ELSEVIER

Fetal growth retardation and death in pantothenic acid-deficient rats is due to impaired placental function

Mei-Chuan Chen, Yosook Song* and Won O. Song

*Department of Food Science and Human Nutrition, Michigan State University, East Lansing, MI USA; *Chonju-Woosuk University, Chonbuk, Korea*

Maternal pantothenic acid (PA) deficiency in rats leads to retarded fetal growth, congenital malformation, and fetal death. The teratogenic effect was reported in mild maternal PA deficiency. We investigated if the adverse outcome of pregnancy in PA deficiency was caused by impaired placental development of functions. Sprague-Dawley pregnant rats (200 to 240 g) were pair-fed AIN-76 purified diets with (controls) or without PA for 3 weeks. We determined the body and organ weights, and PA status of pups and dams; resorption rate, weight and DNA content of placenta; and maternal plasma concentration of progesterone, and placental acetylcholine concentration relative to the placental function. The dams in the PA-deficient group had body and organ weights comparable to the control, produced pups with lower body and organ weights, and had higher resorption rates (57% versus 15%) than the control. The pups were more seriously affected by PA deficiency than the dams, with significantly lower PA and CoA concentration in organs compared with the control pups. Compared with the control, PA-deficient dams had on day 5 of pregnancy, a lower plasma progesterone concentration ($x \pm SEM$: 89.3 ± 8.3 versus 118.3 ± 10.5 nmol/L, $P < 0.05$), and elevated plasma cholesterol (2.57 ± 0.21 versus 1.61 ± 0.11 mmol/L, $P < 0.05$), and on day 15 of pregnancy, elevated placental cholesterol concentration (4.03 ± 0.08 versus 3.54 ± 0.05 μmol/g wet, $P < 0.05$), a lower placental acetylcholine concentration (1.32 ± 0.07 versus 1.54 ± 0.03 nmol/g wet $P < 0.05$), and lower placental PA (free and total) and CoASH concentration. Placental weight and DNA content did not differ significantly between the groups. We conclude that the combination of impaired placental endocrine functions and an inadequate supply of PA for fetal growth explain the retarded fetal growth and death, and more adverse effect to pups than to dams. (J. Nutr. Biochem. 7:451–456, 1996.)

Keywords: pantothenic acid; CoA; growth; development

Introduction

Maternal pantothenic acid (PA) deficiency during embryogenesis in rat has been reported to produce congenital malformation, retarded fetal growth, or fetal death with resorption.[1–4] The teratogenic effect was seen even in mild maternal PA deficiency in which PA concentration in dam's liver decreased only 16% from the level of the control.[4] This suggests that early embryonic development is very sensitive to a relatively mild deficiency of PA.

The requirement of PA as part of CoA, and acyl carrier protein of fatty acid synthase in many metabolic pathways is well understood. However, specific biochemical mechanisms that may link the adverse outcome of pregnancy to PA deficiency are not known. We do not know at present if it is caused by insufficient supply of the vitamin from dam to embryo due to a decreased pool of maternal stores, by impaired placental transport of the vitamin to embryo for its high requirement for fast growth and development, or by impaired placental growth or function either due to altered metabolism or due to high requirement of PA by placenta. Development and endocrine functions of placenta have never been investigated in PA deficiency.

In the present study, placental functions are hypothesized to be modified in PA deficiency based on the following

Address reprint requests to Won O. Song, Ph.D., R.D. at Dept. of Food Science and Human Nutrition, Michigan State University East Lansing, MI 48824–1224 USA
Received November 10, 1995; accepted February 29, 1996.

