TISSUE DISTRIBUTION AND METABOLIC FATE OF $^{35}$S FROM
$^{35}$S-TAURINE IN PREGNANT MICE BY WHOLE-BODY
AUTORADIOGRAPHIC AND
BIOCHEMICAL STUDY

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Tissue distribution and metabolic fate of $^{35}$S from $^{35}$S-taurine in pregnant mice were studied by whole-body autoradiography and biochemical analysis. Whole-body autoradiography was performed at 30 min, 3 and 6 hr after intravenous injection of $^{35}$S-taurine. In the maternal body, high densities were observed in the kidney, liver, bile and digestive tract, while low densities were in the blood and brain. The placenta showed a relatively high density, but the fetus was low density.

As for the biochemical analysis, the mice were decapitated at the same intervals after injection as those for autoradiography and various tissues were removed. These tissues were fractionated into 6% perchloric acid-soluble, -insoluble and lipid fractions. Total radioactivities (cpm) per wet weight (g) were high in the liver, small intestine and kidney and low in the brain, blood and fetus. These data were consistent with those of whole-body autoradiographs. The greatest part (over 98%) of total radioactivities in all tissues was incorporated into the acid-soluble fraction.

The acid-soluble fraction of each tissue was analyzed by thin-layer chromatography. The radioactive spots were examined for taurine and taurocholic acid in the bile, but only for taurine in all other tissues.

The sulfonic amino acid taurine (2-aminoethanesulfonic acid) is biosynthesized in the body from cysteine via cysteine sulfinate (CSA) and hypotaurine, or ingested in the diet. Taurine is a normal constituent of mammalian tissues and is found in particularly high concentrations in excitable tissues such as brain, muscle, and heart, and in the retina (11, 13).

Taurine has several significant roles in some organs and tissues of mammals. Clinically, alterations in taurine content and taurine transporting ability have produced congestive heart failure, genetic cardiomyopathy, epilepsy and ataxia (1, 5, 9). Cats fed a taurine-free diet suffered retinal degeneration and blindness (22, 23). Pharmacologically, taurine has been shown to be antiarrhythmic (21) and inotropic in the heart (7), to be a powerful anticonvulsant agent in experimental epilepsy (10), and to be a sperm motility factor (16). But the mode of its action has not been sufficiently elucidated. Furthermore, there is no definite information concerning the physiological action of taurine, except that taurine enters the bile as a conjugate with cholic acid. But it has recently been shown that taurine may have a functional role as
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a neuromodulator or neurotransmitter in the mature brain (29).

During gestation and development, the activities of cysteine dioxygenase and CSA decarboxylase, which are responsible for synthesis of taurine, were measured and it was found that the activity of cysteine dioxygenase in the maternal liver increased during the late gestational stage, while in fetus and neonate the activities of these enzymes were absent or quite low (2, 8). On the contrary, taurine is extremely high in mammalian milk (20, 30) and a high concentration of taurine was shown in the fetal and neonatal serum and brain (28, 29). It is reasonable to suppose that taurine is synthesized in maternal liver and transferred to the fetus via the placenta and to the neonate via milk (8), and may play a significant role in the fetal and neonatal development (25).

In the present investigation, tissue distribution, transference to fetus, and metabolic fate of radioactive sulfur from $^{35}$S-taurine after intravenous injection were examined in pregnant mice by using whole-body autoradiography and biochemical analysis.

**MATERIALS AND METHODS**

**Animals:** Eighteen albino mice (ICR strain, weighing approx. 60 g) on the 18th day of pregnancy were used in this study. The animals were allowed a normal laboratory diet (CE-2, CLEA JAPAN, INC.) and water ad libitum.

**Labeled compound:** $^{35}$S-taurine (specific radioactivity 38 mCi/mmol) was purchased from Amersham International Limited, England. The radiochemical purity was more than 95% by thin-layer chromatography. The isotope was dissolved in physiological saline (150 μCi/ml) after blow-drying with nitrogen gas.

