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Shuichiro Akahori, Kohei Ejiri, and Kaoru Sekiba

Abstract

The concentrations of taurine in the fetal and neonatal organs, and the maternal organs, plasma and urine of rats between the 15th day of gestation and the 21st day after birth were determined using an automatic amino acid analyzer. In the fetal liver and brain and in the placenta, the taurine concentration was the highest of all ninhydrin positive compounds. In the fetal liver and placenta, the concentrations of taurine increased significantly with the gestational days. Concentrations of taurine in the brain were much higher in the fetus and neonate than that in the adult. Moreover, the total amount of taurine per fetus increased markedly after the 15th day of gestation, and near term, reached almost the same amount as in the adult rat liver. In contrast to this, a significant decrease was observed in the taurine concentration in the maternal liver and muscle near term. The concentration of taurine in the urine of pregnant rats decreased near term, but in the plasma of pregnant rats the concentration of taurine did not change during pregnancy.

KEYWORDS: taurine, developmental change, rat fetus, rat neonate, pregnant rat

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Taurine Concentrations in Fetal, Neonatal and Pregnant Rats

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The concentrations of taurine in the fetal and neonatal organs, and the maternal organs, plasma and urine of rats between the 15th day of gestation and the 21st day after birth were determined using an automatic amino acid analyzer. In the fetal liver and brain and in the placenta, the taurine concentration was the highest of all ninhydrin positive compounds. In the fetal liver and placenta, the concentrations of taurine increased significantly with the gestational days. Concentrations of taurine in the brain were much higher in the fetus and neonate than that in the adult. Moreover, the total amount of taurine per fetus increased markedly after the 15th day of gestation, and near term, reached almost the same amount as in the adult rat liver. In contrast to this, a significant decrease was observed in the taurine concentration in the maternal liver and muscle near term. The concentration of taurine in the urine of pregnant rats decreased near term, but in the plasma of pregnant rats the concentration of taurine did not change during pregnancy.

Key words : taurine, developmental change, rat fetus, rat neonate, pregnant rat

Taurine, 2-aminoethanesulfonic acid, is a simple sulfur-containing compound, which is ubiquitous in nature, and one of the most abundant amino acids present in mammals (1-3). It is found in large concentrations in many animal tissues, and the concentration of taurine in a given tissue vary widely from species to species (2). Moreover the concentration of taurine in an animal tissue changes markedly during development (3-5).

In the human and monkey fetal brain, the concentration of taurine is 4 to 5-fold higher than in the adult brain, and decreases slowly after birth, reaching concentrations found in the adult brain approximately by the time of weaning (6). In addition, taurine is a major constituent of the free amino acid pool of human milk, and may be an important nutrient for the human infant (7-9).

These findings have led to the hypothesis that taurine plays an important role in the developing fetus and infant. However, it takes part in few biochemical reactions, and little is known of its function. The only proven biochemical role for this compound in mammals is its conjugation with bile acids to form bile salts, which are essential for fat absorption (2).

We have studied the metabolism of sulfur-containing amino acids in fetal and neonatal rats (10). The purpose of our study is to clarify the metabolism and function of taurine during the perinatal period. The present paper reports on the concentration of taurine in the fetal, and neonatal organs, and the maternal organs, plasma and urine of rats between the 15th day of gestation and the 21st day after birth.

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Materials and Methods

Animals. Female rats of the Wistar strain weighing 200 g were paired with male animals from 9 p. m. of one day to 9 a. m. of the next. This day was taken as the first day of gestation, if sperm was found in the vaginal smear. These animals were isolated and fed on commercial rat chow (MF of Oriental Yeast CO., Tokyo) and water *ad libitum.* The pregnant rats from the 15th day to the 21st day of gestation and nonpregnant rats were sacrificed by decapitation. The fetus, placenta, liver, brain and skeletal muscle (M. rectus femoris) were quickly removed, and the fetal liver and brain were isolated as well. The liver and brain of neonates were also removed. Blood of pregnant and nonpregnant rats was withdrawn from the carotid artery under ether anesthesia using a heparinized syringe, and plasma was obtained by centrifugation at 1200×g for 10 min. Urine of pregnant rats and nonpregnant rats was collected by a metabolic cage. Nonpregnant rat refers to female rats weighing 200 g which were not paired.

