no animal ever had a tonic seizure. Delivery at term was normal following testing. The pups were not studied—we were interested only in embryonal/fetal survival following seizures, and survival was apparently unaffected.

Enzyme Assays

Four enzymes known or suspected to be important in vitamin B_6 synthesis and degradation were measured using established methods with only minor modifications. These were: aldehyde dehydrogenase (aldehyde:NAD^+ oxidoreductase, EC 1.2.1.3); aldehyde oxidase (aldehyde: oxygen oxidoreductase, EC 1.2.3.1); pyridoxal kinase (ATP:pyridoxal 5'-phosphotransferase, EC 2.7.1.35); and pyridoxamine 5'-phosphate oxidase (pyridoxamine 5'-phosphate: oxygen oxidoreductase [deaminating], EC 1.4.3.5). Tissues were prepared from three groups of mice: virgin controls, pregnant (day 15) and postpartum (morning of delivery). Animals were killed by cervical dislocation, the tissues were removed quickly, and all subsequent preparative steps were performed at 4°.

Enzyme assays were run at 37° in duplicate. Appropriate blanks were used to correct for non-enzymatic reactions as indicated. For timed incubations, the assays were linear for the amount of protein employed over the time interval used. All reagent concentrations given are final concentrations in the reaction mixture. All substrates and coenzymes were prepared daily. Absorbance measurements were made in either a Gilford 240 or Beckman 35 spectrophotometer zeroed against distilled water. Proteins were determined by the Lowry method [10]. Reagents were obtained from Sigma Chemical Co. and Aldrich Chemical Co.

Aldehyde Dehydrogenase

Cytosolic π-AlDH was prepared from the livers of mice as detailed previously [21]. The livers were homogenized in 8 volumes of 0.25 M sucrose and centrifuged at 10,000 g for 10 min. The supernatant fraction was then centrifuged for 60 min at 100,000 g, and the resulting supernatant, designated the cytosolic fraction, was used for assay of AlDH activity. The reaction mixture contained 2.5 mM propionaldehyde, 1.0 mM NAD^+, 1.0 mM pyrazole and liver supernatant (50 μl containing 300–400 μg protein) in 50 mM sodium pyrophosphate, pH 9.6, in a total volume of 1.0 ml. The reaction was initiated by addition of enzyme, and the velocity was monitored by following production of NADH at 340 nm. The oxidation of pyridoxal·HCl was measured in the same system with 1.0 mM pyridoxal·HCl as the substrate. Enzyme

activity was calculated from the initial velocity using the molar extinction coefficient of NADH, 6220 M^{-1} cm^{-1}. Blanks contained no substrate.

Kinetic constants for propionaldehyde and pyridoxal were determined in the presence of 1.0 mM NAD^+. Lineweaver-Burk plots of the data were biphasic, as reported earlier, and kinetic constants were estimated by extrapolation of the linear portions of the curves [21].

Aldehyde Oxidase

The method of Rajagopalan and Handler [16] was used to measure the activity of AO. The cytosolic fraction of liver homogenates served as the source of enzyme. This reaction monitors the conversion of N^1-methylnicotinamide (NMN) to N^1-methyl-6-pyridone-3-carboxamide by the increase in absorbance at 300 nm. The 1.0 ml reaction mixture contained 5.0 mM NMN, 10 μg catalase (to destroy peroxide), 50 μl of the cytosolic fraction and 50 mM potassium phosphate, pH 7.8, containing 0.005% EDTA. The reaction was started by addition of substrate and monitored continuously at 300 nm. Blanks contained no substrate. The production of the pyridone was calculated from its molar extinction coefficient, 4230 M^{-1} cm^{-1}.

Pyridoxal Kinase

Pyridoxal kinase activity was measured in brain homogenates according to the method of McCormick *et al.* [13]. After sacrifice, the brains were quickly removed and the combined cortex, midbrain and cerebellum were homogenized in 2.0 ml of 75 mM potassium phosphate, pH 6.8, using a Potter-Elvehjem homogenizer. The homogenates were centrifuged at 20,000 g for 20 min to remove most of the particulate material. The resulting supernatant fraction was the source of the enzyme.

