Fate of ^{14}C from [1-^{14}C] cystine and ^{35}S from [^{35}S] cysteine in Pregnant Mouse; A Whole-body Autoradiography and Its Biochemical Analysis

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SUMMARY

Metabolic fate of ^{14}C from [1-^{14}C]cystine and ^{35}S from [^{35}S]cysteine, both precursors of taurine, in various tissues and organs of pregnant mice were studied by biochemical and whole-body autoradiographic analyses.

Pregnant mice (17th day of gestation) injected intravenously with the tracers were sacrificed at 30 min, 3 and 6 hour. Total radioactivities (cpm/g • wet weight) of ^{14}C and ^{35}S were high in the kidney, pancreas, Harderian gland and stomach, while low in the brain, skeletal muscle and fetus. The liver and bile showed high radioactivities in ^{35}S, but low radioactivities in ^{14}C. These results were consistent with the autoradiographic results. In the case of fetus, high optical densities of ^{14}C were observed in the lens, and ^{35}S in the cartilage and lens. The radioactivities (cpm/g • wet weight) taken up by the acid-insoluble fraction were high in the pancreas, while low in the liver, kidney and skeletal muscle. By thin-layer chromatography of the acid-soluble fractions, the radioactive spots for cysteine, cysteine sulfinic acid and taurine were detected in the liver, kidney and pancreas.

INTRODUCTION

Recently, taurine has become several significant physiological roles in some tissues. In addition, it has become apparent that taurine is an essential amino acid in fetal and neonatal mammalian development. However, there are no definite information in the physiological and biochemical properties for taurine, except that taurine enters the bile as conjugate with cholic acid. It is generally accepted that cysteine is catabolized mainly to taurine via cysteine sulfinic acid (CSA) and hypotaurine. ^{14}C from [1-^{14}C]cystine could be eliminated from the tissue as $^{14}CO_2$ by the action of CSA decarboxylase, whereas ^{35}S from [^{35}S]cysteine is metabolized to [^{35}S]taurine. According to Yamaguchi *et al.*[1], 30% of cysteine might be metabolized via pyruvate pathway and 70% via taurine pathway. Once cystine has been introduced into tissue in vitro, it is reduced to cysteine in a short time[2].

In our previous studies, tissue distribution of radioactive materials from [^{35}S]taurine in adult rats[3] and from [1-^{14}C]cystine and [^{35}S]cysteine in pregnant mice[4] were investigated by the whole-body autoradiographic method.

In the present work, to obtain further information on functional role of taurine, the metabolic fate of radioactive materials from [1-^{14}C]cystine and [^{35}S]cysteine, both precursors of taurine, in various tissues and organs of pregnant mice were studied by the biochemical analysis and their results were compared with the autoradiographic data.

EXPERIMENTAL

Albino mice (ICR strain, weighing approx. 60 g) on the 17th day of gestation were used in this study. As labeled compounds, L-[^{35}S]cysteine (S.A. 1010 Ci/mmol) and DL-[1-^{14}C]cystine (S.A. 74 mCi/mmol) were used.

Whole-body autoradiography: Pregnant mice were injected intravenously with 50 μCi/mouse of [^{35}S]cysteine and 12.5 μCi/mouse of [1-^{14}C]cystine, respectively. At 30 min., 3 and 6 hour after injection, each of three mice was frozen with dry-ice hexane. Autoradiography of whole-body sagittal cryosections (30 μm thick) was performed in the manner described previously[5,6]. Optical density (OD) of each autoradiograph was measured with a microdensitometer.

Biochemical analysis: Pregnant mice were injected intravenously with 30 μCi/mouse of [^{35}S]cystine and 5 μCi/mouse of [1-^{14}C]cysteine, respectively. 30 min, 3 and 6 hour later, each of three mice was decapitated, and 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 were immediately removed. Ice-cold 6% perchloric acid-soluble, -insoluble and lipid fractions of each organ were obtained by the method described previously[7]. The radioactivity (cpm/g • wet weight) of each fraction was measured using a liquid scintillation counter. As for [^{35}S]cysteine, all calculations of radioactivity measurement allowed for decay of ^{35}S (half-life 87 days).

Thin-layer chromatography (TLC): The acid-soluble fraction of each organ was further analyzed by TLC. Chromatography on a plate coated with Silica Gel G was performed in a developing system containing isopropyl alcohol-folic acid-water (8:1:1, v/v/v). After developing and drying, the silica gel on the plate was scratched up horizontally at 3 mm distance each and the radioactivity was measured with a liquid scintillation counter[7]. Raioactivity of each peak on a chromatogram was corrected with the original radioactivity (cpm/g • wet weight) of the acid-soluble fraction of each organ.