© Elsevier Science Inc. 1996
655 Avenue of the Americas, New York, NY 10010

0955-2863/96/$15.00
PII S0955-2863(96)00078-2

biochemical evidences: a) Progesterone is synthesized in the placenta during pregnancy from cholesterol, which is originated solely from maternal blood via passive diffusion. Lower serum cholesterol concentration has been reported in PA deficiency;[7,8] b) Acetylcholine in placenta is known to regulate transport of amino acids[9] as well as release of placental hormones to circulation.[10] The synthesis of acetylcholine in placenta and brain is regulated by choline acetyltransferase (EC 2.3.1.6.), which is controlled by the flux of acetyl CoA.[11,12] Choline acetyltransferase has Km's for acetyl CoA and CoASH (65 μmol/L and 20 μmol/L, respectively),[13] which are higher than the physiological concentrations of acetyl CoA in brain (2 to 20 μmol/L). Although CoASH level in placenta has never been reported, the concentration and flux of CoASH in placenta are not likely to differ from those in brain; c) Transport of PA in placenta is accomplished by a Na-dependent mechanism.[5] In humans, the Km (9 μmol/L) for the active transport[5] is higher than the physiological blood concentration (0.5 to 1 μmol/L).[6] Transport of PA by rat placenta has never been studied.

The present study was carried out to identify the possible mechanism of the impaired growth and fetal death caused by maternal PA deficiency. Specifically, we determined the body and organ weights and PA status of dams and pups; resorption rate; weight and DNA content of placenta; and progesterone, cholesterol, and acetylcholine concentration relative to the placental function.

Methods and materials

Chemicals

D-pantothenic acid, rabbit serum albumin, CoASH, ATP, Triton X-100 bovine serum albumin, bovine gamma globulin, alkaline phosphatase (EC 3.1.3.1), acyl-CoA synthetase (EC 6.2.1.3), Hoechst 33258, hemoglobin kit, and total cholesterol kit were purchased from Sigma Chemical Company (St. Louis, MO USA). D-[1-^{14}C]-pantothenic acid (1.48-2.22 GBq/mmol), [1-^{14}C]-palmitate (1.48 to 2.22 GBq/mmol), [1,2-^{3}H]-progesterone (185-740 MBq/mmol), and [1-^{14}C]-pyruvate (5-20 mCi/mmol) were from New England Nuclear (Boston, MA USA). Dithiothreitol (DTT) was from Boehringer Mannheim Biochemicals (Indianapolis, IN USA). Soluene was from Packard Instruments Company (Donwer's Grove, IL USA). Safety Solve was from Research Products International (Indianapolis, IN USA), and the progesterone antibody was from Endocrine Sciences (Calabasas Hills, CA USA). Pantetheinase (EC 3.5.1.-) was provided by Dr. Wittwer at the University of Utah, Salt Lake City, UT.

Animals and diet

About 50 female Sprague-Dawley rats (200 to 240 g) were bred with healthy males (280 to 350 g). Each female rat was examined for vaginal smears early each morning by the presence of vaginal plugs, erythrocytes and sperms. The day of positive detection was considered as the first day of gestation. The pregnant rats were then assigned to two to three groups (n = 6 to 8/group) and fed for 3 weeks of pregnancy either an AIN-76 semipurified diet-deficient in PA (<0.8 mg/kg diet), or the same diet supplemented with an adequate amount of PA (12 mg/kg; control diet).

The pregnant rats were housed individually in stainless steel hanging cages until the 20th day of pregnancy and then transferred to plastic-bottom cages. Individual food intake and weights were measured every other day. The room was maintained on a 12-hr light/dark cycle with lights on at 7:00 a.m. Temperature (20 to 22°C) and humidity (68 to 70%) were controlled. The protocol was reviewed and approved by the Michigan State University All-University Committee on Animal Use and Care.

In the first experiment, the pregnant rats were divided into three groups and given: a PA-deficient diet, ad libitum (Group 1); a PA-supplemented control diet, pair-fed with Group 1 (Group 2); a PA-supplemented control diet, ad libitum (Group 3). The second experiment included only groups 1 and 2.

Sample collection

In experiment 1, within 24 hr after pups were born, dams were killed by cardiac puncture (after anesthesia with metophane), and pups by decapitation. Blood was collected into vacutainer tubes containing sodium heparin. Liver and brain were removed from dams and pups, rinsed in ice-cold phosphate buffered saline (pH 7.4), weighed, frozen in dry ice, and stored at −20°C. The blood was measured for hemoglobin and total PA (sum of free PA and all PA derivatives including CoA and its acyl forms). The total PA, free CoA (CoASH), and acetylcholine were measured in liver and brain tissues of both dams and pups.