The incubation mixture contained 1.0 mM ATP, 0.5 mM pyridoxal·HCl, 0.12 mM zinc chloride, brain supernatant (100 μl containing ca 300 μg protein) and 75 mM potassium phosphate, pH 6.0, in a total volume of 1.0 ml. The reaction was initiated by addition of ATP and the production of PLP was measured at 388 nm using the molar extinction coefficient of 4900 M^{-1} cm^{-1}. After a 5 min incubation at 37°, an initial absorbance reading was taken. The tubes were incubated in the dark for one hr, after which a final absorbance reading was made. The absorbance change over this interval minus the blank (no ATP) was used to calculate the enzyme activity.

Pyridoxamine Phosphate Oxidase

Pyridoxamine phosphate oxidase was measured in liver homogenates according to the procedure of Wada and Snell [27] in which PLP, produced from pyridoxamine 5′-phosphate, is measured as its phenylhydrazine derivative. Livers were removed and homogenized in 4 volumes of 50 mM sodium phosphate, pH 8.0. The homogenate was centrifuged at 20,000 g for 20 min and the resulting supernatant was used as the source of enzyme. The reaction mixture contained 0.5 mM pyridoxamine 5′-phosphate and liver supernatant (1.0 ml containing 15–20 mg protein) in 50 mM sodium phosphate, pH 8.0, in a total volume of 1.5 ml. Blanks contained no substrate. The reaction was initiated by the addition of substrate, and was allowed to proceed for 30 min in the dark. It was terminated by the addition of 300 μl of ice-cold 50% (w/v) trichloroacetic acid followed by rapid mixing. After standing for 5 min, the samples were centrifuged for 10 min at 1500 g to sediment the precipitated protein. Four hundred-fifty μl of the clear supernatant was added to 50 μl of phenylhydrazine reagent: 2% (w/v) phenylhydrazine·HCl in 10 N sulfuric acid. After standing for 10 min at room temperature, the absorbance was measured at 410 nm. The amount of PLP produced was calculated from the molar extinction coefficient of the phenylhydrazone, 24,300 M^{-1} cm^{-1}.

Data Analysis

For the seizure data, groups of animals were tested for seizures at days 0, 7, 9, 12, 15 and 18 of pregnancy, at parturition, and days 3, 7, 12 and 20 postpartum. Groups contained 20 animals each except for days 12 and 20 postpartum which contained 18 and 15 mice, respectively. Each animal received two scores: latencies (sec) to myoclonic seizure and to clonic seizure measured from the time flurothyl was injected into the chamber. Data were analyzed by one-way analysis of variance following a natural logarithm transformation of the raw data to correct for nonhomogeneous variances. Differences in individual sample means were detected using the Tukey B test for critical differences [29]. Data comparing control, lactating and non-lactating animals were analyzed by a separate analysis of variance.

Enzyme data were analyzed by one-way analysis of variance followed by the Tukey B post hoc test. Specific activity and total activity measurements were analyzed separately. In all comparisons $p<0.05$ was considered to be significant. Significant F values are reported in the footnotes to the appropriate figure or table.

RESULTS

During pregnancy mice become markedly more susceptible to flurothyl-induced myoclonic and clonic seizures as indicated by the reduced time required for onset of seizures (Fig. 1). The latencies to myoclonus and clonus remained at control levels until day 9 of gestation when myoclonic threshold was significantly reduced ($p<0.05$). On days 12, 15 and 18 of gestation the mice were markedly more prone to flurothyl seizures ($p<0.01$) and the response was quite homogeneous among the animals for both measures. There was no statistical difference in the seizure scores for days 12–18. Parturition (usually at day 20) resulted in a return of seizure susceptibility to control levels. After parturition the latency to myoclonic seizures of the (nursing) females remained at or near control values. The clonic seizure score was lowered on days 12 and 20, but not significantly so because of the large variances in these groups. Still, this suggested that nursing might have had an effect on seizure activity, which was examined more closely.

Table 1 shows the effect of lactation on seizure threshold. Lactating mice were significantly more prone to clonic seizures than either control or non-lactating mice. Myoclonic seizures were not affected by lactation. The effect of lactation on clonic seizures was not examined further.