Determination of taurine. All steps were carried out at 0-4°C. The organs were washed in a buffer containing 0.9% NaCl and 1 mM EDTA immediately after they were removed. They were blotted on filter paper, and weighed. These organs were homogenized with 5 volumes of 4% perchloric acid using a Potter-Elvehjem homogenizer equipped with a Teflon pestle. The precipitated proteins were removed by centrifugation at 1200×g for 10 min. Perchloric acid in the supernatant was eliminated by titration with 5N KOH and centrifugation. The resulting surpernatant was adjusted to pH 2.0 with 3N HCl. Plasma and urine samples were deproteinized by the addition of an equal volume of 20% trichloroacetic acid (TCA). The samples were centrifuged at 1200×g for 30 min, and the supernatant fluid was adjusted to pH 2.0 with 30% NaOH.

Determination of taurine and other amino acids was performed with a Hitachi amino acid analyzer Model 835-50 using the following buffers : 0.155N lithium citrate pH 3.0 at 33°C ; 0.255N, pH 3.7 at 43 and 47°C ; 0.805N, pH 3.3 at 58°C ; 1.00N, pH 4.1 at 58°C and 1.20N, pH 7.0 at 70°C. The deproteinized supernatant (0.5 ml) obtained as above was applied directly to the analyzer.

Results were expressed as μmoles taurine per gram wet weight of tissue, per mg of creatinine in urine and per 100 ml in plasma. The values were expressed as the mean ± S. D. of five separate determinations. The significance of the differences was calculated using Student's t test.

Results

Taurine in fetal and neonatal organs and placenta of rats. The mean concentrations of the 21 amino acids measured in the five fetal brains, fetal livers and placentas of rats at the 21st day of gestation are shown in Fig. 1. In these organs, the most abundant free amino acid was taurine. Glutamine was the second most abundant amino acid in the fetal liver and placenta. In the fetal brain glutamic acid was the second most abundant amino acid. Levels of 5 non-essential amino acids (glutamic acid, aspartic acid, alanine, glycine and glutamine) were all above 0.5 μmoles per gram in these three organs.

The results of the analyses of taurine contents in liver and brain during development are presented in Figs. 2 and 3, respectively. In the rat liver, the concentration of taurine increased during the fetal period, reaching adult levels on the first postnatal day (Fig. 2). The concentrations in the rat liver showed an approximately fourfold increase from the 15th day of gestation to the first postnatal day. On the 3rd postnatal day, the concentrations decreased slightly, and after the 5th postnatal day, they remained constant at the same level as that of adult rats.

In the rat brain, the concentrations of taurine were 7-9 μmoles per gram during the fetal and neonatal periods (Fig. 3), and the maximum concentrations of taurine were found on the 3rd day after birth. The concentrations in the brain of the fetus and

