Ursodeoxycholic acid in the Ursidae: biliary bile acids of bears, pandas, and related carnivores

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Abstract The biliary bile acid composition of gallbladder bile obtained from six species of bears (Ursidae), the Giant panda, the Red panda, and 11 related carnivores were determined by reversed phase liquid chromatography and gas chromatography–mass spectrometry. Bile acids were conjugated solely with taurine (in N-acyl linkage) in all species. Ursodeoxycholic acid (3α,7β-dihydroxy-5β-cholan-24-oic acid) was present in all Ursidae, averaging 1–39% of biliary bile acids depending on the species; it was not detected or present as a trace constituent (< 0.5%) in all other species, including the Giant panda. Ursodeoxycholic acid was present in 73 of 75 American Black bears, and its proportion averaged 34% (range 0–62%). Ursodeoxycholic acid averaged 17% of biliary bile acids in the Polar bear (n = 4) and 18% in the Brown bear (n = 6). Lower proportions (1–8%) were present in the Sun bear (n = 2), Ceylon Sloth bear (n = 1), and the Spectacled bear (n = 1). Bile of all species contained taurine-conjugated chenodeoxycholic acid and cholic acid. In some related carnivores, deoxycholic acid, the 7-dehydroxylation product of cholic acid, was also present. To determine whether the 7β hydroxy group of ursodeoxycholic acid was formed by hepatic or bacterial enzymes, bile acids were determined in hepatic bile obtained from bears with chronic biliary fistulae. Fistula bile samples contained ursodeoxycholic acid, chenodeoxycholic acid, and a trace amount of cholic acid, all as taurine conjugates, indicating that ursodeoxycholic acid is a primary bile acid formed in the liver in Ursidae. Additional evidence for ursodeoxycholic acid being a primary bile acid was provided by fecal bile acid analyses in six bear species. These showed the presence of ursodeoxycholic acid in five of six species and absence of its 3α-hydroxy-7-oxo precursor in all. It is concluded that ursodeoxycholic acid occurs in all Ursidae, but in few other species, and is formed in the liver.—**Hagey, L. R., D. L. Crombie, E. Espinosa, M. C. Carey, H. Igimi, and A. F. Hofmann.** Ursodeoxycholic acid in the Ursidae: biliary bile acids of bears, pandas, and related carnivores. *J. Lipid Res.* 1993. **34:** 1911–1917.

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In 1900–1901, two expeditions set out to explore Greenland (1). The Swedish party was led by Kolthoff and the Danish party by Amdrup. The two groups obtained gallbladders of the Polar bear (*Thalarctos maritimus*) and several other arctic mammals and delivered these to Olof Hammarsten, Professor of Biochemistry, in Uppsala, Sweden. Hammarsten (2, 3) isolated an unknown bile acid from the bile of the Polar bear and named it "ursocholeinsäure", indicating that it was a new bile acid from the bear. Twenty-five years later, Shoda (4) crystallized this bile acid from a commercial preparation of bile of the Black bear (*Ursus americanus*), and Shoda renamed it ursodeoxycholic acid (UDCA), as it was an isomer of deoxycholic acid. Oxidation of UDCA was shown by Iwasaki (5) to yield the same 3,7-dioxo derivative as obtained by oxidation of chenodeoxycholic acid, and he concluded that UDCA is the 7β-epimer of chenodeoxycholic acid. Later, Kanazawa et al. (6) succeeded in synthesizing UDCA from cholic acid. In 1959, UDCA was identified as a minor constituent in human bile by Sjövall (7). Extensive studies by Tammar (8), Haslewood (9), and Hagey (10) on the biliary bile acid composition of vertebrates have shown that although UDCA is present in the biliary bile acids of a number of vertebrates, it generally constitutes < 1–5%. At present, the only species besides the bear known to have an appreciable portion of UDCA in biliary bile acids is the nutria (*Myocastor coypus*), in which UDCA is a dominant primary biliary bile acid (11, 12).

In this paper, we report a systematic examination of the biliary bile acid composition of the family Ursidae. Analyses of the biliary bile acid composition of the Giant

Abbreviations: UDCA, ursodeoxycholic acid; GLC, gas-liquid chromatography; HPLC, high performance liquid chromatography; GC–MS, gas chromatography–mass spectrometry.

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panda (*Ailuropoda melanoleuca*) and Red panda (*Ailurus fulgens*) are also included, as these have been considered to be closely related to the Ursidae. Data on biliary and fecal bile acid composition from a number of additional carnivores have also been included for comparison with those of Ursidae. Biliary bile acids in chronic biliary fistula bears were examined in order to ascertain whether UDCA is formed by hepatic enzymes and is a primary bile acid, or is formed by bacterial enzymes in which case it would be a secondary bile acid (13).