The possibility that π-AlDH could be indirectly responsible for the increased susceptibility to seizures during pregnancy by metabolizing pyridoxal was investigated. The results are shown in Table 2 along with the values for a reference substrate, propionaldehyde. Liver homogenates obtained from pregnant animals show an increased capacity to

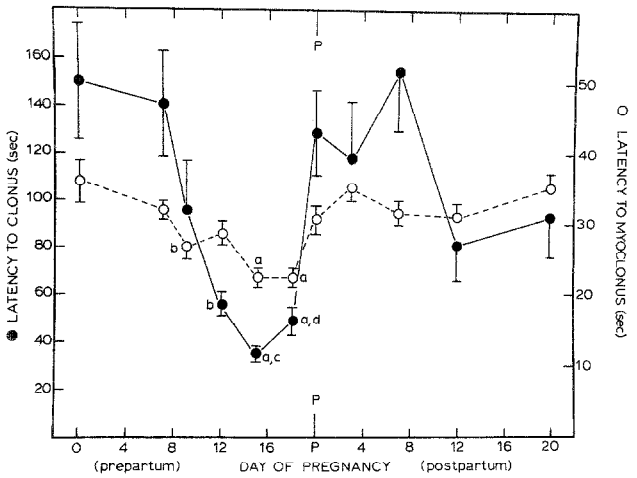

FIG. 1. Influence of pregnancy and lactation on flurothyl-induced seizures in the mouse. Day of pregnancy was timed from observance of vaginal plug. Each value is the mean±SEM of 20 animals per group except the points at days 12 and 20 postpartum which are based on 18 and 15 animals, respectively. P indicates the day of parturition. ●, latency to clonus: $F(2,202)=5.89$, $p<0.001$. ○, latency to myoclonus: $F(2,202)=4.72$, $p<0.001$. [a]Significantly different from control, $p<0.01$ by Tukey B test. [b]Significantly different from control, $p<0.05$ by Tukey B test. [c]Significantly different from day of parturition, $p<0.01$ by Tukey B test. [d]Significantly different from day of parturition, $p<0.05$ by Tukey B test.

TABLE 1

EFFECT OF LACTATION ON SEIZURE SUSCEPTIBILITY

Group	N	Latency to Myoclonus (sec)	Latency to Clonus (sec)
Control	20	35.6 ± 2.7	149.8 ± 23.8*
Lactating	18	30.8 ± 1.9	65.9 ± 10.8
Non-Lactating	11	36.8 ± 3.4	143.9 ± 33.9*

Control mice were virgin females. Lactating and non-lactating mice were seizure tested with flurothyl on the twelfth postpartum day. Lactating mice were nursing 4 pups each. Non-lactating mice had their pups removed on the morning they delivered and were not nursing. Tabled values are mean ± SEM.

Clonus: $F(2,48)=4.66$, $p<0.025$.

*Significantly different from the lactating group, $p<0.05$.

metabolize pyridoxal. The peak of pyridoxal dehydrogenase activity occurs at day 15 of gestation which is coincident with the peak of propionaldehyde dehydrogenase activity and maximal seizure susceptibility. The K_m values are not changed for either substrate during pregnancy. The V_{max} values for propionaldehyde are elevated in the pregnant mouse, but the V_{max} is unchanged for pyridoxal oxidation. Pyridoxal 5′-phosphate was not utilized as a substrate for π-AlDH.

Since aldehyde oxidase is normally considered to be responsible for most of pyridoxal metabolism, its activity was measured and the results are presented in Table 3. The activity of AO is 40- to 200-fold lower than π-AlDH activity at each time point. Aldehyde oxidase activity at parturition is decreased significantly ($p<0.01$) from control. Day 15 levels of AO are lower still, but these are not significantly different from values at parturition.

Two PLP synthetic enzymes were also examined (Table 3). Pyridoxal kinase activity at the time of parturition was significantly ($p<0.01$) elevated over control and day 15. The activity of the enzyme on day 15 was not different from control. Brain was used as the source of enzyme since its activity is highest in that tissue. Attempts to measure pyridoxal kinase in liver homogenates were unsuccessful due to technical difficulties and very low activity.

Pyridoxamine phosphate oxidase activity is present in mouse liver homogenates in only minute quantities. Its activity did not change significantly during pregnancy. A significant ($p<0.05$) increase in total enzyme activity was observed at parturition. Slightly greater protein levels were also found at this time which may partially explain the increase in total activity. The increased protein was probably of mitochondrial or microsomal origin since the concentration of cytosolic protein is not changed during pregnancy on a per gram of liver basis [21].

DISCUSSION

The results of this study demonstrate a clear relationship between pregnancy and susceptibility to chemically-induced seizures in mice. Several years ago it was proposed that the seizures which occur in toxemic pregnancies might result from the deficiency of vitamin B_6 in pregnant women [9]. That hypothesis is supported by this study.