In Experiment 2, blood sample (1 to 1.2 mL/rat) was collected from the tail artery into syringes rinsed with sodium heparin on day 5 (first trimester; critical stage for embryo to implant), day 10 (critical stage for fetal development), and day 15 (earliest stage to detect placenta). On days 15 and 20 of gestation when placenta could be identified, four to seven rats from each group were killed. Blood, liver, brain, and placenta of dams and fetus were collected as in Experiment 1.

The whole blood was centrifuged at 2,000 × g for 15 min for separation of plasma after hemoglobin was measured. Hemoglobin was determined to monitor the effect of hemodilution for the analytes measured.

Analytical methods

Free PA in plasma (100 uL), and free and total PA in liver (0.2 g wet) and placenta (0.2 g wet) were determined by RIA following the method of Wyse et al.[14] and modified by Song et al.[6,18] Briefly, the tissue samples were homogenized in distilled water and incubated with (for total PA) or without (for free PA) an enzyme mixture containing 10 units alkaline phosphatase (EC 3.1.3.1.) and 20 units pantetheinase (EC 3.5.1.-) in 0.1 mol/L tris buffer at 37°C for 12 to 15 hr. The mixture was then deproteinized by equimolar concentration of saturated $Ba(OH)_2$ and 10% $ZnSO_4$, followed by centrifugation at 5,000 × g for 10 min. The resulting supernatant was used for the analysis for PA by RIA. CoASH was quantitated in tissues by modified procedures of Hansford,[15] Allred and Guy,[19] and Knights and Drew.[17]

Progesterone in plasma was determined by RIA described by Endocrine Science Corp. (Calabasas Hills, CA USA). Acetylcholine in brain and placenta was measured by the modified radiochemical assay described by Rivera-Calimlim et al.[19] and Fonnum.[20] Total cholesterol in plasma, liver and placenta was determined by a modified method described by Folch et al.[21] DNA in placenta was determined by a modified procedure of Labarca and Paigen[22] based on the enhanced fluorescence when bisbenzimidazole (Hoechst 33258) binds to DNA.

Statistical analyses

The statistical analyses were performed by the Minitab statistical program. ANOVA, multiple mean comparison tests, and Student's t-tests were used to compare all measurements among groups. Correlation was determined to assess the relationship between the

data of dams, pups, or placenta with the significance level of $P < 0.05$.

Results

Experiment 1

Weight gain, body and organ weights, and food intake of dams and pups. Dams (228 ± 30 g, initial weight) fed the PA-deficient diet, ad libitum (Group 1) consumed during 3-week gestation as much food as those fed a control diet, ad libitum (Group 3) (18 ± 2, 18 ± 1 g/day, respectively). Because food intake did not differ between Groups 1 and 3, we combined the data of two control groups (Groups 2 and 3). The weight gain, organ weights (liver, brain, kidney, and heart) and litter sizes (12.2 versus 12.3) of dams in Group 1 did not differ statistically from those of the control group (*Table 1*).

The birth weight and organ (brain and liver) weights of pups in Group 1 were, however, significantly lower than those of the control group ($P < 0.05$). The birth weight of pups was significantly correlated with their liver weight ($r = 0.79$, $P < 0.01$) and brain weight ($r = 0.58$, $P < 0.01$), but not with the weight gain of dams. The data suggest a clear partition between maternal and fetal metabolism in favor of maternal well-being.

PA status and brain acetylcholine. In dams of both groups, the blood total PA concentration at the beginning of gestation decreased significantly after delivery (*Table 2*). The decrease in Group 1 (2.52 ± 0.38 to 0.77 ± 0.10 μmol/L) was, however, significantly more than that in the control group (2.58 ± 0.18 to 1.45 ± 0.19 μmol/L) with no differences in hemoglobin content. Dams in Group 1 after delivery had significantly lower liver total PA and CoASH concentrations, than the control group ($P < 0.05$). Brain of dams in Group 1 had lower total PA and CoASH concentrations than those of the control group ($P < 0.05$) with no difference in acetylcholine content.

