WHOLE-BODY AUTORADIOGRAPHIC STUDY OF [U-¹⁴C] GALACTOSE IN MICE AND ITS BIOCHEMICAL ANALYSIS¹

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Tissue and organ distribution and metabolic fate of radioactive carbon from [U-¹⁴C] galactose in mice were studied by whole-body autoradiography and biochemical analysis.

Autoradiography was performed before and after 6% perchloric acid treatment at various intervals after intravenous injection of [U-¹⁴C] galactose. At 5 min after injection, high radioactivities were observed in the kidney, small intestine and liver. The radioactivities in almost all organs were markedly decreased with the acid treatment. At 30 min after injection, the kidney and small intestine had relatively high radioactivities which were retained by the acid treatment.

As for the biochemical analysis, at various intervals after intravenous injection of [U-¹⁴C] galactose, the mice were decapitated, and the blood, liver, kidney, small and large intestines, myocardium and brain were removed. These organs were fractionized into 6% perchloric acid-soluble, -insoluble and lipid parts. Total radioactivity (cpm) per gram of wet weight was the highest in the kidney. The incorporation rates of the radioactivity into the acid-soluble fraction were high in all organs at early intervals, while at late intervals, the rates into the acid-insoluble fraction became relatively high in the kidney, small and large intestines. These results were consistent with those obtained from whole-body autoradiographs.

Acid-soluble fractions of the blood, kidney, liver and brain were analyzed by paper chromatography. The radioactive spots for galactose and glucose were observed in all organs examined, but the changes of radioactive values with the passage of time were different in each organ. Furthermore, the radioactive spots for R_{Glucose} value 0.28 were detected in these organs.

Galactose is C-4 epimer of glucose and is derived from lactose. Ingested lactose is absorbed in the small intestine and then hydrolyzed by β -galactosidase (lactase) into glucose and galactose. It is generally accepted that galactose is conveyed to the liver, where it is converted to glucose by the Leloir pathway (18). In the previous studies, enzyme activities belonging to this pathway have been found in the liver (3, 5, 8, 33), kidney (3, 5, 8, 16, 20, 25, 34), intestine (23), brain (21, 32, 40) and myocardium (21) etc. On the other hand, it has been reported that galactose is an important constituent of the glycoproteins, glycolipids and mucopolysaccharides (1, 4, 10, 13, 14, 15, 22).

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In the previous paper (30), the distribution of radioactive carbon of [U- ^{14}C] galactose in mice was investigated by whole-body autoradiography, which was performed before and after 6% perchloric acid treatment of cryosection.

Therefore, in order to elucidate the metabolic fate of radioactive carbon from [U- ^{14}C] galactose after intravenous injection, biochemical analyses were employed for paper chromatography in the present work.

MATERIALS AND METHODS

Animals: Twenty four adult male albino mice of the ICR strain (22 to 25 g) were used in this study. The animals were fed a normal laboratory diet (CE-2, CLEA JAPAN, INC.) *ad libitum*.

Labeled compound: D-[U- ^{14}C] galactose (specific radioactivity, 95.0 and 57.0 mCi/mmol) was purchased from the Amersham International Limited, England. The radiochemical purity was more than 99% by paper chromatography.

Whole-body autoradiography: Nine mice were injected intravenously with 5.0 μCi of D-[U- ^{14}C] galactose in 0.2 ml of physiological saline. At 5, 15 and 30 min after injection, each of the three animals were anesthetized with ethyl ether and frozen in dry-ice acetone. Whole-body sagittal cryosections of animals were cut with a cryomicrotome (LKB 2250, Sweden) (29, 35). Autoradiography was performed before and after 6% perchloric acid treatment of sections (designated "untreated" and "treated autoradiograph") in the manner described before (41).

The optical densities of autoradiographs were measured with a microdensitometer (PDM 5, Konishiroku Photo Co., Japan) (42). Densitometrical values of autoradiographs were corrected with ^{14}C standards (set number C864, CFR. 10, Amersham, UK) and corrected values were expressed as autoradiographic density (AD). AD value 0 was represented by the blank of ^{14}C standards (30).

Some H-E stained whole-body sections were also prepared for tissue identification of autoradiographs (31).

Biochemical analysis: Fifteen mice were injected intravenously with 5.0 μCi of D-[U- ^{14}C] galactose in 0.2 ml of physiological saline containing 50 mg of cold D-galactose.