Vitamin B_6 is a cofactor in the synthesis and/or degradation of the neurotransmitters norepinephrine, dopamine, serotonin and GABA. It was postulated that vitamin B_6 deficiency results in increased seizure susceptibility because of abnormally low levels of these neurotransmitters [18].

Flurothyl was used to induce seizures because this agent provides a clear distinction between myoclonus and clonus, a valuable feature not shared with other seizure-inducing agents. Greer and Alpern [7] reported that myoclonus and clonus were pharmacologically distinguishable entities and suggested that myoclonus was mediated by dopaminergic pathways and clonus by GABA and acetylcholine pathways. Our data (Fig. 1) show that both myoclonus and clonus are affected by pregnancy and imply a generalized reduction in levels or turnover of all the neurotransmitters, as would be predicted [18]. Our hypothesis is supported by a recent report [25] which showed that the plasma concentration of norepinephrine in normal pregnancies decreased significantly as pregnancy advanced. Plasma norepinephrine levels in pre-eclamptic pregnancies were even lower (significantly) than those found in normal pregnancies.

The cause of the vitamin B_6 deficiency in pregnancy is not known. Shunting of PLP to the fetus occurs to some extent [1] and cord levels of PLP are indicative of the state of vitamin B_6 nutriture during pregnancy. It was reported that the activity of pyridoxal kinase was depressed in placentas from toxemic pregnancies when compared to placentas obtained from normal pregnancies [5,9] and these investigators suggested that the synthesis of PLP was, therefore, compromised in toxemic pregnancies. Our data show no change in pyridoxal kinase activity until after parturition when its activity increases significantly. In one study [5] the activity of pyridoxamine phosphate oxidase in toxemic placentas

TABLE 2

CYTOSOLIC π-ALDEHYDE DEHYDROGENASE ACTIVITY IN LIVER DURING PREGNANCY

Substrate	Control	Day 15	Parturition
Propionaldehyde*	[14]	[14]	[16]
Specific activity	23.99 ± 1.77	59.20 ± 2.15§	45.05 ± 2.62¶§
Total activity	1258 ± 82	3293 ± 104§	2515 ± 127¶§
K_{m_1} ($\times 10^5$ M)‡	18.8	19.5	22.0
V_{max_1}	39.3	66.8	65.3
K_{m_2} ($\times 10^5$ M)‡	3.62	3.44	2.94
V_{max_2}	29.3	42.2	38.2
Pyridoxal·HCl†	[12]	[15]	[15]
Specific activity	7.30 ± 0.55	11.08 ± 0.61§	10.11 ± 0.42§
Total activity	387 ± 37	642 ± 35§	565 ± 22§
K_{m_1} ($\times 10^5$ M)‡	163	186	253
V_{max_1}	46.5	35.9	46.1
K_{m_2} ($\times 10^5$ M)‡	13.2	10.8	20.3
V_{max_2}	13.7	10.2	10.3

Tabled values are mean ± SEM. Numbers per group for specific and total activities are given in brackets. K_m and V_{max} values are the average of 2 or 3 separate determinations.

*Propionaldehyde dehydrogenase activity is expressed as nmoles NADH formed per min per mg protein (specific activity), $F(2,41)=58.9$, $p<0.001$, or per gram liver, wet weight (total activity), $F(2,41)=86.0$, $p<0.001$.

†Pyridoxal dehydrogenase activity is expressed as nmoles NADH formed per min per mg protein (specific activity), $F(2,39)=12.6$, $p<0.001$, or per gram liver, wet weight (total activity), $F(2,39)=16.1$, $p<0.001$.

‡K_m and V_{max} values were determined in the presence of 1.0 mM NAD·V_{max} values are in units of nmoles NADH formed per min per mg protein.

§Significantly different from control, $p<0.01$ by Tukey B test.

¶Significantly different from day 15, $p<0.01$ by Tukey B test.

TABLE 3

VITAMIN B_6 ENZYME ACTIVITIES DURING PREGNANCY

Enzyme Activity	Control	Day 15	Parturition
Aldehyde Oxidase*	[14]	[20]	[17]
Specific activity	0.595 ± 0.068	0.280 ± 0.026§	0.368 ± 0.020§
Total activity	31 ± 3	16 ± 1§	21 ± 1§
Pyridoxal Kinase†	[10]	[10]	[10]
Specific activity	22.92 ± 1.24	21.88 ± 1.34	35.95 ± 1.89§¶
Total activity	493 ± 32	532 ± 36	708 ± 35§¶
Pyridoxamine Phosphate Oxidase‡	[8]	[7]	[7]
Specific activity	0.110 ± 0.022	0.158 ± 0.043	0.217 ± 0.042
Total activity	1.79 ± 0.34	2.75 ± 0.70	3.96 ± 0.62#

Tabled values are mean ± SEM. Numbers per group are given in brackets.

