Bile Acid Composition in Some Desert Rodents[1] (37374)

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Sterol metabolism in the Mongolian gerbil, *Meriones unguiculatus,* which is a rodent indigenous to desert, differs from that of the laboratory white rat, *Rattus rattus.* Gerbils on a cholesterol supplemented diet developed hypercholesteremia (1) and maintained this state for several weeks with no evidence of atheromatous lesions (2). In contrast, feeding cholesterol to white rats showed no significant change in serum cholesterol. Also, dietary cholesterol was shown to be associated with atheroma formation in white rats (3).

Since it is well known that bile acids constitute the major pathway of cholesterol metabolism in gerbils and other mammals (4), bile acid composition was studied in gerbils (5, 6) and other laboratory rodents (6–8). In view of the apparent difference in bile acid composition of mammals and the fact that we are not aware of any data on wild desert animals, we report in this paper the bile acid composition in seven species of desert rodents which span three rodent families and differ to varying extents in ecologic distribution and food habits.

Materials and Methods. Seven species of desert rodents, representing three families, were trapped using live traps from the Mohave Desert near Las Vegas, Nevada. Ecologic distribution and food habits of the species studied are shown in Table I. All animals were sacrificed within 12 hr of capture; bile was obtained from the gall bladder of each animal and then frozen until analysis. The bile acids were extracted and hydrolyzed, as described by Yousef *et al.* (7). The bile acids were separated using gas chromatography, and their identification and quantification was made as previously described (7, 9). The gas chromatograph used was the Packard 7401, equipped with dual glass columns. A standard quantitated mixture of 5β-cholanoic acid, cholesterol, lithocholic acid (LCA), deoxycholic acid (DOCA), chenodeoxycholic acid (CDOCA), ursodeoxycholic acid (UDOCA), and cholic acid (CA) was analyzed with at least every five samples of unknown bile acid mixture. A typical example of separation of the standard mixture is shown in Fig. 1A. A known amount of 5β-cholanoic acid was added as internal standard to all samples of unknown bile extraction.

Results. The average percentage of the different bile acids of all species studied is shown in Table II. A typical chromatogram comparing bile acid spectra of the bile acid pool isolated from the gall bladder of all species studied is shown in Fig. 1B–H. In the family Heteromyidae, cholic acid (CA) was the primary bile acid found in all species and constituted from 48 to 74% of the total pool of the bile acids. Chenodeoxycholic acid (CDOCA) is the other primary bile acid which was also present in the other four species studied (Fig. 1B–E); however, the ratio CA:CDOCA differs from species to species. The CDOCA contributed from 10 to 17% of the total pool of bile acids. Secondary metabolic products of CA and CDOCA were identified and included: deoxycholic acid (DOCA), ursodeoxycholic acid (UDOCA), 3β, 12α-dihydroxycholanic acid, (DHCA) and lithocholic acid (LCA). Considerable amounts of all these secondary

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TABLE I. Ecologic Distribution and Food Habits of Rodents in Southern Nevada.

Family, species and common name	Sample size	Av body wt (g)	Ecologic distribution	Food habits
Sciuridae				
Ammospermophilus leucurus (Antelope ground squirrel)	4	80	Widespread in desert below 1800 m	Omnivorous diet consisting of green vegetation, seeds, arthropods and small vertebrates (10, 11)
Spermophilus tereticaudus (Round-tailed ground squirrel)	3	88	Localized in sandy habitats in low desert below 900 m	
Heteromyidae				
Perognathus formosus (Long-tailed pocket mouse)	3	15	Widespread in desert up to 1700 m	Diet of seeds with supplement of green vegetation during reproductive season (11, 12, 13)
Dipodomys microps (Chisel-Toothed kangaroo rat)	3	49	Localized in high desert between 1600 and 1800 m	
Dipodomys merriami (Merriam's kangaroo rat)	3	33	Widespread in desert below 1600 m	
Dipodomys deserti (Desert kangaroo rat)	5	88	Localized in sandy habitats in low desert below 1200 m	
Cricetidae				
Neotoma lepida (Desert pack rat)	5	100	Localized in rocky or wash habitats in desert below 1800 m	Diet of succulent vegetation including cacti where available