In order to observe the changes of radioactivity of ^{14}C with the passage of time after intravenous injection of [U- ^{14}C] galactose in more detail, the survival intervals were set up at 1, 5, 15, 30 and 60 min. Namely, at 1, 5, 15, 30 and 60 min, each of the three animals were decapitated and the blood, liver, small and large intestines, myocardium and brain were immediately removed. They were stored in a deep freezer (-80°C) until use. The samples were weighed and homogenized vigorously with an Ultra-turrax dispenser (IKA-WERK, West Germany) in 5 volumes of ice-cold 6% (v/v) perchloric acid. After centrifugation at $8,000 \times g$ for 15 min at 4°C , the sediments were rehomogenized in 1.0 ml of 6% perchloric acid and re-centrifuged as described above. Both supernatants were combined and the residues were dissolved in 1.0 to 2.0 ml of 1 N 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 centrifuged at $600 \times g$ for 5 min at room temperature 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 a Beckman liquid scintillation counter.

Paper chromatography: The acid-soluble fraction of the blood, kidney, liver and brain, which were removed at 1, 5, 15, 30 and 60 min after injection, was analyzed by paper chromatography. The acid-soluble fraction was neutralized with cold 40% (w/v) KOH, and the pH was adjusted by using indicator paper. After sedimentation (overnight), the precipitated KClO_4 was discarded (37, 38). Ascending chromatography on a Toyo No. 51B strip was carried out and ethyl acetate-pyridine-water (12: 5: 4, v/v/v) was used as the solvent system. In each sample, 10,000 cpm was spotted to the strip. Chromatograms were developed for 18 hr. The strips were cut horizontally at 3 mm distance each and the radioactivity was measured with a Beckman liquid scintillation counter (11). Original total acid-soluble fraction of each organ was diluted with distilled water for setting the constant radioactivity (10,000 cpm) for paper chromatography, as described above. Radioactivity of each peak on paper chromatogram thus obtained was corrected with the original radioactivity of the acid-soluble fraction of each organ.

RESULTS

Whole-body autoradiography

At 5 min after intravenous injection of $[\text{U-}^{14}\text{C}]$ galactose, the highest radioactivity was observed in the kidney (Fig. 1a). The activity in the urine, small intestine and liver was also high, while in the brain the activity was low (Fig. 1a).

Figs. 1a, b. Untreated (a) and treated (b) autoradiographs of mouse at 5 min after i.v. injection of $[\text{U-}^{14}\text{C}]$ galactose. Black-rimmed photographs on right side show the kidney. $\times 1.0$

Note a high AD in the kidney, liver and small intestine of the untreated autoradiograph, and significant low ADs of the treated autoradiograph as compared with that of the untreated one.

FIG. 2. Untreated autoradiograph of mouse at 15 min after i.v. injection of $[U-^{14}C]$ galactose. $\times 1.0$
Note a high AD in the kidney and a relatively high AD in the liver, small intestine and Harderian gland.

FIGS. 3a, b. Untreated (a) and treated (b) autoradiographs of mouse at 30 min after i.v. injection of $[U-^{14}C]$ galactose. Black-rimmed photographs on right side show the kidney. $\times 1.0$
Note a high AD in the urine and a relatively high AD in the kidney and the small intestine of the untreated autoradiograph, and a retained higher AD in the kidney and the small intestine of the treated autoradiograph as compared with those of other organs and tissues.

FIGS. 4a-c. AD values in each organ of the untreated (—) and treated (...) autoradiographs after i.v. injection of $[U-^{14}C]$ galactose, presented by star diagram. a. 5 min. b. 15 min. c. 30 min. (the mean values of three mice)

The treated autoradiograph, however, showed a marked decrease of the radioactivity in almost all organs (Fig. 1b), and their activities became less than 50% of those in the untreated autoradiographs (Fig. 4a). This indicates that a large part of ^{14}C -galactose metabolites was incorporated into the acid-soluble substances. Five to 30 min after injection, the activities were reduced in many organs, although the activities in the salivary glands and Harderian gland increased in the first 15 min (Figs. 2, 4b). At 30 min after injection, relatively high activities were observed in the kidney, small intestine, mucous salivary glands and Harderian gland (Figs. 3a, 4c). In these organs, the activities were retained by acid treatment, indicating that radioactive carbon was mainly incorporated into the acid-insoluble substances (Figs. 3b, 4c).