Compared with dams, pups in both groups had two to three times higher blood total PA concentrations, and 40 to 60% lower total PA and CoASH contents in liver and brain. Compared with pups in the control group, pups in the deficient group had significantly lower concentration of total PA and CoASH in blood and liver ($P < 0.05$). The high gradient in blood total PA concentration between pups to dams indicates indirectly that the active transport of PA by placenta is intact, but cannot make up for the low blood concentrations in PA-deficient dams. Although the brain of PA-deficient pups had significantly lower total PA and acetylcholine concentration, CoASH concentration did not differ between the two groups.

Table 1 Weight gain, and body and organ weights of dams and pups[1]

	Pantothenic acid status	
	Deficient	Adequate[2]
	Dams	
Weight gain (g/d)	6.78 ± 0.39[a]	7.59 ± 0.20[a]
Liver (g wet)	10.27 ± 0.52[a]	10.63 ± 0.32[a]
Brain (g wet)	1.58 ± 0.02[a]	1.58 ± 0.02[a]
Kidney (g wet)	1.83 ± 0.03[a]	1.82 ± 0.04[a]
Heart (g wet)	0.98 ± 0.04[a]	0.95 ± 0.02[a]
	Pups	
Birth wt (g)	5.75 ± 0.24[a]	6.41 ± 0.14[b]
Liver (g wet)	0.23 ± 0.00[a]	0.26 ± 0.01[b]
Brain (g wet)	0.21 ± 0.00[a]	0.22 ± 0.00[b]

[1]Values are means ± SEM for deficient group ($n = 5$) and control group ($n = 13$). Values in the same row with different superscripts are significantly different ($P < 0.05$).
[2]The data represent a pool of pair-fed and ad libitum controls because food intake did not differ between the two control groups.

Table 2 Total PA, CoASH, and Ach concentration in blood, liver, and brain of dams and pups[1]

	Pantothenic acid status	
	Deficient	Adequate[3]
	Dams	
At the beginning of gestation		
Blood PA[2] (total, μmol/L)	2.52 ± 0.38[a]	2.58 ± 0.18[a]
Hb (g/L)	150.7 ± 7.0[a]	154.4 ± 3.1[a]
After delivery		
Blood PA[2] (total, μmol/L)	0.77 ± 0.10[a]	1.45 ± 0.19[b]
Liver PA (total, nmol/g wet)	430.68 ± 12.60[a]	521.62 ± 35.04[b]
CoASH (nmol/g wet)	121.73 ± 11.77[a]	168.09 ± 10.19[b]
Brain PA (total, nmol/g wet)	57.00 ± 3.49[a]	72.60 ± 6.82[b]
CoASH (nmol/g wet)	30.60 ± 2.69[a]	51.42 ± 4.32[b]
Brain Ach (nmol/g wet)	0.41 ± 0.04[a]	0.49 ± 0.03[a]
Hb (g/L)	141.2 ± 8.0[a]	144.6 ± 5.6[a]
	Pups	
Blood PA[2] (total, μmol/L)	1.75 ± 0.14[a]	3.90 ± 0.21[b]
Liver PA (total, nmol/g wet)	187.20 ± 10.28[a]	326.86 ± 17.57[b]
CoASH (nmol/g wet)	71.80 ± 6.08[a]	113.28 ± 6.30[b]
Brain PA (total, nmol/g wet)	16.12 ± 2.46[a]	48.65 ± 5.03[b]
CoASH (nmol/g wet)	11.86 ± 0.85[a]	11.37 ± 0.70[a]
Brain Ach (nmol/g wet)	0.22 ± 0.02[a]	0.27 ± 0.01[b]
Hb (g/L)	128.2 ± 4.7[a]	122.0 ± 3.2[a]

[1]Values are means ± SEM for deficient group ($n = 5$) and control group ($n = 13$). Values in the same row with different superscript letter are significantly different ($P < 0.05$).
[2]Blood does not contain CoA.
[3]The data are a pool of pair-fed and ad libitum controls because food intake did not differ between the two groups.