MATERIALS AND METHODS

Samples

Gallbladder bile from bears and related carnivores was obtained from deceased animals at the San Diego Zoo under an approved protocol of the Zoological Society of San Diego. Bile samples from 11 North American Black bears that were killed by accident or by hunting in Maine and Minnesota were analyzed by GLC at the Brigham and Women's Hospital, Boston, MA. An additional 57 HPLC analyses of American Black bears, 3 Polar bears, and 4 North American Brown bears (*Ursus arctos;* also known as grizzly bear) were provided by the National Fish and Wildlife Forensics Laboratory, Ashland, OR. This laboratory also provided HPLC analyses of bile obtained from 23 bears with chronic biliary fistulae maintained in the Peoples Republic of China. Details on the maintenance of these animals has been published in the popular press (14). Capsules (n ≈ 20) of "bear bile" were purchased in traditional Chinese pharmacies. Bile from the sloth bear (*Melursus ursinus*) was provided by the Philadelphia Zoo (Penrose research laboratory). Bile samples were dispersed in several volumes of reagent grade isopropanol immediately after collection to prevent bacterial degradation. Fecal samples were frozen immediately after collection and kept at −20°C until analysis.

Analyses

Bile acid conjugates were analyzed by HPLC of gallbladder bile essentially as described by Rossi, Converse, and Hofmann (15). The method uses an octadecylsilane column (RP C-18) with elution at 0.75 ml/min with an isocratic 50 mM KH_2PO_4-K_2HPO_4 buffer, apparent pH 5.4, in methanol-water 68.2:31.8 (v/v). The effluent was monitored at 205 nm (amide bond of conjugated bile acids) to quantify bile acids. Peaks were tentatively assigned by comparison of the relative retention times with those of known standards. Biliary bile acids were also analyzed by GC-MS using a Hewlett-Packard 5890 Gas Chromatograph-5970 MSD, controlled by an HP/UX Chem Station program. The 30-meter capillary column was a Supelco 0.25-mm ID SPB-35 (35% phenyl methyl silicone) operated at 275°C (isothermal). A splitless injection was used with an injection temperature of 295°C and interface temperature of 290°C. Helium served as the carrier gas with a column head pressure of 6 pounds per square inch. To prepare for GC-MS, bile acids were deconjugated chemically (1.0 N NaOH, 130°C, 4 h). Deconjugated bile acids were esterified with methanol (diazomethane) and acetylated by the perchloric acid-catalyzed method of Roovers, Evrard, and Vanderhaeghe (16); bile acids were incubated for 1.5 h in 2 ml of the acetylation fluid (14 ml acetic acid, 10 ml acetic anhydride, 0.1 ml perchloric acid), and then extracted with ethyl acetate; the ethyl acetate phase was then reduced to dryness.

The unconjugated bile acids, which constitute > 90% of all fecal bile acids, were analyzed in six bear species. Samples were refluxed for 1 h in methanolic sodium hydroxide solution (85 ml methanol, 10 ml of 10 N NaOH), and after the addition of water, neutral sterols were extracted with hexanes. The remaining aqueous fraction was acidified (pH < 2) with dilute hydrochloric acid and the unconjugated bile acids were extracted into ethyl acetate. Fecal bile acids were then derivatized and examined by GC-MS as described above.

TABLE 1. HPLC analysis of the biliary bile acids present in gallbladder bile of adult bears (Ursidae)

			Bile Acid Composition, %			
Common Name	Latin Name	N	3α7α[a]	3α7β	3α7α12α	3α12α[b]
Black bear	*Ursus americanus*	7	29.6 ± 5.2	38.8 ± 7.4	31.0 ± 5.2	0.6 ± 0.37
Brown bear	*Ursus arctos*	6	5.7 ± 1.5	18.6 ± 7.3	75.7 ± 14.3	0.0
Polar bear	*Thalarctos maritimus*	4	19.6 ± 7.8	17.4 ± 6.9	62.8 ± 14.4	0.2 ± 0.2
Sun bear	*Helarctos malayanus*	2	63.6 ± 8.7	8.6 ± 3.1	27.8 ± 5.6	0.0
Sloth bear	*Melursus ursinus*	1	31.3	1.4	67.3	0.0
Spectacled bear	*Tremarctos ornatus*	1	23.6	6.3	70.1	0.0

Bile acids were present in bile as the taurine (N-acyl) conjugates. Bile acid composition has been normalized to 100% and is expressed as mean ± standard error. Bile acids shown in Tables 1-4 comprise > 95% of the total bile acids for all of the species listed.

[a] The position of the hydroxyl substituents is indicated. 3α, lithocholic acid; 3α7α, chenodeoxycholic acid; 3α7β, ursodeoxycholic acid; 3α7α12α, cholic acid; 3α12α, deoxycholic acid.

[b] Deoxycholic acid (3α12α) is a secondary bile acid formed by 7α-dehydroxylation of cholic acid in the intestine.