Biochemical analysis

Organ samples obtained from the mice injected with ^{14}C -galactose were fractionized into the acid-soluble, -insoluble and lipid parts. Figure 5 shows the change with the passage of time in the total radioactivity (cpm) per gram of wet weight in each organ after injection. The highest level was seen in the kidney during the entire period of this examination. Following the kidney, a high level was observed in the liver at 1 and 5 min, and in the small intestine at 15 to 60 min. Each organ had a radioactive peak at 1 or 5 min, except the brain which showed a peak at 15 min.

FIG. 5. Total radioactivity (cpm) per gram of wet weight in each organ at various intervals after i.v. injection of $[\text{U-}^{14}\text{C}]$ galactose.

TABLE 1. *Incorporation rate (%) of radioactivity into the acid-soluble (S), -insoluble (I) and lipid (L) fractions in each organ after i.v. injection of [^{14}C] galactose. Incorporation rate (%) = [radioactivity (cpm) of each fraction/total radioactivity (cpm) in each organ] $\times 100$*

ORGAN		min after injection				
		1	5	15	30	60
BLOOD	S	97	99	90	95	76
	I	2	1	9	5	17
	L	1	0	1	0	7
LIVER	S	96	93	88	89	91
	I	3	6	11	10	8
	L	1	1	1	1	1
KIDNEY	S	91	86	73	68	61
	I	7	13	26	30	36
	L	2	1	1	2	3
SMALL INT.	S	68	80	60	53	45
	I	24	18	38	44	51
	L	8	2	2	3	4
LARGE INT.	S	85	83	70	66	36
	I	13	16	26	28	60
	L	2	1	4	6	4
BRAIN	S	98	92	81	77	74
	I	1	7	5	13	22
	L	1	1	14	10	4
MYOCARDIUM	S	93	95	94	86	70
	I	6	3	3	6	29
	L	1	2	3	8	1

As shown in Table 1, at 1 and 5 min, the incorporation rate [(radioactivity (cpm) of each fraction/total radioactivity (cpm) in each organ) $\times 100$] of the radioactivity into the acid-soluble fraction was high in all organs examined. At 30 and 60 min, the blood, liver, brain and myocardium retained high incorporation rates of the radioactivity into the acid-soluble fraction, whereas the kidney, small and large intestines showed relatively high rates into the acid-insoluble fraction. The rates into the lipid fraction were low in almost all organs at all intervals, but relatively high in the brain at 15 and 30 min.

Paper chromatography

Figure 6 shows the paper chromatograms of the acid-soluble fraction of the blood, kidney, liver and brain. Galactose, galactose-1-phosphate, UDP-galactose and glucose were used as the markers. As shown in Fig. 6a, the blood had radioactive spots for galactose at 1 and 5 min, for galactose and glucose at 15 and 30 min, and for glucose at 60 min after injection. Radioactivities of these carbohydrates decreased with time after injection. Figure 6b shows the kidney. The radioactive spots in the kidney were detected for galactose and glucose at 1 to 30 min, and the level of radioactivity for glucose became higher than that for galactose with time after injection. At 60 min, the radioactivity for these carbohydrates was a trace amount. As shown in Fig. 6c, the radioactive spots in the liver were detected for galactose and glucose at 1 and 5 min, and for glucose at 15 and 30 min. These

FIG. 6.

FIG. 6.

spots, however, were not detected at 60 min. Figure 6d shows the brain. In the brain, the radioactive spots were detected for galactose at 1 min, and for galactose and glucose at 5 and 15 min. After 30 min, these spots were not detected at 30 and 60 min. In these organs the radioactive spot for $R_{\text{Glucose}}(R_G)$ value 0.28 was detected. R_G is the relative ratio of the migrated distance from origin to each radioactive spot to that of glucose. Furthermore, relatively high radioactivities were retained at origin in these organs except for the blood.

DISCUSSION

The distribution and metabolic fate of radioactive carbon from [U- ^{14}C] galactose was examined by whole-body autoradiography and biochemical analysis.

The total radioactivity of each organ was presented as the counts/min (cpm) per gram of wet weight (Fig. 5). The kidney had the highest radioactivity at all intervals after injection, and the liver and the small intestine also had high activities. These results of biochemical analysis were consistent with those obtained by untreated whole-body autoradiographs (Figs. 1, 2, 3). In the cat and rabbit, the kidney was capable of metabolizing galactose (17, 25), and Wells *et al.* (43) showed that the kidney of the chick had the greatest oxidizing capability of various tissues. They claimed that the kidney might contribute significantly to the overall metabolism of galactose in these animals (17, 25, 43). McNamara *et al.* (20) reported that the kidney cortex of rat oxidized about twice the amount of galactose per unit weight as did the liver.