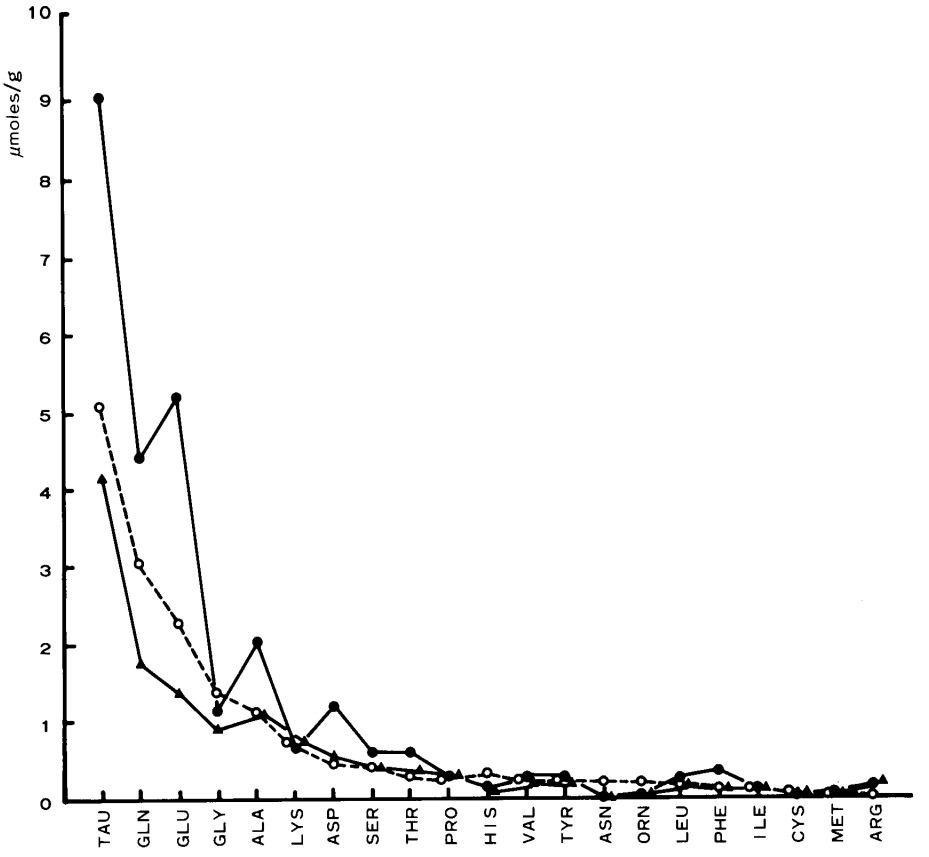
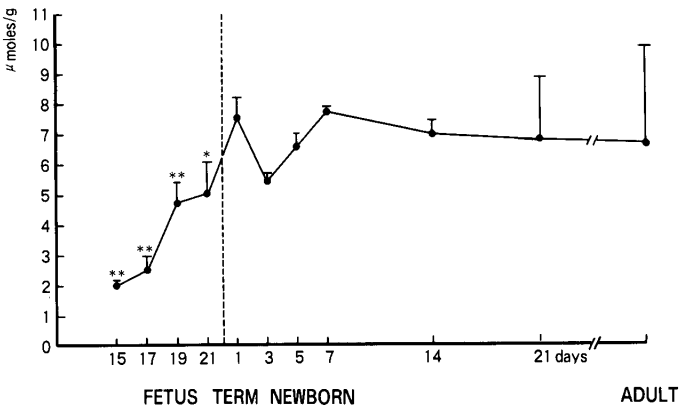


Fig. 1 Free amino acid concentrations of fetal brain, fetal liver and placenta of rat on the 21st day of gestation. Amino acid concentrations are expressed as μmole per gram wet weight. Each point represents the mean value of 5 separate determinations of pooled livers and brains from 12 fetal rats and 12 pooled placentas. Fetal rat brain, (●) ; fetal rat liver, (○) ; placenta, (▲).

Fig. 2 Concentrations of taurine in the rat liver during development. Taurine concentrations are expressed as μmole per gram wet weight. Each point represents the mean value of 5 separate determinations of pooled livers from 26 fetal and 6 neonatal rats. Vertical lines indicate the SD. Taurine concentrations which differ significantly from values of the mature rat are indicated by * (*$p < 0.05$, **$p < 0.01$).