RESULTS

Whole-body autoradiography: In the dam, at 30 min. after i.v. injection of [1-^{14}C]cystine and [^{35}S]cysteine, the highest OD was observed in the pancreas and then the kidney, small and large intestines, stomach, Harderian gland, mammary gland, liver and salivary gland were followed (Fig. 1). These organs showed the decrease of ODs with time, particularly marked decrease in the pancreas and kidney, except for the Harderian gland and salivary gland having a peak of OD at 3 hour in [^{35}S]cysteine. The brain and skeletal muscle showed low ODs of ^{14}C and ^{35}S. The placenta gave slightly low ODs of ^{14}C and ^{35}S than those of the maternal blood at 30 min. (Fig. 1) and showed the decrease of ODs with time. In the case of fetus, ODs of ^{14}C and ^{35}S showed a peak at 3 hour or a gradual increase. High ODs of ^{14}C were observed in the lens, and ^{35}S in the cartilage and lens (Figs. 2, 3).

Biochemical analysis: Organ samples obtained from the pregnant mice after injection were

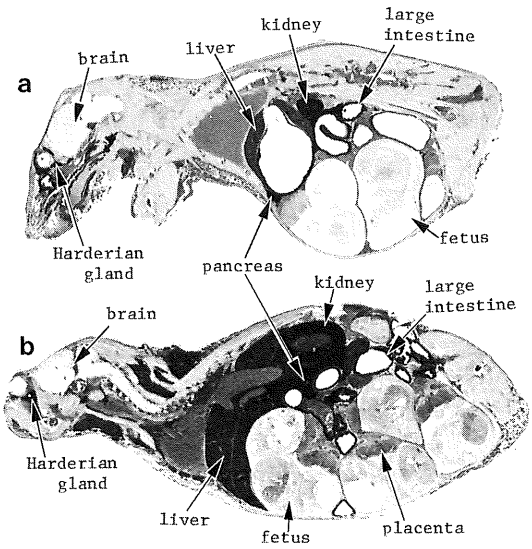

Fig. 1. Whole-body autoradiographs of 17th day pregnant mice at 30 min. after i.v. injection of [1-^{14}C]cystine (a) and [^{35}S]cysteine (b). The high density in the pancreas, kidney and Harderian gland, and low in the brain. ×1.3.

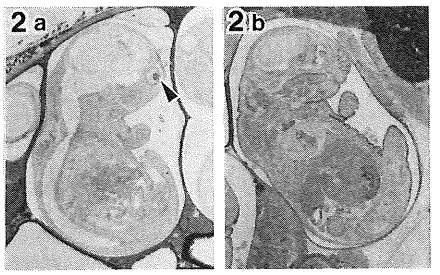

Fig. 2. Enlarged autoradiographs of the fetuses at 30 min. (a) and 6 hour (b) after i.v. injection of [1-^{14}C]cystine. The high density in the lens (arrow) at 30 min. after injection. ×2.7.

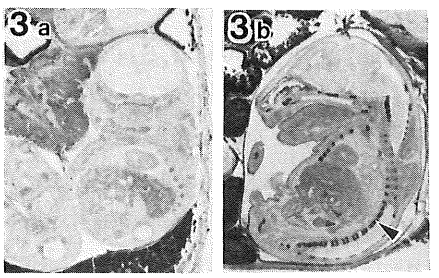

Fig. 3. Enlarged autoradiographs of the fetuses at 30 min. (a) and 6 hour (b) after i.v. injection of [^{35}S]cysteine. The high density in the cartilage (arrow) at 6 hour after injection. × 2.7.

fractionated into the acid-soluble, -insoluble and lipid fractions. Table 1 shows the total radioactivity (cpm/g • wet weight) of organ at each interval after injection of [1-^{14}C]cystine and [^{35}S]cysteine. At 30 min., the radioactivities of ^{14}C and ^{35}S were high in the kidney, pancreas, Harderian gland and stomach. The liver and bile were high in the radioactivities of ^{35}S, while low in ^{14}C. The radioactivities of ^{14}C and ^{35}S in these organs were decreased with time, except for that of the Harderian gland having a peak at 3 hour in ^{35}S, and that of the bile presenting a gradual increase of ^{14}C. On the other hand, the brain and fetus showed low radioactivities in ^{35}S and ^{14}C throughout the experimental periods, but in the fetus the radioactive peak was observed at 3 hour.