**Whole-body autoradiography:** Nine mice were injected intravenously with 30 μCi of $^{35}$S-taurine in 0.2 ml of physiological saline. At 30 min, 3 and 6 hr after injection, three groups of three animals each were anesthetized with ethyl ether and then frozen in a mixture of dry-ice and hexane. The frozen animals were embedded in 6% (w/v) carboxymethyl cellulose paste. Whole-body sagittal cryosections (25 μm thick) of animals were cut with a cryomicrotome (LKB cryostat 2258-PM, Sweden). Whole-body autoradiography were performed in the manner described previously (24, 36). As a section supporter, Scotch tape (Type 810, Minnesota mining & Manufacturing Co., USA) was used. The sections adhering to the tape were freeze dried, and then applied to Medical X-ray films (Fuji Photo Co., Japan) in a dark room. After exposure in a dark box for 7 days, the films were developed by Rendol (20°C) for 5 min and fixed by Fujifix (20°C) for 20 min. Optical densities of the autoradiographs were measured with a microdensitometer (PDM-5 Sakura microphotometer, Konishiroku Photo. Co., Japan) (38). The densitometrical value of the autoradiograph was expressed as autoradiographic density (AD), and AD value 0 was represented by the density of the background of X-ray film. Standard deviations in the present work were within 10%. Some H-E stained whole-body sections were also prepared for tissue identification (26).

**Biochemical analysis:** Nine mice were injected intravenously with 30 μCi of $^{35}$S-taurine in 0.2 ml of physiological saline. At 30 min, 3 and 6 hr after injection, three groups of three animals each were decapitated and the following organs were immediately removed; the blood, brain, salivary gland, Harderian gland, thymus,
myocardium, liver, pancreas, spleen, kidney, stomach, small and large intestines, bile, skeletal muscle, mammary gland, placenta and fetuses. These organs were stored in a deep freezer (−80°C) until use. The samples were weighed and homogenized in ice-cold 6% perchloric acid with an Ultra-turrux disperser (Yamato Scientific Co., Japan). After centrifugation at 8,000 × g for 30 min at 4°C, the sediments were rehomogenized in 2.0 ml of 6% perchloric acid and recentrifuged as described above. The supernatants obtained after centrifuging twice were combined and the residues were dissolved in 2.0 ml of 1N sodium hydroxide. An equal volume of chloroform-methanol (2 : 1, v/v) was added to the supernatants and the sodium hydroxide solution of the residues, respectively. The mixture was vigorously shaken and centrifuged at 1,500 × g for 15 min at 4°C in order to separate lipids, and then the chloroform layer of the mixture was obtained. The radioactivity in an aliquot (0.2 ml) of each fraction (the perchloric acid-soluble, -insoluble and lipid fractions) was measured using an Aloka liquid scintillation counter. All calculations of radioactivity measurement allowed for decay of 35S (half-life 87 days) and were referred to the day of measurement of the specific radioactivity of the 35S-taurine.

Thin-layer chromatography: The acid-soluble fraction of each organ was analyzed by thin-layer chromatography. The acid-soluble fraction was neutralized with 40% (w/v) KOH, and the pH was adjusted by using indicator paper. After sedimentation (overnight), the precipitated KClO₄ was discarded. Ascending chromatography on a plate coated with 0.25 mm silica gel G was performed in a developing system containing isopropyl alcohol : folic acid : water (8 : 1 : 1, v/v/v). The developed area of the plates had a radius of 10 cm. After developing and drying, horizontal scratches were made in the silica gel on the plate 3 mm apart, and the radioactivity was measured with an Aloka liquid scintillation counter (6, 27). Radioactivity of each peak on the chromatogram was corrected with the original radioactivity (cpm/gram of wet weight) of the acid-soluble fraction of each organ.