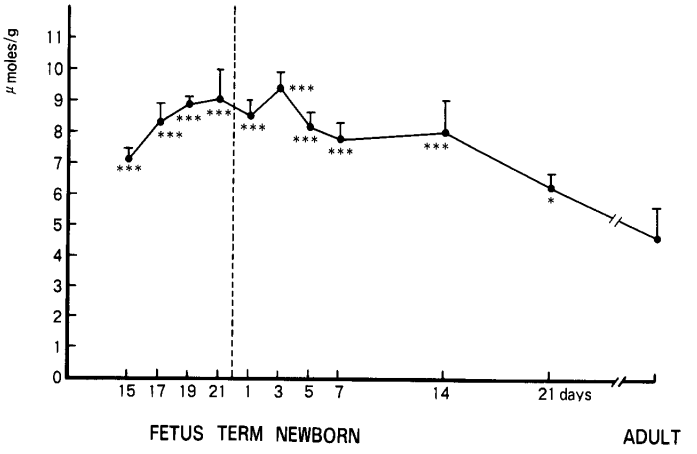


Fig. 3 Concentrations of taurine in the rat brain during development. Taurine concentrations are expressed as μmoles per gram wet weight. Each point represents the mean value of 5 separate determinations of pooled brains from 26 fetal and 6 neonatal rats. Vertical lines indicate the SD. Taurine concentrations that differ significantly from values of the mature rat are shown by * (***$p < 0.001$, *$p < 0.05$).

neonate were twice the amount of the adult level.

Fig. 4 gives the taurine concentration in the rat placenta at different stages during the late period of gestation. Taurine showed a steady increase until the last day studied. The concentration of taurine doubled from the 15th day to the 21st day of gestation.

Total amounts of some free amino acids in the rat fetus during development are shown in Fig. 5. The most abundunt ninhydrin-positive compound in the rat fetus was taurine

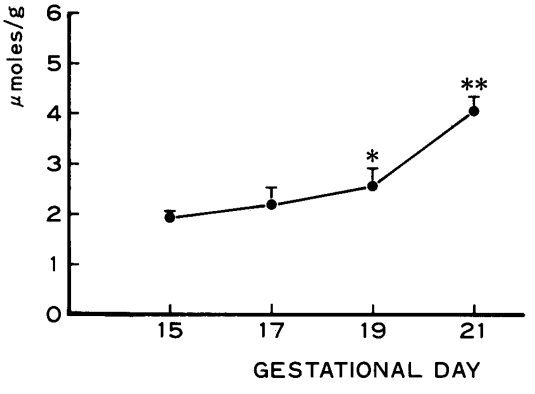


Fig. 4 Concentrations of taurine in the rat placenta during pregnancy. Taurine concentrations are expressed as μmole per gram wet weight. Each point represents the mean value of 5 separate determinations of 12 pooled placentas. Vertical lines indicate the SD. Taurine concentrations which differ significantly from values on the 15th day of pregnancy are shown by * (*$p < 0.05$, **$p < 0.01$).

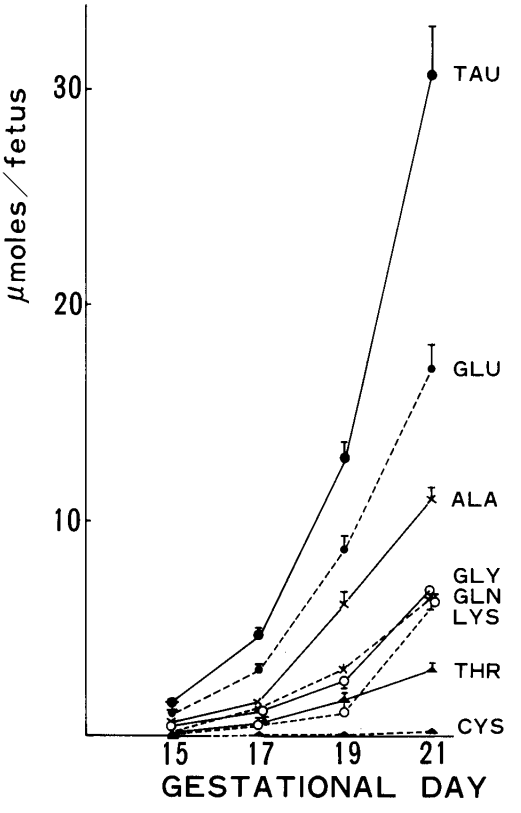


Fig. 5 Total amounts of some free amino acids in the rat fetus during development. Each point represents the mean value of 5 separate determinations. Vertical lines indicate the SD. Total amounts of each free amino acid are expressed as μmoles per fetus.