*Aldehyde oxidase activity is expressed as nmoles N^1-methylnicotinamide oxidized per min per mg protein (specific activity), $F(2,48)=16.6$, $p<0.001$, or per gram liver, wet weight (total activity), $F(2,48)=15.9$, $p<0.001$.

†Pyridoxal kinase activity is expressed as nmoles pyridoxal 5′-phosphate formed per hour per mg protein (specific activity), $F(2,27)=26.6$, $p<0.001$, or per gram brain, wet weight (total activity), $F(2,27)=11.2$, $p<0.001$.

‡Pyridoxamine phosphate oxidase activity is expressed as nmoles pyridoxal 5′-phosphate formed per hour per mg protein (specific activity), $F(2,19)=2.3$, NS, or per gram liver, wet weight (total activity), $F(2,19)=3.91$, $p<0.05$.

§Significantly different from control, $p<0.01$ by Tukey B test.

¶Significantly different from day 15, $p<0.01$ by Tukey B test.

#Significantly different from control, $p<0.05$ by Tukey B test.

was also found to be (non-significantly) reduced compared to normal placentas. Again, our data show a modest increase in this enzyme activity in liver after parturition. These results may not be directly comparable (or contradictory) since we studied brain and liver enzymes while the previous reports concerned placental enzymes which were reduced only in abnormal pregnancies. Table 3 shows that during pregnancy in the mouse no change occurs in two major PLP synthetic enzymes until after parturition when the activity increases significantly (pyridoxal kinase) or marginally (pyridoxamine phosphate oxidase). The increase in PLP synthetic machinery over control coincides with a return of seizure susceptibility to control levels—an enticing observation which may simply be fortuitous.

Less is known concerning the metabolism of vitamin B_6 in pregnancy than is known about its synthetic enzymes. The activity of pyridoxal phosphate phosphatase was measured in normal and pre-eclamptic placentas and found to be unchanged [5]. There are no reports in the literature concerning the activity of AO in pregnancy. Table 3 shows convincing evidence that the activity of this enzyme, which is thought to be responsible for the majority of pyridoxal oxidation, is reduced by half at the beginning of the third trimester in mice and by 40% after parturition. If no other system were involved, then, it would seem that PLP would be maintained at normal or greater concentration during pregnancy. This discrepancy might be answered by the suggestion that π-AlDH, an enzyme which is induced in pregnancy, metabolizes a greater than normal fraction of pyridoxal.

Table 2 shows that the liver of a pregnant animal has more pyridoxal dehydrogenase activity than control. Pyridoxal is a poor substrate for π-AlDH when compared to propionaldehyde, but it can be oxidized nonetheless. Pyridoxal is not a preferred substrate for π-AlDH since there is no change in its V_{max} during pregnancy, whereas a preferred substrate such as propionaldehyde has an elevated V_{max}. A similar result was found for some other aromatic aldehyde substrates (i.e., 4-carboxybenzaldehyde) by us previously [21] and by others for the phenobarbital-induced (ϕ-AlDH) enzyme in rat liver [4]. The increased pyridoxal dehydrogenase activity parallels that of propionaldehyde dehydrogenase, indicating that π-AlDH is indeed responsible for this elevated activity. The role of π-AlDH in pregnancy remains elusive, but it is likely that the increased oxidation of pyridoxal represents nothing more than a coincidental reaction of an enzyme with broad substrate specificity. Further experimentation is necessary to determine the role of π-AlDH in the control of steady-state levels of vitamin B_6 normally and during pregnancy. Our feeling is that it might contribute to, but not fully explain, the reduction in circulating PLP during pregnancy. We have not yet measured vitamin B_6 levels in our pregnant animals. However, the pregnancy-associated increase in π-AlDH activity [21] correlates very well with the pregnancy associated decline in PLP [19]. It may be possible to attenuate seizure susceptibility and restore normal neurotransmitter system activity by selectively inhibiting π-AlDH and/or supplementing the diet of pregnant mice with some form of vitamin B_6. Since the level of π-AlDH returns to control by day 12 postpartum [21], it is unlikely that it contributes to the increased sensitivity to clonus at that time. We have not examined this observation further to date.