RESULTS

Whole-body autoradiography

In the maternal bodies, at 30 min after intravenous injection of 35S-taurine, high densities were observed in the kidney, liver and digestive tract (corpus of stomach, small and large intestines) (Fig. 1a). AD values of these organs were over 0.90 AD (Fig. 2). These organs showed a decrease of AD values with time, except for the stomach where AD values gradually increased. In the kidney, the outer zone of the medulla showed a much higher density compared with other regions of the kidney throughout the experimental periods, particularly at 3 to 6 hr (Fig. 1b). The bile showed a marked increase of AD value at 30 min to 3 hr, and retained a high AD value at 6 hr (Figs. 1a-c, 2). The blood and brain showed very low densities, being less than 0.20 AD, throughout the experimental periods (Figs. 1a-c, 2).

In the placenta, a relatively high AD value (0.77 AD) was observed at 30 min after injection, but the AD value decreased gradually with time (Figs. 1b–c, 2). The AD values in the fetal organs and tissues were significantly low at 30 min after injection, but the uptake of the radioactive sulfur was observed in the liver, intestine, kidney, myocardium, retina and nasal mucosa at 3 to 6 hr (Figs. 3a–e, 4).
Whole-body autoradiographs of 18th-day pregnant mice at 30 min (a), 3 hr (b) and 6 hr (c) after i.v. injection of 35S-taurine. ×1.1. Note the high densities in the kidney, liver, stomach, small and large intestines. Bile showed a low density at 30 min, but a high density at 3 and 6 hr.
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Biochemical analysis

a) Fractionations

Organ samples obtained from the pregnant mice injected with $^{35}$S-taurine were fractionated into the acid-soluble, -insoluble and lipid fractions. Table 1 shows the total radioactivity (cpm) per gram of wet weight in each organ at each survival period after injection. At 30 min, high radioactivities were seen in the liver, small intestine and kidney. These organs showed a marked decrease in activity with time. The Harderian gland, salivary gland and pancreas had a peak in radioactivity at 3 hr. On the other hand, levels of radioactivities in the brain, blood and fetus were low during the entire period of this experiment.

The incorporation rates [(radioactivity (cpm) of each fraction/total radioactivity (cpm) in each organ) × 100] of the radioactivity into the acid-soluble fraction were very high (over 98%) in all organs for every experimental period.
Figs. 3a–e. Enlarged autoradiographs of the fetuses at 30 min (a), 3 hr (b, c) and 6 hr (d, e) after i.v. injection of $^{35}$S-taurine. Note the low density of the fetal tissues and organs at 30 min and relatively high densities in the liver, intestine, myocardium, retina and nasal mucosa at 3 and 6 hr. $\times 2.0$

b) Thin-layer chromatography

Figures 5a–c show the thin-layer chromatograms of the acid-soluble fractions of the liver, kidney and bile. As markers, taurine, isethionic acid and taurocholic acid were used. As shown in Figs. 5a–b, the liver and kidney had a radioactive spot only for taurine and the level of radioactivity was highest at 30 min. The other organs, except for the bile, showed similar results as the liver and kidney. In the bile (Fig. 5c), the radioactive spots were detected for taurine and taurocholic acid at 30 min to 6 hr. Radioactivity of taurocholic acid was about 2 to 3 times higher than that of taurine.

DISCUSSION

Tissue distribution, transference to fetus and metabolic fate of radioactive sulfur from $^{35}$S-taurine in various organs and tissues of pregnant mice were examined by whole-body autoradiography and biochemical analysis.

The thin-layer chromatograms of the acid-soluble fractions of almost all organs ex-
Fig. 4. AD values of organs in fetuses of pregnant mice at 30 min, 3 and 6 hr after i.v. injection of $^{35}$S-taurine. White columns: 30 min, hatched columns: 3 hr, black columns: 6 hr. Data are means for 3 animals.