at any stage of development studied. Taurine contents were 1.5 times higher than glutamic acid and 2 times higher than alanine. Like other amino acids, the fetal body pools of taurine expanded rapidly during fetal development. In one fetal body, there was 30.7 μmoles of taurine on the 21st day of gestation.

Taurine in the brain, liver, muscle, plasma and urine of pregnant rats. Fig. 6 shows

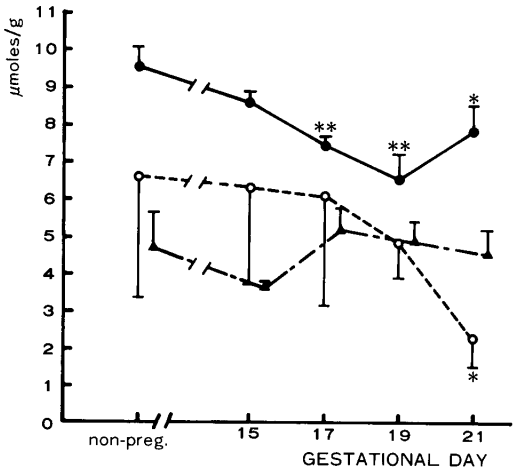


Fig. 6 Concentrations of taurine in brain, liver and muscle of pregnant rats. Taurine concentrations are expressed as μmole per gram wet weight. Each point represents the mean value of 5 separate determinations. Vertical lines indicate the SD. Taurine concentrations which differ significantly from values of nonpregnant rats are shown by * (*$p < 0.05$, **$p < 0.01$). Muscle, (●) ; liver, (○) ; brain, (▲).

the changes in taurine concentrations in the maternal rat brain, liver and muscle with the progress of gestation. On the 15th day of gestation, the concentration of taurine in the liver of pregnant rats did not differ from that of the nonpregnant rats. Taurine concentrations remained constant until the 17th day of gestation and decreased gradually on the 20th day. The taurine concentration fell on the 21st day to a value as low as 1/3 of that in nonpregnant rats. The concentrations of taurine in the muscle of the pregnant rats began to fall significantly on the 17th day of gestation. The mean taurine concentration in the muscle of nonpregnant rats was 9.6 μmoles per gram wet weight. The values in pregnant rats were as follows : 17th day, 7.5 ; 19th day, 6.6 and 21st day, 7.9. Taurine concentrations on the 17th, 19th and 21st days of gestation were significantly lower than in nonpregnant rats.

On the other hand, the concentration of taurine in the brain of pregnant rats did not differ from that of the nonpregnant rats and remained constant during pregnancy. Pregnancy itself appeared not to affect the brain taurine concentrations.

Taurine concentrations expressed as μmole/mg of creatinine in the urine of pregnant rats are given in Fig. 7. The mean taurine concentration in the urine of nonpregnant rats was 4.1 μmoles/mg of creatinine. The value in pregnant rats was about 3.0 μmoles/mg of creatinine during the late period of pregnancy and was significantly