Table 1. Total radioactivity ($x10^{-3}$ cpm/g·wet weight) in each organ after i.v. injection of [1-^{14}C] cystine and [^{35}S] cysteine

Organs	[1-^{14}C] cystine			[^{35}S] cysteine		
	30 min.	3 hour	6 hour	30 min.	3 hour	6 hour
Brain	19	18	10	96	104	101
Salivary gland	145	123	88	1542	1562	2235
Liver	138	107	65	3560	3220	2532
Kidney	676	301	144	4878	3176	2479
Small intestine	161	148	96	1556	1405	2027
Large intestine	190	164	97	1347	1884	2026
Pancreas	1281	540	154	3111	1678	1686
Blood	146	71	34	530	520	275
Myocardium	106	70	31	807	814	653
Spleen	220	209	78	857	892	962
Harderian gland	478	344	212	2604	3200	2208
Stomach	306	280	211	2393	1614	1906
Thymus	172	169	59	1209	1031	824
Skeletal muscle	56	21	18	354	204	277
Placenta	96	75	29	441	761	511
Fetus	36	52	33	169	221	166
Mammary gland	100	67	57	310	665	386
Bile	72	92	105	2047	1235	1070

Data are means for three animals.

In order to compare with ^{14}C and ^{35}S radioactive data in different organs, we calculated the ratios in radioactivity of each organ relative to that of the blood, and presented them as relative ratio in this report (Table 2). In the liver, kidney, salivary gland, small and large intestines, the relative ratio of ^{35}S was significantly higher than that of ^{14}C, vice versa in the pancreas at 30 min. and 3 hour.

As shown in Table 3, the incorporation rates [(radioactivity (cpm) of each fraction / total radioactivity (cpm) in each organ) $\times$ 100] of ^{14}C and ^{35}S into the acid-insoluble fraction were high (over 50%) in the pancreas, stomach, large intestine and salivary gland. In contrast to these organs, the liver, kidney and skeletal muscle showed low incorporation rates into the acid-insoluble fraction and high rates (over 80%) into the acid-soluble fraction. In the pancreas, unlike other organs, the incorporation rates of ^{35}S and ^{14}C into the acid-insoluble fraction were decreased with time. The rates of ^{35}S and ^{14}C into the lipid fraction were markedly low (below 3%) in almost all organs at all intervals examined, while relatively high (15%) in the Harderian gland.

Thin-layer chromatograms of the acid-soluble fractions of the liver, kidney and pancreas were shown in Fig. 4. Cystine, cysteine, cysteine sulfinic acid (CSA), hypotaurine and taurine were used

Table 2. Relative ratio of the total radioactivity in each organ to that of the blood after i.v. injection of [1-^{14}C] cystine and [^{35}S] cysteine

Organs	[1-^{14}C] cystine			[^{35}S] cysteine		
	30 min.	3 hour	6 hour	30 min.	3 hour	6 hour
Brain	0.1	0.3	0.3	0.2	0.2	0.4
Salivary gland	1.0	1.7	2.6	2.9	3.0	8.1
Liver	0.9	1.5	1.9	6.7	6.2	9.2
Kidney	4.6	4.2	4.2	9.2	6.1	9.0
Small intestine	1.1	2.1	2.8	2.9	2.7	7.4
Large intestine	1.3	2.3	2.9	2.5	3.6	7.4
Pancreas	8.8	7.6	4.5	5.9	3.2	6.1
Blood	1.0	1.0	1.0	1.0	1.0	1.0
Myocardium	0.7	1.0	0.9	1.5	1.6	2.4
Spleen	1.5	2.9	2.3	1.6	1.7	3.5
Harderian gland	3.3	4.8	6.2	4.9	6.2	8.0
Stomach	2.1	3.9	6.2	4.5	3.1	6.9
Thymus	1.2	2.4	1.7	2.3	2.0	3.0
Skeletal muscle	0.4	0.3	0.5	0.7	0.4	1.0
Placenta	0.7	1.1	0.9	0.8	1.5	1.9
Fetus	0.2	0.7	1.0	0.3	0.4	0.6
Mammary gland	0.7	0.9	1.7	0.6	1.3	1.4
Bile	0.5	1.3	3.1	3.9	2.4	3.9

Data are means for three animals.

as the markers. As shown in Fig. 4a, the liver had radioactive spots of ^{14}C and ^{35}S for CSA at 30 min. and the amount of CSA decreased with time. At 3 and 6 hour, ^{35}S spot was detected for taurine. In the kidney (Fig. 4b), ^{14}C spots were CSA and cysteine, and these radioactive levels were decreased with time. ^{35}S spots were detected for CSA, taurine and cysteine at 30 min, and only for taurine at 3 and 6 hour. In the pancreas (Fig. 4c), the radioactive spots of ^{14}C and ^{35}S were detected for CSA, and its radioactivity of ^{14}C was decreased with time, while that of ^{35}S was constant throughout the experimental periods.

