Placental Transport of Phenylalanine in the Rat: Maternal and Fetal Metabolism (35366)

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The children of mothers with phenylketonuria are almost invariably mentally retarded and have a high frequency of congenital abnormalities (1). Dietary treatment of the mother with a restricted protein intake during pregnancy has been reported to prevent the retardation in one case (2), but additional well controlled data are mandatory before such therapy can be recommended. Because the mammalian placenta actively transports amino acids, blood levels are from 1.5 to 2.4 times higher in the fetus than in the mother (3–7), suggesting that maternal blood should be below 8 mg/100 ml. In this way fetal levels would be below 15 mg/100 ml, the value above which damage may occur to the brain.

Another factor which may influence brain damage is the integrity of the blood–brain barrier to phenylalanine in the fetus. Yet another consideration is whether the excess phenylalanine affects the early developmental structure of the brain so that mental retardation results. Phenylalanine hydroxylase is absent in most mammalian livers until near birth (9) so that phenylalanine metabolism by this pathway is absent. The minor phenylalanine transaminase pathway is similarly inactive prior to birth (10). Partly balancing this is the greater incorporation of phenylalanine into protein in the rapidly growing fetus.

This study was designed to study phenylalanine transport across the placenta and subsequent metabolism by the fetus.

Materials and Methods. Pregnant female albino rats of known gestational age[1] were used within the last 5 days of pregnancy, *i.e.*, 16 to 20 days. The mothers were anesthetized by the intraperitoneal administration of Nembutal (30 mg/kg) after an overnight fast but with water available. A tracheostomy was performed after the female was strapped supine on a continuously warmed surface in order to maintain a free airway. Twenty to 100 mg of L-phenylalanine were injected/kg of body weight to the female after a control blood specimen was drawn. Maternal blood was collected initially and after each hour for 3 hr, from the cut end of the tail from which the last inch had been amputated. Between collections blood loss was prevented by ligature.

Fetuses were removed from one uterine horn sequentially as needed starting from the distal end. The cut end of the horn was ligated and the whole uterus was returned to the abdominal cavity which was closed with clips between removal of the fetuses. When one horn was emptied, the other horn was used. Any overt intra-uterine bleeding caused the horn to be discarded. The collected blood from 2 fetuses was allowed to clot, centrifuged, and the phenylalanine content of the serum was assayed by the method of McCaman and Robins (11). Each time a specimen of pooled fetal blood was obtained, a maternal specimen was collected simultaneously.

Results. The control levels of the fetal serum phenylalanine were always equal to or above that found in the maternal serum as is shown by the fetal–maternal ratio in Table I. These ratios are slightly higher than those previously reported (3–8) especially in the earlier pregnancies. There was only a slightly significant difference in ratios ($p<.10$) between the 17 day pregnancy (fetal–maternal

[1] Purchased from Sprague–Dawley or the Holtzman Company in Madison, Wisconsin.

TABLE I. Ratio[a] of Phenylalanine in Fetal and Maternal Serum in Fasting Rats.[b]

Stage of pregnancy (days)	No.	Mean	Range
16	17	3.2	1.2–6.3
17	14	3.5	1.4–7.5
18	14	2.4	1.0–4.5
19	13	2.8	1.1–6.5
20	14	2.6	1.4–5.4

[a] Each value represents the ratio of fetal serum phenylalanine (mg/100 ml) to maternal serum phenylalanine (mg/100 ml).

[b] The differences approach significance ($p<0.10$ >0.05) only when comparing 17 day pregnancies (3.5) to 18 day pregnancies (2.4).

ratio 3.5:1) and the 18-day pregnancy (ratio 2.4:1). This drop in fetal phenylalanine at 18 days may be due to greater incorporation of phenylalanine into protein associated with a marked increase in fetal size.

A typical experiment is shown in Fig. 1. The control fetal serum phenylalanine of 4.9 mg/100 ml is compared with the maternal level of 2.3 mg/100 ml. After 100 mg/kg of L-phenylalanine was injected, the maternal blood phenylalanine level rose to 10.8 mg/100 ml and then fell so that at 3 hr it was almost back to the base line level. The fetal level of phenylalanine, however, increased more slowly and although it was only slightly higher than the maternal value after 1 hr, it fell less rapidly so that after 3 hr the fetal serum phenylalanine was still 8.3 mg/100 ml. Thus, the fetal–maternal ratio

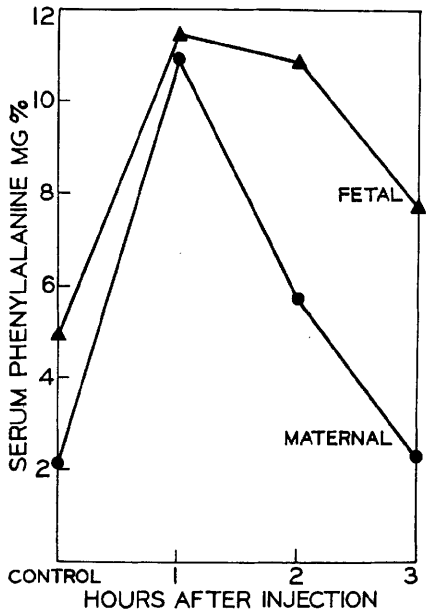

FIG. 1. Simultaneous serum phenylalanine in adult female rat and her fetuses before and 1, 2, and 3 hr after 100 mg/kg of L-phenylalanine was injected into the maternal subclavian vein.

was considerably higher at 3 hr than the control ratio (3.3 compared to 2.3).