Seizure control in pregnant epileptics is often unpredictable, with many women suffering increased incidence of seizures [17]. Anticonvulsant medication may have to be increased markedly and some of the most frequently prescribed anticonvulsants (phenytoin, trimethadione and phenobarbital) are potent teratogens [20]. A 2- or 3-fold increase in congenital malformations has been attributed to in utero exposure to anticonvulsants. Stumpf and Frost [24] pointed out methodological problems in some of the studies which may overemphasize the teratogenic potential of anticonvulsants, yet it is clear that epileptic women do run a higher risk of giving birth to a malformed infant than do non-epileptics.

Our study suggests that pregnancy and seizure susceptibility are linked. It is tempting to speculate that it might be possible to improve the efficacy of continued anticonvulsant therapy in at least a select group of pregnant epileptics by judicious use of vitamin B_6 supplementation. A similar suggestion has been made by others to prevent and/or improve the outcome of toxemic pregnancies [5, 11, 26]. In one report [5] the suggestion was offered to use pyridoxal as the form of vitamin B_6 supplementation. Considering the data presented in Table 2, this might not be the best choice. Pyridoxamine might be preferred because it is active itself as a coenzyme (when phosphorylated via pyridoxal kinase), and its enzymatic transformation to PLP via pyridoxal kinase [13] and pyridoxamine phosphate oxidase [27] should be relatively unaffected in the pregnant animal (Table 3). It has already been shown that pyridoxine is not effective in raising PLP levels in pregnant rats [19], but other forms of the vitamin were not tested.

We are making the assumption that enzyme changes which occur in the pregnant mouse mirror those which occur in pregnant women. We recognize that it is not always valid to take data from one species and extrapolate them to another. It is not known if an induction of π-AlDH occurs in pregnant women, and since our experience suggests that this induction occurs only in the liver, it would be a difficult and ethically problematic task to replicate these data in humans. Pre-eclampsia/eclampsia is a condition peculiar to pregnant women and other higher primates, but the mouse might be useful in investigating basic enzyme and metabolic changes which occur in pregnancy. Interestingly, the values we obtained for pyridoxamine phosphate oxidase and pyridoxal kinase are similar to those found in human placental tissue. Clearly, more study is needed to ascertain the importance of π-AlDH in normal and pre-eclamptic pregnancies. Our working hypothesis is that the observed increase in seizure susceptibility is the result of altered function of brain neurotransmitter systems subsequent to a deficiency of the coenzyme form of vitamin B_6 due to increased metabolism by a pregnancy-associated enzyme, π-aldehyde dehydrogenase.

REFERENCES

1. Brin, M. Abnormal tryptophan metabolism in pregnancy and with the oral contraceptive pill. II. Relative levels of vitamin B_6-vitamers in cord and maternal blood. *Am. J. clin. Nutr.* **24:** 704–708, 1971.

2. Brophy, M. H. and P. K. Siiteri. Pyridoxal phosphate and hypertensive disorders of pregnancy. *Am. J. Obstet. Gynec.* **121:** 1075–1079, 1975.