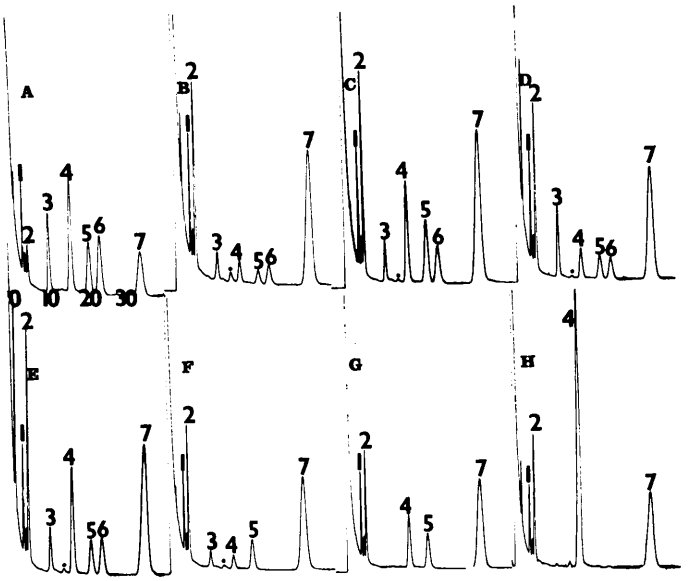

FIG. 1. A typical gas chromatogram of the trifluoracetates of bile acid methyl esters. Operating temperatures were: Column, 225°; injection, 225°; and detector 240°. The carrier gas was helium and its flow was 37.5 cc/min. Chart speed was 10 min/2.5 cm. The letters A to H represent the following: A: standard mixture; B: *D. microps*; C: *D. merriami*; D: *D. deserti*; E: *P. formosus*; F: *A. leucurus*; G: *S. tereticaudus*; H: *N. lepida*. The numbers 1 to 7 represent the following: 1: 5 β-cholanic acid; 2: cholesterol; 3: Lithocholic acid; 4: deoxycholic acid; 5: Chenodeoxycholic acid; 6: Ursodeoxycholic acid; 7: Cholic acid. The closed circles (●) represent a tentative identification (standard is not available) for 3 β, 12 α-dihydroxycholanic acid.

metabolites were present in the three species of kangaroo rats (*Dipodomys*); however, DHCA and LCA were detected only in trace amounts in the bile acid pool of the pocket mouse, *P. formosus*.

In the family Sciuridae, CA and CDOCA were the primary bile acids, and the ratio of CA:CDOCA was 3.9 and 2.7 for *A. leucurus* and *S. tereticaudus*, respectively. Small amounts of LCA and DHCA were present, although DOCA was found to contribute 6–15% of the total bile acid pool. In both species UDOCA was not found, as shown in Fig. 1F and G.

The family Cricetidae was represented by only one species, *N. lepida*. The chromatogram of the bile acid spectra, shown in Fig. 1H, indicated the presence of CA as the primary bile acid and DOCA as a secondary metabolic product of the bile acid pool.

For an overall analysis of the bile acid pool, as extracted from the gall bladder of the seven species studied, we considered CA, DOCA and DHCA as the 12α-hydroxylated bile acids and then calculated the ratio of 12α-hydroxylated: non-12α-hydroxylated bile acids. The ratio was as low as 2 in *D. merriami* and *D. deserti* and as high as 100 in *N. lepida* (Table II). The percent similarity in bile acid spectra of desert rodents was calculated as shown in Table III. It is evident there was a difference not only in the total bile acid constituents, but also in the relative proportions of each bile acid secreted.

Discussion. Data presented in Table II and Fig. 1 indicated that the bile acid content of desert rodents, as obtained from gall bladders, is both complex and variable. In all studies involving mammals, CA is known to be the principal bile acid synthesized directly from cholesterol in the liver (6). Desert rodents are similar in this respect (Table II). It is also known that CDOCA is the other primary bile acid secreted in the other rodents previously studied (5–7). However, the ratio CA:CDOCA differs from species to species. With the ex-

TABLE II. Percentage of Different Bile Acids Obtained from the Bile of Desert Rodents.

Species	CA	CDOCA	DOCA	UDOCA	DHCA	LCA	CA/CDOCA ratio	12α-hydroxylated non-12α-hydroxylated bile acids
Dipodomys microps	74 ± 7	11 ± 2.0	5 ± 0.3	4 ± 0.2	3 ± 0.1	3 ± 0.3	6.7 ± 1.4	4.6 ± 0.9
Dipodomys deserti	55 ± 6	12 ± 1.5	5 ± 0.4	9 ± 0.3	7 ± 0.4	12 ± 1.1	4.6 ± 0.9	2.0 ± 0.4
Dipodomys merriami	48 ± 10	17 ± 3.4	16 ± 5.0	13 ± 2.1	2 ± 0.4	6 ± 1.5	2.8 ± 0.9	2.0 ± 0.4
Perognathus formosus	56 ± 4	10 ± 1.3	20 ± 2.4	12 ± 0.9	trace[a]	2 ± 0.4	5.6 ± 1.1	3.2 ± 0.9
Spermophilus tereticaudus	59 ± 6	22 ± 2.4	15 ± 1.3	—	trace	2 ± 1.2	2.7 ± 0.9	2.8 ± 1.2
Ammospermophilus leucurus	75 ± 10	19 ± 1.4	6 ± 0.4	—	trace	trace	3.9 ± 1.1	4.3 ± 0.8
Neotoma lepida	42 ± 8	- trace	58 ± 7.1	—	—	—	100 ± 0.0	100 ± 0.0