In Table II, the fetal–maternal ratios of serum phenylalanine are shown for various loads of phenylalanine at different stages of pregnancy at 1, 2, and 3 hr after the injection. As shown, at lower dosages of phenylalanine and earlier in pregnancy the fetal–maternal ratio varies little with time after injection. Later in pregnancy and especially

TABLE II. Fetal–Maternal Serum Phenylalanine Ratio[a] at 1, 2, and 3 hr After Intravenous Injection of Phenylalanine into the Maternal Rat Subclavian Vein.

		Days of pregnancy														
		16			17			18			19			20		
Dose (mg/kg)	(hr):	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
20		3.2	3.0	3.3	2.8	3.4	2.6	2.0	2.8	2.2	2.1	2.0	1.7	—	—	—
30		2.1	1.4	2.1	—	—	—	—	—	—	2.2	3.1	2.6	1.9[b]	2.6[b]	3.5[b]
60		2.3	3.4	2.6	3.9[b]	4.8[b]	5.0[b]	2.9	2.5	2.5	1.8[b]	2.9[b]	3.1[b]	2.4	3.5	2.6
100		1.7	1.7	2.3	—	—	—	—	—	—	2.6[b]	2.2[b]	4.2[b]	1.9[b]	2.6[b]	3.3[b]

[a] Each value represents the ratio of fetal serum phenylalanine (mg/100 ml) to maternal serum phenylalanine (mg/100 ml). Each value shown is the mean of at least three experiments.

[b] Values which show a correlation coefficient between the ratio and time greater than 0.5.

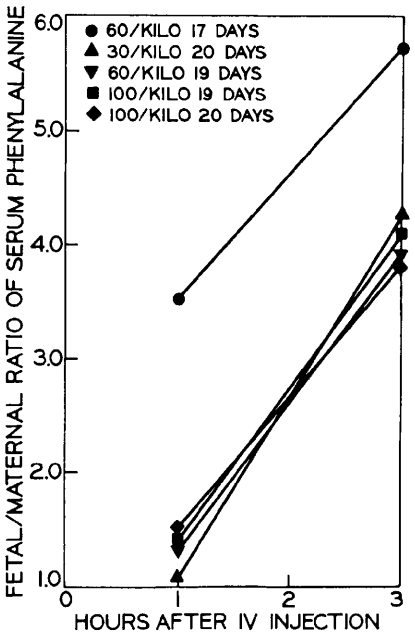

FIG. 2. Regression lines obtained by the method of least squares from the fetal–maternal ratio of serum phenylalanine at 1, 2, and 3 hr after an intravenous load of phenylalanine at various stages of pregnancy.

with higher loads (60 and 100 mg/kg) the ratio rises substantially with time. In those values marked with "*b*" the correlation coefficient between the ratio and time after injection is greater than 0.5. These values have been plotted by the method of least squares in Fig. 2, and demonstrate that for pregnancies of 19 or 20 days loads of 30 to 100 mg/kg produce a consistent regression line.

Discussion. These results indicate that late in pregnancy the fetal rat is not able to metabolize, as rapidly as its mother, a load of phenylalanine delivered by the placenta. In addition the fetal blood level of phenylalanine is always higher than the maternal. It must be recalled that the rat has 40 times the phenylalanine hydroxylase activity of humans (12) and this may explain why the levels achieved in the maternal blood are not high even with a 100 mg/kg load. Thus, if these data apply in the human situation, it may be most dangerous to perform a heterozygote test for phenylketonuria with added phenylalanine in a pregnant woman for the fetal blood will be elevated to approximately twice that of the maternal and will fall much more slowly, thus exposing the fetus to a higher and more prolonged hyperphenylalaninemia.

These data suggest that, if low phenylalanine dietary therapy of pregnant phenylketonuries is ever to be attempted, the monitoring of the diet will require that maternal blood should be drawn 30–60 min postprandially if the maximum amount of phenylalanine reaching the fetus is to be accurately known.

Summary. The fetal–maternal ratios of serum phenylalanine in the rat have been measured in the fasting state and 1, 2, and 3 hr after intravenous phenylalanine loading. Resuts indicate that fetal levels are always higher than maternal and that the fetus metabolizes the transplacental load less rapidly than its mother. This suggests that any woman with hyperphenylalaninemia who becomes pregnant should have her blood phenylalanine maintained below 8 mg/100 ml of blood at all times if the fetal brain is to remain undamaged.

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