DISCUSSION

The total radioactivities (cpm/g • wet weight) of ^{14}C and ^{35}S were high in the kidney and pancreas at 30 min. after injection and markedly decreased with time. By contraries, high radioactivity of ^{35}S and low radioactivity of ^{14}C were observed in the liver at 30 min., and these activities decreased gradually thereafter. These results were consistent with those of the whole-body autoradiography.

In the liver, the incorporation rates of ^{14}C and ^{35}S into the acid-soluble fraction were significantly

Table 3. Incorporation rate of radioactivity into the acid-insoluble fraction of each organ after i.v. injection of [1-^{14}C] cystine and [^{35}S] cysteine

Organs	[1-^{14}C] cystine			[^{35}S] cysteine		
	30 min.	3 hour	6 hour	30 min.	3 hour	6 hour
Brain	16	28	30	23	25	29
Salivary gland	30	62	58	25	23	26
Liver	17	14	20	7	4	6
Kidney	9	19	35	10	7	12
Small intestine	39	44	47	16	20	22
Large intestine	46	55	62	30	30	23
Pancreas	67	55	34	50	43	31
Blood	24	59	74	14	8	20
Myocardium	19	44	55	11	9	14
Spleen	28	45	53	22	19	26
Harderian gland	25	40	40	25	10	17
Stomach	48	55	55	32	32	28
Thymus	27	50	61	14	25	28
Skeletal muscle	7	5	11	9	13	8
Placenta	18	47	52	12	25	36
Fetus	25	42	65	3	1	7
Mammary gland	31	46	54	12	18	20
Bile	3	29	24	5	9	17

The values represent percent of the radioactivity in the acid-insoluble fraction to the total radioactivity of each organ.

Data are means for three animals.

high (over 80%) at all intervals examined. On the TLC of the acid-soluble fraction of ^{35}S, a large amount of CSA was detected at 30 min. and taurine began to appear in large amount at 3 hour. From these results, it is suggested that synthetic rate from cysteine to taurine might be slow. In the paper chromatographic data of the rat liver reported by Awapara *et al.*[8], at 10 min. after i.v. injection of [^{35}S]cysteine, large amounts of cysteine had accumulated and taurine began to appear only at end of 30 min. However, the relative ratio of ^{35}S was much higher than that of ^{14}C at 30 min. This may show that a large amount of cysteine taken up by the liver was rapidly metabolized within 30 min. Namely, ^{14}C from [1-^{14}C]cystine was removed as $^{14}CO_2$ and ^{35}S from [^{35}S]cysteine was metabolized to [^{35}S]taurine. [^{35}S]taurine was then conjugated with cholic acid and present in the bile which showed relatively high activities at 30 min.

In the kidney, the incorporation rates of ^{35}S and ^{14}C into the acid-soluble fraction were significantly high (over 80%) at all intervals. On the TLC of acid-soluble fraction of ^{35}S, a large amount of cysteine, CSA and taurine was detected at 30 min. This indicates that cysteine taken up by the kidney might be metabolized to taurine, and eliminated into the urine. This is supported by the facts