TABLE 1. Total radioactivity (cpm) per gram of wet weight in each organ at 30 min, 3 and 6 hr after i.v. injection of $^{35}$S-taurine. Data are means for 3 animals.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Time after iv injection</th>
<th>30 min</th>
<th>3 hour</th>
<th>6 hour</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>× 10^{-3} cpm/g-wet weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>blood</td>
<td></td>
<td>43</td>
<td>52</td>
<td>35</td>
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<tr>
<td>brain</td>
<td></td>
<td>69</td>
<td>63</td>
<td>56</td>
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<tr>
<td>salivary gl.</td>
<td></td>
<td>308</td>
<td>415</td>
<td>327</td>
</tr>
<tr>
<td>Harderian gland</td>
<td></td>
<td>1224</td>
<td>1395</td>
<td>1167</td>
</tr>
<tr>
<td>Liver</td>
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<td>1904</td>
<td>1551</td>
</tr>
<tr>
<td>kidney</td>
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<td>1829</td>
<td>1354</td>
<td>968</td>
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<tr>
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<td>514</td>
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<tr>
<td>fetus</td>
<td></td>
<td>11</td>
<td>27</td>
<td>23</td>
</tr>
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</table>
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amind, except for the bile, indicated a radioactive spot only for taurine (Fig. 5a-b). These data clearly show that almost all of the silver grains (radioactivity) on the whole-body autoradiograph reveal $^{35}$S-taurine and not radioactive metabolites of this sulfur-containing amino acid. The rate of turnover of taurine is extremely slow in various animals. The half-life of taurine is 18.6 days in mouse, 15 days in human heart (13), 7 days in rat skeletal muscle and several days or more in rat visceral organs and brain (12).

High AD values at 30 min after injection were observed in the liver and kidney, but AD values of these organs decreased with time (Figs. 1a-c, 2). These results in the autoradiographs were consistent with those obtained by biochemical analysis (Table 1). The liver is thought to be one of the main organs in taurine metabolism, since the activities of cysteine dioxygenase and CSA decarboxylase, which are responsible for conversion of cysteine to taurine, were highest in the liver among various rat organs (39). The only known function of taurine in liver is conjugation with bile salts to form predominantly taurocholic acid. In the present work, this is confirmed by the detection of radioactive spots for taurine and taurocholic acid with TLC in the acid-soluble fraction of the bile (Fig. 5c). In the bile, AD value was low at 30 min, followed by a marked increase of AD value at 3 hr to 6 hr (Figs. 1a-c, 2). However, in the biochemical analysis, the radioactivity in the bile was constant at relatively high level throughout the experimental period (Table 1). This discrepancy between autoradiographic and biochemical data at 30 min is not understood. According to Iwata et al. (14), the excretion of radioactivity in bile following oral administration of $^{35}$S-taurine in rats increased with the passage of time, and taurine was excreted as the conjugates with cholic acid, muricholic acid or dihydrocholic acid.

In the rat kidney, the intravenously injected $^{35}$S-taurine was absorbed from the blood at a faster rate than various other organs, such as liver, heart and intestine etc. The maximum appeared at 15 min after the injection and then the concentration began to decrease (3). In the present study, the incorporation rate of $^{35}$S into the acid-soluble fraction of the kidney was very high and a radioactive spot only for taurine was detected by TLC (Fig. 5b). These results were supported by the fact that taurine is non-metabolizable in the renal cortex of mouse and rat and is free within the cell after uptake without being incorporated into a cell protein fraction (4). Portman and Mann (19) showed that the radioactivity in protein-free extracts of kidney and liver from rats 24 hr after injection with $^{35}$S-taurine was largely accounted for as taurine.

In the kidney, a much higher density was observed in the outer zone of medulla consisting of the rectal part of the proximal tubule, the loop of Henle, the rectal part of the distal tubule and the collecting duct (Fig. 1b). The function of these sites is the reabsorption of Na$^+$, Cl$^-$ and H$_2$O. It is, therefore, assumed that taurine may be closely related to the osmotic regulation. Thurston et al. (34, 35) suggested that taurine was involved in the osmoregulation of the mammalian heart and brain.