According to the present data of treated autoradiographs and biochemical analysis, the incorporation rate of the ^{14}C into the acid-insoluble fraction was high in the kidney, small and large intestines. On the other hand, it has been shown that the labeled carbon from ^{14}C -galactose was incorporated into glycoprotein in the proximal convoluted tubule cells of the kidney (22, 25) and into the constituent of mucin after having been taken up by the cells of intestinal glands and goblet cells (14, 22).

In the liver and the kidney, at 1 and 5 min after injection, radioactive spots for galactose and glucose were detected (Figs. 6b, 6c), indicating the high conversion rate of galactose into glucose. By the Leloir pathway, galactose was predominantly metabolized in the liver, where the enzymes had high activity (3, 8, 12, 33). Galactose could be converted into glycogen or released as glucose, pyruvate and lactate (6, 26). Because of the high radioactivity for origin or R_G 0.28, it is assumed that in the liver these metabolites may be present in the glycogen or glucose form. It has been reported that after incubation of rat kidney cortical slices with galactose, phosphorylated intermediates were found and that the glucose in the cortical slices incubated in [1- ^{14}C] galactose became labeled and $^{14}\text{CO}_2$ released from the media came from galactose that had been converted into glucose (20).

Figs. 6a-d. Paper chromatograms of the acid-soluble fractions of the blood (a), kidney (b), liver (c) and brain (d). The radioactivity (cpm/g-wet weight) of each peak on paper chromatogram was corrected with the original radioactivity of the acid-soluble fraction of each organ. Arrows indicate the markers: GAL-1-P (Galactose-1-phosphate), UDP-GAL (Uridine diphosphate galactose), GAL (Galactose), GLC (Glucose).

In the blood, total radioactivity was the highest at 1 min after injection, and decreased with the passage of time (Fig. 5). By paper chromatogram of the acid-soluble fraction of the blood, a radioactive spot for galactose was detected at 1 through 30 min, presenting a decrease of radioactivity with the passage of time. And at 60 min, radioactivity of this spot was trace. However, 5 min thereafter, radioactivities for glucose and unknown metabolites (for R_G 0.28) were again detected (Fig. 6a). These facts suggest that ^{14}C injected into the blood is taken up and metabolized in certain other organs and recirculates in the blood. Segal *et al.* (27) reported that in adults galactose in the blood was undetectable at 15 min after unlabeled galactose infusion, whereas after intravenous injection of ^{14}C -galactose, the blood ^{14}C went up simultaneously and remained at relatively high levels for a long time thereafter, as shown in the present work, indicating that metabolites of galactose might be present in the blood. According to Bassett *et al.* (2), no galactose remained in the blood at 75 min after the injection of galactose in normal adults. Cohn *et al.* (6) found that after an intravenous injection of radioactive galactose to normal adults, 50% of the radioactivity was found in the body glucose pools within 30 min. These findings may be in good agreement with our present results.

In the brain of whole-body autoradiography (Fig. 4) and biochemical analysis, the highest radioactivity was observed at 15 min after injection. And incorporation rate into the acid-soluble fraction was very high through the entire period of examination (Table 1). By paper chromatography at 1 min, high radioactivity was detected only for galactose, whereas 5 min thereafter high activity for unknown metabolites (for R_G 0.28) was observed (Fig. 6d). Galactose can be taken up into the brain across the blood-brain barrier by the same carrier mechanism of glucose (7, 9, 24). The presence of the enzymes of the Leloir pathway in rat and chick brain has been well documented (3, 5, 8, 19, 40). On the other hand, low galactose-1-P uridyltransferase activity in the rat brain has been reported by Musick *et al.* (21). Therefore, galactose metabolism in the brain might be limited. Vrba (36), Vrba *et al.* (39) and Shimada *et al.* (28) showed the rapid incorporation of ^{14}C from ^{14}C -glucose into free amino acids in rat and mouse brains. Our present findings may be consistent with the data of these reports and suggest that most of ^{14}C may be taken up into the brain after being converted into glucose in certain organs (30) and then metabolized further into such free amino acids as glutamate, aspartate, GABA, alanine and glycine.

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