[a] Trace is less than 0.1%.

ception of one species, *N. lepida,* which does not produce CDOCA, this conclusion can be extended to include the other six desert species used in our study. Among desert species, only *D. microps* and *A. leucurus* secrete considerably more CA than other species, even in the same family. Although Noll *et al.* (5) reported a CA:CDOCA ratio of 23:1, the highest ratio shown in Table II, excluding *N. lepida,* is 6.7. This suggests sterol metabolism differs in various species, and this difference does not appear to be related to ecology, nutrition or ecological distribution of these species. However, it is possible this difference is due to the activity of the endocrine system. Beher *et al.* (6) have shown that hormonal changes alter the bile acid pool spectra.

In *N. lepida* CDOCA was not detected in the bile acid pool. This finding suggests a block in the metabolic pathway of cholesterol to CDOCA. Therefore, *N. lepida* represents an excellent experimental model for biochemical studies related to the biokinetics of CA synthesis.

The secondary metabolic products of CA and CDOCA showed clearly a different pattern among the seven species studied. The presence of DOCA was a prominent feature of all species. The secondary metabolic product, UDOCA, was found in the family Heteromyidae but was not detected in the families Sciuridae and Cricetidae (Table II). This difference does not seem to be related to ecologic distribution but may be related to phylogenetic and/or nutritional differences. Heteromyids are highly gramnivorous, whereas ground squirrels are omnivorous and pack rats are herbivorous (Table I). The presence of small amounts of DHCA and LCA in five species offers additional evidence for a difference in sterol metabolism in these species. The absence of LCA in *N. lepida* is not surprising since the precursor CDOCA was not detected in the bile acid pool.

The overall picture of our data indicates that 12α-hydroxylated bile acid is more dominant as compared to non-12α-hydroxylated bile acids. The ratio of these two types of bile acids is higher in the desert species (Table II) as compared to data reported on

TABLE III. Percent Similarity[a] in Bile Acid Spectra of Desert Rodents.

	Dipodomys merriami	*Dipodomys microps*	*Perognathus formosus*	*Ammospermophilus leucurus*	*Spermophilus tereticaudus*	*Neotoma lepida*
Dipodomys deserti	82	81	81	73	74	47
Dipodomys merriami		73	88	71	82	58
Dipodomys microps			76	91	73	47
Perognathus formosus				71	83	62
Ammospermophilus leucurus					83	45
Spermophilus tereticaudus						57
Neotoma lepida						

[a] Percent similarity represents the sum of the percents (relative proportions) of bile acids shared by two species.

white rats (7). These differences appear to be unrelated to such factors as phylogenetic relationships, nutrition, or general environment, as the ratio varied from 2 to 100 in the desert species studied. Another significant difference between desert rodents and white rats is the bile and acid pool of white rats contained bile acids hydroxylated at the 6-position, namely α and β muricholic acid (7), whereas, these bile acids were not detected in desert rodents. This is in agreement with Noll *et al.* (5) who also failed to identify any muricholic acids in gerbil bile. However, Beher *et al.* (6) reported the presence of muricholic acid in the bile acid pool of the gerbil.

Our knowledge of bile acid composition in different rodents is limited to only a few species and families. These include *Rattus* and *Mus* (Muridae), *Meriones, Cricetus* and *Neotoma* (Cricetidae), *Ammospermophilus* and *Spermophilus* (Sciuridae) and *Dipodomys* and *Perognathus* (Heteromyidae). The bile acid composition for a family is based on examination of a limited number of genera and species. More comparative data on bile acid composition are needed at species, generic and family levels. Only after analysis of such data can realistic and worthwhile ideas be developed concerning possible evolutionary and adaptive patterns for bile acid metabolic pathways in the Rodentia be formulated.

Summary. Seven species of desert rodents representing three families were used to investigate bile acid composition of the bile acid pool isolated from the gall bladder. Cholic acid (CA) was the common primary bile acid among all species. The other primary acid chenodeoxycholic acid (CDOCA) was detected in all species but one.

The ratio CA:CDOCA differed from species to species. The secondary metabolic products of CA and CDOCA showed a different pattern among the seven species and deoxycholic acid was a prominent feature of all species. Additional studies are needed to fully understand the role of ecologic distribution, phylogeny, and nutrition on bile acid composition.

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