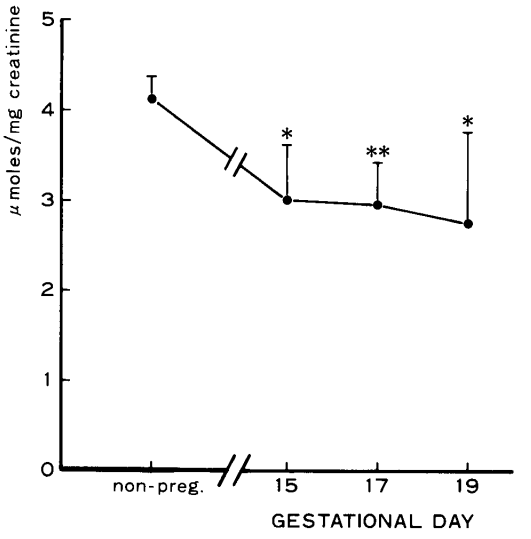


Fig. 7 Concentrations of taurine in the urine of pregnant rats. Taurine concentrations are expressed as μmole per mg creatinine. Each point represents the mean value of 5 separate determinations. Vertical lines indicate the SD. Taurine concentrations which differ significantly from values of nonpregnant rats are shown by * (*$p < 0.05$, **$p < 0.01$).

lower than that of nonpregnant rats.

The concentrations of taurine in plasma in pregnant rats were not significantly different from the values in nonpregnant rats (Fig. 8).

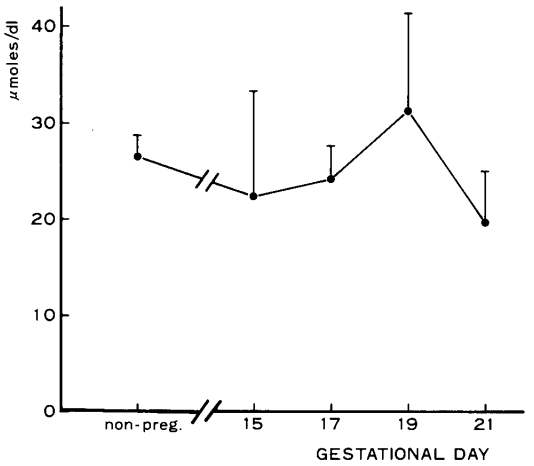


Fig. 8 Concentrations of taurine in the plasma of pregnant rats. Taurine concentrations are expressed as μmole per dl. Each point represents the mean value of 5 separate determinations. Vertical lines indicate the SD. Taurine concentrations of the pregnant rats are not different from the values of nonpregnant rats.

Discussion

The major pathway for the biosynthesis of taurine in mammalian tissues has been elucidated (1, 2, 11). The pathway has been demonstrated uneqivocally to operate in mammalian liver and brain, although its efficiency varies greatly from species to species (2). The capacity of the immature human infant to convert cystathionine to cysteine is limited, owing to delayed maturation of cystathionase (12). Activity of cysteine-sulfinic acid decarboxylase is usually lower in fetal and in newborn tissues than in adult tissues (13), so the capacity to convert cysteine to taurine is limited in the human infant. Thus, it is suggested that cysteine may be essential amino acids and taurine may be an important amino acids both for the fetus and the newborn infant (8, 9, 12, 13). Taurine and cysteine are highly concentrated in human milk (8). These amino acids are thus supplied to the infant via milk.

In the rat brain and liver, the activity of cysteine sulfinic acid decarboxylase, which is the limiting factor for the regulation of taurine biosynthesis, is low during the fetal and neonatal periods (14, 15). The activity of cystathionase in the rat brain is low throughout all stages of development (16). Moreover, no activity of cysteine dioxygenase is detected in the fetal liver (17). These facts suggest that the ability to synthesize taurine is limited to the early development of the rat brain and liver. As reported here, the high concentrations of taurine in the fetal rat liver and brain, where taurine is not synthesized, are striking. In the fetal liver and brain, taurine was present in higher concentrations than any other ninhydrin positive compound. In the rat liver, taurine increased during the fetal period, reaching adult levels on the first postnatal day.

The only known function of taurine in the liver is conjugation with bile acids to form predominantly taurocholic acid (2). In the rat, the fetal hematopoietic tissue in the liver is replaced by adult parenchymatous tissue within a few days of birth (18). Coinciding with the histological changes of biliary canalicular proliferation and maturation, which are observed during the late fetal and early neonatal periods, taurocholate secretion begins during the period between the 19th and 21st day of gestation, and continues to develop during late gestation and the first day after birth (19). Developmental changes in hepatic taurine in the rat are perhaps consistent with the taurocholate secretion in the fetal liver.