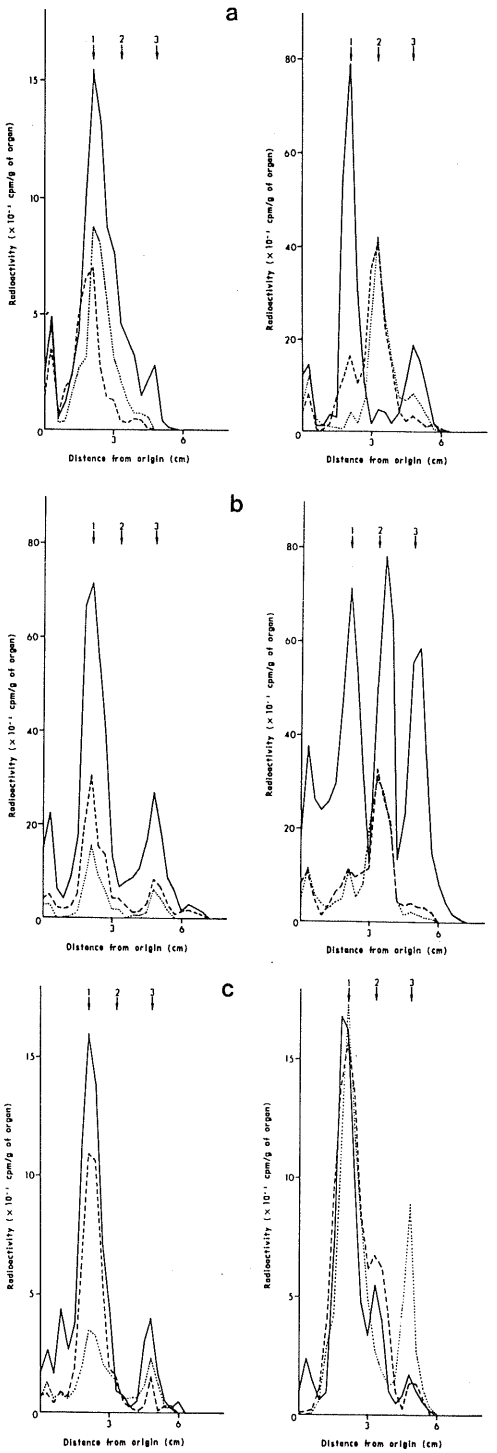

Fig. 4. Thin-layer chromatograms of the acid-soluble fractions of the liver (a), kidney (b) and pancrease (c) after i.v. injection of [1-^{14}C]cystine (left) and [^{35}S]cysteine (right). (——): 30 min, (------): 3 hour, (·····): 6 hour. Arrows indicate the markers; 1: CSA, 2: taurine, 3:cysteine.

that OD of ^{35}S in the urine were significantly high, and the radioactivity (cpm/g • wet weight) and OD of the kidney were markedly decreased with time. However, CSA decarboxylase of the mouse kidney shows high activity, while cysteine dioxygenase activity is negligible[1,9]. From these enzymic data, the high radioactivity in the kidney might be contributed to CSA and/or taurine metabolized in certain other organs such as the liver.

In the pancreas, the relative ratio of ^{14}C was significantly higher than that of ^{35}S within 3 hour after injection. This is consistent with the result of whole-body autoradiography. In addition, it was ascertained in the previous whole-body autoradiographic study[1] that the pancreas showed low OD of ^{35}S from [^{35}S]taurine. These findings suggest that ^{14}C and ^{35}S incorporated into the pancreas could be present as cysteine or CSA form. The incorporation rate into the acid-insoluble fraction was significantly high in the pancreas. It is conceivable, therefore, that ^{14}C and ^{35}S into the acid-insoluble fraction might be incorporated into the pancreatic juice. On the TLC of the acid-soluble fraction of ^{14}C and ^{35}S, radioactive spot was detected for CSA. Its radioactive amount of ^{35}S was constant throughout the experimental periods, while that of ^{14}C was decreased with time. Relevant to the Rf value of CSA and hypotaurine, little difference could be seen in TLC. Therefore, there is a possibility that most of the activity of ^{35}S for CSA could be hypotaurine.

In the salivary, Harderian and mammary gland, which secrete saliva, lipid and milk respectively, total radioactivity and OD of ^{35}S had a peak at 3 hour or 6 hour after injection, while those of ^{14}C decreased with time. These results may suggest that after decarboxylated in certain other organs such as the liver, a large amount of ^{35}S might be incorporated into these organs after 30 min.

Various fetal tissues and organs showed a peak at 3 hour in total radioactivity and in OD of ^{14}C and ^{35}S. In the fetal tissues, high ODs of ^{14}C were observed in the lens, and ^{35}S in the cartilage and lens. The liver, kidney, intestine, retina and myocardium showed relatively high ODs in ^{14}C and ^{35}S. In addition, little activities were observed in the fetal tissues after i.v. injection of [^{35}S]taurine and [1-^{14}C]CSA in pregnant mice (unpublished). Fetal cysteine dioxygenase and CSA decarboxylase activities have been reported to be significantly low[10]. From these findings, it is conceivable that ^{14}C from [1-^{14}C]cystine and ^{35}S from [^{35}S]cysteine might be transfered to fetus via placenta mostly as cysteine form, not as CSA or taurine.

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