Experiment 2

Resorption rate, placenta weight, and DNA content. Dams in PA-deficient group (Group 1) and the control group (Group 2) had resorption rates of 57% and 15%, respectively with mean litter sizes of 7 and 11, respectively. The average placental (±SEM) weight of Group 1 and the control group did not differ on day 15 (0.22 ± 0.05 g versus 0.24 ± 0.04 g, respectively), nor on day 20 (0.46 ± 0.00 g versus 0.46 ± 0.04 g, respectively) of gestation. DNA content per placenta did not significantly differ between PA-deficient and control groups on day 15 (0.51 ± 0.15, 0.59 ± 0.07 mg, respectively) and on day 20 (0.77 ± 0.11, 0.61 ± 0.06 mg, respectively).

Progesterone and cholesterol in plasma and tissues of dams. On day 5 of gestation (the critical stage for embryo to implant in uterus), dams in the deficient group had a significantly lower plasma progesterone concentration ($P < 0.05$) compared with the control group (*Table 3*). Although the PA-deficient dams had a lower mean plasma progesterone concentration than the control throughout the pregnancy, statistically significant difference ($P < 0.05$) was seen only at the early stage of pregnancy (day 5). In contrast, the deficient group had a significantly higher plasma (days 5 and 15) and placental cholesterol concentration did not, however, differ between the two groups. The correlation between plasma cholesterol and placental cholesterol concentrations was positive ($r = 0.73$, $P < 0.05$).

Placental acetylcholine, total PA and CoASH. Acetylcholine concentration in placenta was more than 3 times higher than that in brain of dams. On day 15 of gestation, placental acetylcholine concentration of PA-deficient group was significantly lower than that of the control group ($P < 0.05$) (*Table 4*). Towards the delivery (day 20), the difference between the two groups became insignificant.

The differences in total PA and CoASH concentration in placenta between the two groups were significant on both days 15 and 20 ($P < 0.05$). Compared with brain, placenta contained lower concentration of total PA and CoASH, while higher (four to seven times) concentration of acetylcholine. Correlation between total PA concentration in placenta and free PA in plasma was positive ($r = 0.87$, $P < 0.05$).

Discussion

Hypophagia and declined feed efficiency are one of the prominent early signs of PA deficiency. The signs have consistently been observed in most animals within 2 to 3 weeks after being fed a PA-deficient diet.[1,2,23] The signs usually accompany reduced organ weight with or without reduced CoA concentrations.[24,25] In contrast, the pregnant rats in the present study that were fed a PA-deficient diet for 3 weeks had as much food intake, weight gain, and organ weights as the dams fed the control diet. This new observation was confirmed through the repeated experiments. The findings suggest that metabolic and endocrine changes occurring during pregnancy prevent the early sign of PA deficiency in rats.

Pups produced by these PA-deficient dams had, however, significantly lower body and organ weights, and resorption rate than those of the control group. The common notion that normal fetal growth and development are protected at the cost of maternal tissue store could not be supported in PA deficiency. Pups were more susceptible than dams to PA deficiency. The lower body and organ weights of pups and high incidences of resorption in PA-deficient group compared with the control group have previously been reported.[1–4] Giroud[4] reported that the PA-deficient pregnant rats whose liver PA concentration was 40% of the normal value resulted in fetal deaths. We observed that pup's birth weight was significantly ($P < 0.01$) correlated with its organ weights ($r = 0.79$ for liver; $r = 0.58$ for brain), but not with weight gain of the dams. This means that the birth outcome cannot be predicted by weight gain nor by the biochemical parameter of dams in the case of PA deficiency. These observations also indicate that maternal PA deficiency is likely to affect metabolism of placenta and/or fetus directly.