In the mature brain, taurine may function

as a neurotransmitter or neuromodulator (20, 21), and it has been implicated in epilepsy, both in animal models and in man (22, 23). The concentration of taurine in the brain of the fetal and neonatal rat was approximately 2-fold of that in the mature brain. Brain growth in the rat is greatest during the period between 17th days of gestation and the 5th day after birth (24). This period corresponds with that during which the taurine concentration is high. Higher concentrations of taurine in the fetal and neonatal brain tissue than in the corresponding adult tissue have been observed in other mammalian species (6, 25). In these mammalian species, the decrease in the concentration of taurine in neonatal and infant brain correlated highly with age, reaching concentrations similar to those found in the mature brain at approximately the end of weaning (6). The high concentration of taurine in fetal brain and its slow postnatal decrease suggest that taurine may be associated in some way with brain development *per se*, in addition to any functional role it may play in the mature brain.

As a result of the accumulation of taurine in the fetal liver and brain, the total taurine content of rat fetuses increased rapidly during fetal development. Taurine was the most abundant ninhydrin positive compound in the rat fetus during fetal development. On the 21st day of gestation, 30.7 μmoles of taurine was present in one fetal body. Incidentally, the total taurine per liver of nonpregnant rats was 46.7 μmoles. On the 21st day of gestation, the fetal body pool of taurine was thus approximately equal to the taurine pool of nonpregnant rat livers. Taurine, which is not incorporated into proteins and is the end product of sulfur-containing amino acids, accumulates in the rat fetus like any other essential amino acid. From these observations, it would appear that taurine plays an important role during fetal development.

In the rat placenta, taurine is present in the highest concentration of all ninhydrin positive compounds, and it increases during pregnancy. In the human term placenta, the most abundant placental free amino acid is taurine, present in concentrations 40% higher than glutamic acid, the amino acid present at the next highest concentration (26). The reason for the high concentration of taurine in the placenta remains an enigma. It may be the result of the transport of taurine from the mother to the fetus, or taurine may have a role in placental metabolism.

A large quantity of taurine accumulates in the fetal organs and placenta during pregnancy. The question arises regarding the source of taurine in the fetus and placenta. As previously mentioned, the ability to synthesize taurine is limited to fetal rat organs. Taurine can cross the placenta. In the rhesus monkey, for example, taurine is efficiently concentrated in the fetal circulation in spite of any change in the maternal plasma concentration that might occur postprandially (27). Other data indicate that the radioactivity from maternally injected ^{35}S-taurine is transferred to the fetus in both pregnant rodents and primates (28, 29). These findings indicate that taurine is transported through the placenta, and it is presumed to be transported by the β-amino acid transport site like β-alanine (2). Therefore, the hypothesis is advanced that the source of the high concentration of taurine in the fetus is the mother. Taurine must be transferred to the fetus from the mother via the placenta.

From our observations, the concentrations of taurine in the pregnant rat liver and skeletal muscle significantly decreased in the late period of pregnancy. Moreover, urinary taurine excretion of pregnant rats decreased during pregnancy. These findings suggest that the pregnant rat minimizes the loss of taurine by decreasing urinary excre-

tion. The maternal liver, which is the main producing site of taurine, and the maternal muscle, which contains 75% of the total body taurine, may be the source of the high concentration of taurine in the fetus (2).

In conclusion, our results tend to confirm that a large quantity of taurine accumulates in the rat fetus during development, and the taurine pool of the maternal rat liver and muscle may be the source of the fetal taurine pool. The physiological significance of taurine during this period of rapid fetal rat growth is not known, but it seems to be associated with many important physiological systems that deserve continued study.

Acknowledgments. The author is deeply indebted to professor Toshihiko Ubuka, Department of Biochemistry, Okayama University Medical School, for his suggestions on this study.

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