In Vivo Incorporation of ^{14}C into Liver and Kidney Sterols from Parenterally Administered [2-^{14}C]D,L-Mevalonic Acid[1] (37644)

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Elwood and Van Bruggen (1) reported that the parenteral administration of [2-^{14}C]D,L-mevalonic acid to rats was followed by an inordinately high incorporation of ^{14}C into renal nonsaponifiable lipid. An average of 67% of the total body nonsaponifiable lipid radioactivity was recovered in the kidney. Duncan and Best (2) confirmed this observation and utilized the high radioactivity of renal sterols attained following the iv administration to rats of [2-^{14}C]D,L-mevalonic acid to determine the effects of thyroid status and of triparanol on the turnover of kidney cholesterol. It is known, however, that renal sterol synthesis from acetate is meager, considerably less than by liver (3–6), and the peculiar pattern of mevalonic acid metabolism may be a reflection of the capacity of the kidney tubular cells to concentrate small amounts of mevalonic acid. Moreover, Hellstrom *et al.* (7) have shown that only small amounts of ^{14}C from parenterally administered [2-^{14}C]mevalonate are found in rat kidney cholesterol, the greatest quantity of ^{14}C-labeled nonsaponifiable lipid was in the cholesterol precursors squalene and lanosterol.

All of these studies have utilized rats as the experimental subjects and the question which arises is whether this peculiar pattern of ^{14}C-sterol distribution occurs only in rats. In this report we indicate the intrarenal distribution of and the species differences in the formation of ^{14}C-labeled kidney digitonin precipitable sterols following the parenteral administration of [2-^{14}C]D,L-mevalonic acid.

Methods. [2-^{14}C]D,L-mevalonic acid as the dibenzylethylenediamine salt (DBED salt, New England Nuclear Corp., Boston, Mass.) was dissolved in 0.154 *M* NaCl solution and administered iv in a dose of 1.0 μCi ^{14}C/kg body weight to several dogs weighing 8–10 kg and sheep weighing 15–18 kg. Euthanasia was performed at 24 hr, and at 4, 7 or 9, and 10 days after the administration of the mevalonic acid by exsanguination following pentobarbital sodium (30 mg/kg) induced anesthesia. The kidneys and segments of liver were quickly removed and placed into iced beakers and the kidney medulla was isolated from the cortex. Blood samples were taken immediately prior to euthanasia for the extraction of plasma cholesterol. Methods used for the isolation and radioassay of digitonin precipitatible sterols have been described by Gans and Cater (8). Sterol specific radioactivity is expressed as the specific radioactivity of tissue or plasma cholesterol.

Gerbils and hamsters were given the saline solution of [2-^{14}C]D,L-mevalonic acid DBED salt intraperitoneally in a dose of 0.2 μCi/g body weight. Euthanasia was performed by stunning and exsanguination at 24 hr and at 1, 2, 3, and 4 wk after the administration of the labeled mevalonic acid. From each animal the liver and kidneys were quickly removed and placed into iced beakers and the medulla was dissected free from each kidney. One gerbil and one hamster were each given the [2-^{14}C]D,L-mevalonic acid intrapleurally and euthanasia and sterol extractions were performed 24 hr after the administration of the mevalonic acid. The extraction and radioassay of kidney and liver digitonin precipitatible sterols was performed as outlined for dogs and sheep.

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TABLE I. Relative Specific Radioactivties (SRA) of Kidney, Liver, and Plasma Digitonin Precipitatible Sterols in Dogs and Sheep Following the Intravenous Administration of [2-^{14}C] D,L-Mevalonic Acid.

Species	Tissue ratio[a]	Days after administration of ^{14}C-labelled mevalonic acid				
		1[b]	4	7	9	10
Dog	KC/Pl	10.0 ± 0.7	4.2		3.9	4.2
	KM/Pl	0.8 ± 0.1				
	L/Pl	1.3 ± 0.1	1.1		1.2	1.2
Sheep	KC/Pl	5.6 ± 0.8		10.0		7.0
	KM/Pl	0.9 ± 0.2		1.5		1.3
	L/Pl	1.1 ± 0.1		1.1		1.2

[a] Ratio of SRA kidney cortex (KC), kidney medulla (KM), or liver (L) cholesterol/SRA Plasma (Pl) cholesterol.

[b] $N = 4$ for dogs and sheep at 1 day and $N = 2$ for each species at days 4, 7, 9, and 10. Where appropriate, the data are expressed as the average ± 1 SE of the mean.

Results. Twenty-four hr after the administration of the ^{14}C-labeled mevalonic acid to dogs, the specific radioactivity (SRA) of kidney digitonin precipitatible sterols expressed as cholesterol was 10-fold greater than the SRA of plasma cholesterol (Table I). Liver cholesterol SRA was only 29% greater than that of plasma cholesterol and the SRA of kidney medullary cholesterol was less than the SRA of plasma cholesterol (Table I). A comparable pattern of ^{14}C-sterol distribution was found in sheep, but the SRA of kidney digitonin precipitatible sterol was 5.6-fold greater than the SRA of plasma cholesterol 24 hr after the ^{14}C-mevalonic acid had been administered. At later time periods the ratio SRA kidney cortex cholesterol: SRA plasma cholesterol decreased in the dog but increased in sheep while in both dogs and sheep the ratios for the SRA of liver cholesterol:plasma cholesterol and SRA kidney medulla cholesterol:plasma cholesterol in sheep remained near unity.