Progesterone is important to maintain early stage of embryogenesis. Plasma concentration of the hormone was significantly lower in PA-deficient dams than in the control dams on day 5 of gestation ($P < 0.05$), whereas weight and DNA content of the placenta did not differ significantly between the two groups. This indicates that dietary PA deficiency during 3-week pregnancy did not impair growth of

Table 3 Progesterone and total cholesterol concentration in plasma, placenta and liver of dams[1]

Length of gestation (days)	Pantothenic acid status	
	Deficient	Adequate
	Plasma progesterone (nmol/L)	
5	89.3 ± 8.3[a]	118.3 ± 10.5[b]
10	129.2[2]	147.2[2]
15	147.6 ± 25.4[2]	58.9 ± 5.3[a]
20	161.0[2]	182.0 ± 20.0
	Plasma cholesterol (mmol/L)	
5	2.57 ± 0.21[a]	1.61 ± 0.11[b]
10	2.04 ± 0.09	2.07[2]
15	2.18 ± 0.19[a]	1.17 ± 0.10[b]
20	2.28 ± 0.16[a]	2.05 ± 0.13[a]
	Placental cholesterol (μmol/g wet)	
15	4.03 ± 0.08[a]	3.54 ± 0.05[b]
20	4.60 ± 0.98[a]	5.09 ± 0.08[a]
	Liver cholesterol (μmol/g wet)	
15	4.19 ± 0.13[a]	3.98 ± 0.26[a]
20	4.11 ± 0.23[a]	3.78 ± 0.16[a]

[1]Means ± SEM, $n = 3$ to 8 for each group. Different superscripts in the same row denote significant difference ($P < 0.05$).
[2]$n = 2$.

Table 4 Placental acetylcholine, pantothenic acid, and CoA concentration[1]

Length of gestation (days)		Pantothenic acid status	
		Deficient	Adequate
		Acetylcholine (nmol/g wet)	
15		1.32 ± 0.07[a]	1.54 ± 0.03[b]
20		1.57 ± 0.10[a]	1.30 ± 0.08[a]
		Placenta pantothenic acid (nmol/g wet)	
15	Free	2.50 ± 0.40[a]	13.36 ± 1.34[b]
	Total	7.42 ± 0.25[a]	19.24 ± 1.85[b]
20	Free	2.25 ± 0.33[a]	8.01 ± 0.93[b]
	Total	6.72 ± 0.21[a]	8.19 ± 0.53[b]
		Placenta CoASH (nmol/g wet)	
15		4.92 ± 0.12[a]	5.88 ± 0.92[b]
20		4.47 ± 0.09[a]	6.08 ± 0.27[b]

[1]Values are means ± SEM, $n = 3$ to 8 for each group. Different superscripts in the same row denote significant differences *($P < 0.05$).

placenta nor other maternal organs, but its endocrine function was modified.

It is known that most of the cholesterol for the steroidogenesis by the placenta is derived from the maternal blood.[26–28] Our data rule out the possibility that the blood level of cholesterol, the precursor for progesterone, is the cause of the low steroidogenesis in placenta of PA-deficient dams, because these rats had an elevated cholesterol concentration in plasma and placenta at day 5 of pregnancy. A positive correlation between plasma and placental cholesterol concentrations ($r = 0.73$, $P < 0.05$) also indirectly suggest a normally functioning placental uptake of cholesterol. This observation with pregnant rats is in contrast to our previous observations with non-pregnant rats[29] and others,[7,8,36,37] which showed reduced levels of blood and liver total cholesterol when deficient in PA. The data further suggest that hormonal and metabolic changes accompanying pregnancy inhibit the decrease in serum cholesterol seen in PA-deficient non-pregnant rats.

Placenta synthesizes, stores, and releases acetylcholine into maternal and fetal circulation.[30–32] We observed, interestingly, that placental acetylcholine concentration was more than three times higher than that in brain of rats. Placental acetylcholine in the PA-deficient dams was lower than that in the controls on day 15 of gestation ($P < 0.05$), during the critical period of fetal development. Acetylcholine concentration in rat placenta has never been quantitated or reported in relation to PA nutriture. CoASH concentration in the placenta of dams in PA-deficient and control groups (4.92 and 5.88 nmol/g wet, respectively) was, as suspected, much lower than the reported Km of choline acetyltransferase (EC 2.3.1.6.) for CoA (20 μmol/L).[13] Brain acetylcholine concentrations were about 20% lower in PA-deficient dams and pups compared with those in the control group. The differences in brain acetylcholine content of newborn pups between the two groups was statistically significant ($P < 0.05$), whereas the differences in dams were not, due to a large variation. Although no similar studies have been reported, others[33] have linked a decreased brain acetylcholine concentration to PA deficiency in young and old rats. Because rat brain grows fastest at approximately the eighth day after birth[34] and because it has an increased ability to synthesize and store acetylcholine after birth,[35] the significant difference in acetylcholine content of the brain observed at birth might not persist during later stages of development.