3. Cheyney, M. C. and G. H. Beaton. Assessment of vitamin B_6 nutriture in pregnancy in the rat. *Proc. Soc. exp. Biol. Med.* **120:** 692–696, 1965.
4. Deitrich, R. A., A. C. Collins and V. G. Erwin. Genetic influence upon phenobarbital-induced increase in rat liver supernatant aldehyde dehydrogenase activity. *J. biol. Chem.* **247:** 7232–7236, 1972.
5. Gaynor, R. and W. B. Dempsey. Vitamin B_6 enzymes in normal and pre-eclamptic human placentae. *Clinica chim. Acta* **37:** 411–416, 1972.
6. Greer, C. A., H. P. Alpern and A. C. Collins. Increased CNS sensitivity to flurothyl as a measure of physical dependence in mice following morphine, phenobarbital, and ethanol treatment. *Life Sci.* **18:** 1375–1382, 1976.
7. Greer, C. A. and H. P. Alpern. Mediation of myoclonic seizures by dopamine and clonic seizures by acetylcholine and GABA. *Life Sci.* **21:** 385–392, 1977.
8. Hamfelt, A. and L. Hahn. Pyridoxal phosphate concentration in plasma and tryptophan load test during pregnancy. *Clinica chim. Acta* **25:** 91–96, 1969.
9. Klieger, J. A., C. H. Altshuler, G. Krakow and C. Hollister. Abnormal pyridoxine metabolism in toxemia of pregnancy. *Ann. N.Y. Acad. Sci.* **166:** 288–296, 1969.
10. Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193:** 265–275, 1951.
11. Lumeng, L., R. E. Cleary, R. Wagner, P.-L. Yu and T.-K. Li. Adequacy of vitamin B_6 supplementation during pregnancy: A prospective study. *Am. J. clin. Nutr.* **29:** 1376–1383, 1976.
12. McClearn, G. E., J. R. Wilson and W. Meredith. The use of isogenic and heterogenic mouse stocks in behavioral research. In: *Contributions to Behavior-Genetic Analysis: The Mouse as a Prototype,* edited by G. Lindzey and D. D. Thiessen. New York: Appleton-Century-Crofts, 1970, pp. 2–22.
13. McCormick, D. B., M. E. Gregory and E. E. Snell. Pyridoxal phosphokinases. I. Assay, distribution, purification, and properties. *J. biol. Chem.* **236:** 2076–2084, 1961.
14. Molony, C. J. and A. H. Parmalee. Convulsons in young infants as a result of vitamin B_6 deficiency. *J. Am. Med. Ass.* **154:** 405–406, 1954.
15. Petersen, D. R., S. S. Panter and A. C. Collins. Ethanol metabolism in the pregnant mouse. *Drug Alcohol Depend.* **2:** 409–420, 1977.
16. Rajagopalan, K. V. and P. Handler. Aldehyde Oxidase. In: *Methods in Enzymology* vol. 9, edited by W. A. Wood. New York: Academic Press, 1966, pp. 364–368.
17. Ramsey, R. E., R. G. Strauss, B. J. Wilder and L. J. Willmore. Status epilepticus in pregnancy: Effect of phenytoin malabsorption on seizure control. *Neurology* **28:** 85–89, 1978.
18. Schlesinger, K. and R. A. Schreiber. Interaction of drugs and pyridoxine deficiency on central nervous system excitability. *Ann. N.Y. Acad. Sci.* **166:** 281–287, 1969.
19. Sloger, M. S. and R. D. Reynolds. Effects of pregnancy and lactation on pyridoxal 5′-phosphate in plasma, blood and liver of rats fed three levels of vitamin B_6. *J. Nutr.* **110:** 1517–1524, 1980.
20. Smith, D. W. Teratogenicity of anticonvulsive medications. *Am. J. Dis. Child.* **131:** 1337–1339, 1977.
21. Smolen, A., D. R. Petersen and A. C. Collins. Liver cytosolic aldehyde dehydrogenase activity in the pregnant mouse. *Devl Pharmac. Ther.* **3:** 31–49, 1981.
22. Stanulović, M. and S. Chaykin. Aldehyde oxidase: Catalysis of the oxidation of N^1-methylnicotinamide and pyridoxal. *Archs Biochem. Biophys.* **145:** 27–34, 1971.
23. Stanulović, M., V. Jeremić, V. Leskovac and S. Chaykin. New pathway of conversion of pyridoxal to 4-pyridoxic acid. *Enzyme* **21:** 357–369, 1976.
24. Stumpf, D. A. and M. Frost. Seizures, anticonvulsants, and pregnancy. *Am. J. Dis. Child.* **132:** 746–748, 1978.
25. Tunbridge, R. D. G. and P. Donnai. Plasma noradrenaline in normal pregnancy and in hypertension of late pregnancy. *Br. J. Obstet. Gynec.* **88:** 105–108, 1981.
26. Wachstein, M. and L. W. Graffeo. Influence of vitamin B_6 on the incidence of pre-eclampsia. *Obstet. Gynec.* **8:** 177–180, 1956.
27. Wada, H. and E. E. Snell. The enzymatic oxidation of pyridoxine and pyridoxamine phosphates. *J. biol. Chem.* **236:** 2089–2095, 1961.
28. Webb, O. L. and R. L. Russell. Diurnal chemoconvulsive responses and central inhibition. *Archs int. Pharmacodyn. Thér.* **159:** 471–476, 1966.
29. Wike, E. L. *Data Analysis: A Statistical Primer for Psychology Students.* Chicago: Aldine-Atherton, 1971.