The data obtained from the experiments which utilized gerbils and hamsters are analyzed in terms of kidney cortex and liver cholesterol SRA (Fig. 1) since no plasma samples were taken. It is assumed that the SRA of liver cholesterol is an approximation of the SRA plasma cholesterol because studies in a large number of laboratory animals (2–4) as well as in human subjects (9) indicate that dynamically the liver-plasma cholesterol pool comprises a single compartment. The ratio SRA kidney cortex cholesterol: SRA liver cholesterol 24 hr after the administration of [2-^{14}C]D,L-mevalonic was 34 for gerbils and 18.7 for hamsters (Fig. 1). A comparable pattern of ^{14}C distribution was observed in the gerbil and hamster which received the ^{14}C-mevalonic acid intrapleurally indicating that the magnitude of the difference between kidney and liver sterol SRA was not a peculiarity of the ip route of injection. If the data from dogs and sheep were similarly expressed, the ratio SRA kidney cortex cholesterol:SRA liver cholesterol would have been 7.8 and 5.3, respectively. Three weeks after the administration of the ^{14}C-labeled mevalonic acid the ratio SRA kidney cortex cholesterol:SRA liver cholesterol was 7.5 for gerbils and 4.7 for hamsters (Fig. 1).

Kidney medullary cholesterol SRA of hamsters was 70% that of liver cholesterol 24 hr after the [2-^{14}C]D,L-mevalonic acid had been given and the ratios between the SRA of cholesterol from kidney medulla and from liver approximated unity at the later time intervals. Samples of cholesterol from gerbil kidney medullae were inadvertently lost.

The apparent half life of kidney cortex digitonin precipitatible sterol (expressed as cholesterol) was 5.5 days in hamsters and 4.3 days in gerbils. Liver cholesterol of hamsters had an apparent half life of 10.8 days; the data obtained from gerbils did not permit an estimation of liver cholesterol half-life.

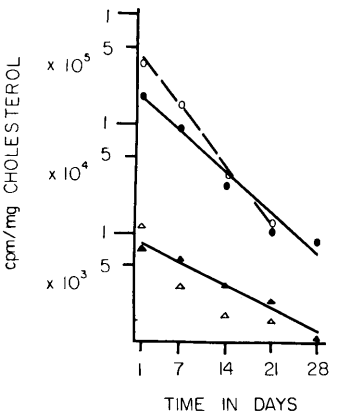

FIG. 1. Specific radioactivity of gerbil and hamster digitonin precipitatible sterols, expressed as cholesterol, following the intraperitoneal injection of [2-^{14}C]D,L-mevalonic acid DBED salt. Solid circles, hamster kidney cortex sterols; solid triangles, hamster liver sterols; open circles, gerbil kidney cortex sterols; open triangles, gerbil liver sterols. TIME IN DAYS refers to time in days following the administration of the ^{14}C-labeled mevalonic acid.

Discussion. The high *in vivo* incorporation of ^{14}C from a tracer dose of [2-^{14}C]D,L-mevalonic acid into kidney digitonin precipitatible sterol occurs in dogs and small ruminants such as the sheep as well as in laboratory rodents. The phenomenon, therefore, would appear to be a general characteristic of the *in vivo* metabolism of a tracer dose of [2-^{14}C]D,L-mevalonic acid but one which involves only the kidney cortex. The cells of the kidney medulla clearly are not capable of forming ^{14}C-sterols of high specific radioactivity from ^{14}C-labeled mevalonic acid. There are quantitative differences, among the species studied, with regard to the magnitude of ^{14}C incorporation into kidney cortical sterols. Small laboratory rodents such as the rat (2), gerbil and hamster form renal cortical sterols of considerably greater radioactivity relative to that of liver and plasma sterols than do dogs and sheep. These quantitative differences bear no relationship to cholesterol concentrations since in all species studied in this laboratory as in others (2, 10) the cholesterol concentration in the kidney (cortex) averages between 4.0–4.8 mg/g wet weight.

Kidney cholesterol equilibrates slowly with the liver-plasma compartment (11, 12) and its turnover rate would equal or more likely be slower than that of liver and plasma cholesterol. The relatively short half life of kidney cortical "cholesterol" in gerbils and hamsters suggests that what was being measured was a specific sterol pool with a more rapid turnover rate than that of the kidney cholesterol compartment which ultimately equilibrates with liver-plasma cholesterol. Hellstrom *et al.* (7) indicate that the ^{14}C-labeled cholesterol precursors which are formed in the kidney from [2-^{14}C]mevalonate do not readily become incorporated into kidney cholesterol. Rather, these ^{14}C-labeled cholesterol precursors may preferentially leave the kidney to be transformed into cholesterol by the liver. Our results would be compatible with this interpretation and further designate these cholesterol precursors as the kidney cortical sterols with a more rapid turnover rate than liver, plasma, or kidney cortical cholesterol. It should be clear, however, that the apparent half life of hamster liver cholesterol may be an overestimation if the quantity of ^{14}C-labeled cholesterol precursors which leave the kidney is significant.

Summary. Twenty-four hr after the iv administration of [2-^{14}C]D,L-mevalonic acid to dogs and to sheep, the specific radioactivity of digitonin precipitatible sterol expressed as cholesterol in the kidney cortex was respectively 10.0- and 5.6-fold greater than the specific radioactivity of plasma cholesterol. Cholesterol of the kidney medulla, by contrast, attained a specific radioactivity which was 20% and 10% less than that of plasma cholesterol. Parenteral administration of [2-^{14}C]D,L-mevalonic acid to gerbils and to hamsters resulted in kidney cortical sterol specific radioactivities, 24 hr after the mevalonic acid had been given, which were, respectively, 34- and 18.7-fold greater than the specific radioactivity of liver cholesterol. Hamster kidney medullary cholesterol, at the same time interval attained a specific radioactivity 30% less than that of liver cholesterol. The apparent half life of the ^{14}C-labeled kidney cortical sterols was 4.3 days in gerbils and 5.5 days in hamsters while the apparent half life of hamster liver cholesterol was 10.8 days.

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