Our data suggest that the fetal growth retardation and death observed in PA-deficient rats were caused by a combination of inadequate placental endocrine function as demonstrated by low level of plasma progesterone at the critical stage for embryogenesis, and decreased synthesis of placental acetylcholine during the important stage for fetal development, plus the inadequate placental transfer of PA to fetus.

Acknowledgments

The project was partly supported by Michigan Agricultural Experiment Station and Korea Science and Engineering Foundation.

References

1 Nelson, M.M. and Evans, H.M. (1946). Pantothenic acid deficiency and reproduction in the rat. *J. Nutr.* **31,** 497–507

2 Nelson, M.M., Wright, H.V., Baird, C.D.C., and Evans, H.M. (1957). Teratogenic effects of pantothenic acid deficiency in the rat. *J. Nutr.* **62,** 395–402

3 Lefebrversp Boisselot, J. (1951). Role teratogene de la deficinecy en acide pantothenique chez le rat. *Ann. Med.* (Paris) **52,** 225

4 Giroud, A. (1970). *The nutrition of embryo.* pp. 107-108, Springfield, Charles C. Thomas

5 Grassl, S.M. (1992). Human placental brush-border membrane Na^+-pantothenate cotransport. *J. Biol. Chem.* **267,** 22902–22906

6 Song, W.O., Wyse, B.W., and Hansen, R.G. (1985). Pantothenic acid status of pregnant and lactating women. *J. Am Diet. Assoc.* **85,** 192–198

7 Perry, W.F., Hawkins, W.W., and Cumming, G.R. (1953). Adrenal function in pantothenic acid deficiency. *Am. J. Physiol.* **172,** 259–264

8 Remer, T. and Pietrzik, K. (1989). Effects of pantothenate deficiency on steroid hormone secretion in rats. I. Assessment of the state of function of the adrenal cortex. *J. Clin. Biochem. Nutr.* **6,** 1–14

9 Sastry, B.V.R., Barnwell, S.L., and Moore, R.D. (1983). Factors affecting the uptake of α-amino acids by human placental villus: acetylcholine, phospholipid methylation. Ca^{++} and cytoskeletal organization. *Trophoblast Res.* **1,** 81–100

10 Harbison, R.D., Olubadewo, J., Dwivedi, C., and Sastry, B.V.R. (1975). Proposed role of the placental cholinergic system in the regulation of fetal growth and development. In *Basic and Therapeutic Aspects of Perinatal Pharmacology,* pp. 107–120, Raven Press, Ltd., New York USA

11 Tucek, S. (1988). Choline acetyltransferase and the synthesis of acetylcholine. In *The Cholinergic Synapse, Handbook of Experimental Pharmacology,* (V.P. Whittaker, ed.), Vol. 86, pp. 125–165, Springer-Verlag, Berlin, Heidelberg

12 Tucek, S. and Cheng, S.C. (1970). Precursors of acetyl groups in acetylcholine in the brain in vivo. *Biochim. Biophys. Acta* **208,** 538–540

13 Schuberth, J., Sollenberg, J., Sundwall, A., and Sorbo, B. (1965). Determination of acetylcoenzyme A in brain. *J. Neurochem.* **12,** 451–454

14 Wyse, B.W., Wittwer, C., and Hansen, R.G. (1979). Radioimmunoassay for pantothenic acid in blood and other tissues. *Clin. Chem.* **25,** 108–111

15 Hansford, R.G. (1974). The control of tricarboxylate cycle oxidations in Blowfly flight muscle. The steady state of concentrations of CoA, acetyl CoA and succinyl CoA in flight muscle and isolated mitochondria. *Biochem. J.* **142,** 509–519

16 Allred, J.B. and Guy, D. (1969). Determination of CoA and Acetyl CoA in tissue extract. *Anal. Biochem.* **29,** 293–299