In the blood, AD value was very low throughout the experimental periods (Figs. 1a-c, 2). Similar results were reported by Moriyama et al. (18): when a pregnant rat

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Figs. 5a–c. Thin-layer chromatograms of the acid-soluble fractions of the liver (a), kidney (b) and bile (c). The radioactivity of each peak on the chromatogram was corrected with the original radioactivity (cpm/g wet weight) of the acid-soluble fraction of each organ. TLC was performed on silica gel G with a solvent system of isopropyl alcohol : folic acid : water (8 : 1 : 1).
was injected with $^{14}$C-taurine, the radioactivity in the maternal blood decreased markedly between 3 to 15 min after injection and retained a low level thereafter. These findings indicate that the radioactivity injected into the blood is transferred rapidly to such organs as the liver, kidney, intestines etc.

The AD value of the small intestine was high at 30 min and decreased gradually thereafter (Figs. 1a–c, 2). According to Kimura et al. (15), in the rat the absorption of taurine was negligible in the stomach and large intestine and was rapid in the small intestine. But the corpus of the stomach and large intestine showed high AD values in the present study (Figs. 1a–c, 2).

The maternal nasal mucosa had a relatively high AD value at 30 min after injection and retained the same level of AD value during the entire experimental period (Fig. 2). In the fetus, the nasal mucosa showed a higher AD value than other fetal tissues at 3 to 6 hr (Figs. 3b, e, 4). Lindquist et al. (17) showed that using whole-body autoradiography of $^{14}$C-taurine in pregnant and adult male mice, a high concentration was observed in the adult and fetal nasal mucosa at 20 min to 24 hr after injection followed by accumulation in the olfactory bulb at longer survival times. They speculate that taurine is taken up by the nasal mucosa and transported to the olfactory bulb and that taurine is incorporated into a peptide, which may, as carnosine, be a neurotransmitter or neuromodulator in the olfactory system. Sturman et al. (32) observed high uptake of $^{35}$S-taurine in the olfactory bulb of the kitten and suggested that taurine may play a special role in the olfactory bulb. But in the present investigation, AD values in the brain, containing olfactory bulb, were significantly low (Figs. 1a–c, 2).

It is well known that in rat placenta, taurine was present in higher concentration than any other amino acid and its concentration increased significantly with the increase in the age of the fetus (2). But it is not clear whether taurine fulfills any function in the placenta or not. It is also known that the radioactivity from maternally injected $^{35}$S-taurine is transferred to the fetus in both the pregnant rat and monkey (31, 33). Stegink et al. (28) speculated that taurine should be able to cross the placenta because taurine is able to cross the blood-brain barrier (BBB) in adult and neonate animals (3, 30, 37) and most substances that cross the BBB also transverse placental capillary walls. In the present investigation, AD value was relatively high in the placenta at 30 min, but very low in the fetal organs and tissues (Fig. 3a). At 3 to 6 hr, some fetal tissues, such as the liver, intestine, kidney, myocardium, retina and nasal mucosa, showed a slight increase of AD values (Figs. 3b–e, 4). These autoradiographic results were coincident with those of biochemical analysis (Table 1). These data may indicate that $^{35}$S (mostly in the form of taurine) in the placenta taken up from the blood is accumulated in large amounts and small amounts are transferred to various fetal tissues. As for the fetal tissues and organs, the following metabolic patterns might be suggested. Since no activity of cysteine dioxygenase, the enzyme responsible for synthesis of taurine from cysteine, has been observed in the fetal liver, it is difficult to consider that during the embryonic period the activity of this enzyme is high in other tissues and organs (8). From these data, it is reasonable to postulate that taurine carried from the maternal blood may be accumulated in the placenta and form a large pool there. As need demands, taurine may be transferred to the fetus through the placental barrier and distributed to each fetal organ which may demand taurine.
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