17 Knights, M. and Drew, R. (1988). A radioisotopic assay of picomolar concentrations of coenzyme A in liver tissue. *Anal. Biochem.* **168,** 94–99

18 Song, W.O., Wyse, B.W., and Wittwer, C. (1996). Modern analytical methods for pantothenic acid. In *Analytical Methods for Fat and Water Soluble Vitamins* (Y. Haroon, ed.), John Wiley and Sons, Inc. (In press)

19 Rivera-Calimlim, L., Hartley, D., and Osterhout D. (1988). Effects of ethanol and pantothenic acid on brain acetylcholine synthesis. *Br. J. Pharmacol.* **95,** 77–82

20 Fonnum, F. (1975). A rapid radiochemical method for the determination of choline acetyltransferase. *J. Neurochem.* **24,** 407–409

21 Folch, J., Lees, M., and Sloane Stanley, G.H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226,** 497–509

22 Labarca, C. and Paigen, K. (1980). A simple, rapid, and sensitive DNA assay procedure. *Anal. Biochem.* **102,** 344–352

23 Barboriak, J.J., Krehl, W.A., and Cowgill G.R. (1957). Pantothenic acid requirement of the growing and adult rat. *J. Nutr.* **62,** 13–21

24 Hurley, L.S. and Volkert, N.E. (1965). Pantothenic acid and coenzyme A in the developing guinea pig liver. *Biochim. Biophys. Acta* **104,** 372–376

25 Reibel, D.K., Wyse, B.W., Berkich, D.A., and Neely, J.R. (1982).

Coenzyme A metabolism in pantothenic acid-deficient rats. *J. Nutr.* **112,** 1144–1150
26 Hellig, H., Gattereau, D., Lefebvre, Y., and Bolte, E. (1970). Steroid production from plasma cholesterol. I. Conversion of plasma cholesterol to placental progesterone in humans. *J. Clin. Endocr.* **30,** 624–631
27 Brannian, J.D., Kurz, S.G., and Shiigi, S.M. (1994). Decline in fluorescent low density lipoprotein (LDL) uptake by small and large porcine luteal cells with advancing age of the corpus luteum. *Biol. Reprod.* **50,** 204–209
28 Albrecht, E.D. and Pepe, G.J. (1990). Placental steroid hormone biosynthesis in primate pregnancy. *Endocrine Rev.* **11,** 124–150
29 Chevaux, K.A. (1993). Adrenocortical function and cholesterol in pantothenic acid deficiency. Master's thesis, Michigan State University, East Lansing, MI USA
30 Gude, N.M., Krishna, B.R., King, R.G., Brennecke, S.P., and Boura, A.L.A. (1989). Effects of opioids on acetylcholine output of perfused human placental cotyleda. *Placenta* **10,** 445–452
31 Brennecke, S.P., Chen, S., King, R.G., and Boura, A.L.A. (1988). Human placental acetylcholine content and release at parturition. *Clin. Exp. Pharmacol. Physio.* **15,** 715–725
32 Chen, Y.S., Brennecke, S.P., and King, R.G. (1992). Human placental choline acetyltransferase activity at parturition. *Placenta* **13,** 255–264
33 Rivera-Calimlim, L. (1981). Pantothenic acid and brain acetylcholine. *Clin. Pharmacol. Ther.* **35,** 269
34 Dobbing, J. (1972). Vulnerable periods of brain development. In *Lipids, Malnutrition and the Developing Brain* (A.V. Muralt, ed.), pp. 9–29, Associated Scientific Publishers, Amsterdam
35 Parnavelas, J.G., and Dori, I. (1986). A transient population of cholinergic neurons in the developing rat cerebral cortex. *Neurosci. Lett. Suppl.* **24,** S47
36 Carter, C.W. and Hockaday, T.D. (1962). Liver lipids and ketone-body formation in rats deficient in pantothenate. *Biochem. J.* **84,** 275–280
37 Ershoff, B.H., Slater, R.B., and Gaines, J.G. (1953). Effect of pantothenic acid deficiency on pituitary-adrenal function in the rat. *J. Nutr.* **50,** 299–316