RESULTS

Biliary bile acids in the Ursidae

The biliary bile acid compositions of the Ursidae are summarized in **Table 1.** The major biliary bile acids in each bear were UDCA, chenodeoxycholic acid, and cholic acid, all as taurine conjugates. Deoxycholic acid, the major bacterial biotransformation product of cholic acid, was absent in four species of bears and present in trace levels only in the remaining two species.

Table 2 shows the biliary bile acid composition for the dominant three bile acids in 75 North American Black bears. The proportion of UDCA averaged 47% (range 0–78%). UDCA was completely absent in only 2 bears; both bears were obtained by hunting in the Northeastern United States (Study B). HPLC analysis of the biliary bile acids of 23 bears with chronic bile fistulae gave the following results (mean ± SEM): UDCA, 76.1 ± 2%; chenodeoyxcholic acid, 21.3 ± 1.7%; and cholic acid, 2.6 ± 0.73%. All were present solely as taurine conjugates. The biliary fistula bears are a mixture of genera, but are likely to consist primarily of Asiatic Black bears (*Selenarctos thibetanus*) (14). Compared to the composition of bears with an intact enterohepatic circulation, the proportion of cholic acid in the biliary bile acids of bile fistula bears was markedly reduced.

Five neonates from three different species were also examined but are not included in Table 1. Results are as follows: Brown bear (n = 3), chenodeoxycholic acid, 14.0 ± 2.8%; UDCA, 5.7 ± 0.9%; cholic acid, 80.3 ± 3.4; Polar bear (n = 1), chenodeoxycholic acid, 21.8%; UDCA, 10.5%; and cholic acid, 67.7%; and Sloth bear (n = 1), chenodeoxycholic acid, 27.1%; UDCA, 0.3%; and cholic acid, 72.6%. Deoxycholic acid was not observed in any of the neonates. Although the percentage of UDCA observed in neonates was lower than that found in adults, the differences for this small sample size were not statistically significant.

The fecal bile acid composition for bears is shown in **Table 3.** The amount of UDCA in feces closely paralleled the amount of UDCA in bile, and secondary bile acids were observed only in the Black, Polar, and Brown bears. None of the animals examined contained significant (> 0.5%) levels of 3α-hydroxy-7-oxo-5β-cholan-24-oic acid, which has been shown to be converted to UDCA by the mammalian liver (17, 18) and which is an intermediate in the microbial epimerization of chenodeoxycholic acid to UDCA (19–21).

TABLE 2. Biliary bile acid composition (gallbladder bile) of the dominant three bile acids in the American Black bear (*Ursus americanus*)

Study	N	3α7α	3α7β	3α7α12α
A[a]	7	29.8 ± 5.2	39.0 ± 7.4	31.2 ± 5.2
B[b]	11	27.6 ± 3.5	17.5 ± 4.1	54.9 ± 7.4
C[c]	57	15.9 ± 1.4	30.0 ± 3.0	54.1 ± 3.1

See Table 1 for abbreviations. All bile acids were amidated with taurine.
[a] Zoo and hunted bears; HPLC analyses by L. R. Hagey, 1983–1991.
[b] Hunted bears; GLC analyses by M. C. Carey and H. Igimi, 1981–1985.
[c] Hunted bears; HPLC analyses by E. Espinosa, 1990–1991.

TABLE 3. GC-MS analysis of the fecal bile acid composition of bears (Ursidae)

Species	N	Fecal Bile Acid Composition, %				
		3α7α	3α7β	3α	3α7α12α	3α12α
Black bear	1	19.1	35.7	0.0	29.4	15.8
Polar bear	2	7.4	27.7	0.0	16.0	48.9
Brown bear	1	17.5	16.3	18.7	29.7	17.8
Sun bear	3	74.6	2.5	0.0	26.2	0.0
Sloth bear	1	32.5	4.6	0.0	62.9	0.0
Spectacled bear	4	64.6	0.0	0.0	35.4	0.0

See Table 1 for abbreviations. The biliary bile acid spectrum of the conjugated fraction (< 2% of fecal bile acids) was analyzed and found to be identical.

Biliary bile acid composition in pandas and related carnivores

The biliary bile acid composition of the Red panda, Giant panda, and 11 other representative carnivores from six families is shown in **Table 4.** Unlike what was observed in bears, UDCA was present in only trace proportions (0–0.5%). (Data are not given in Table 4 because of the trace proportion.) The biliary bile acid profile of the Giant panda (n = 5) was characterized by the presence of only chenodeoxycholic acid (61.7%) and cholic acid (38.3%); UDCA and deoxycholic acid were both absent. Cholic acid was the predominant bile acid in all carnivores. As in the Ursidae, all bile acids were amidated exclusively with taurine.

DISCUSSION

The analyses reported in this paper lead to four major conclusions: *1)* UDCA is present in all species of Ursidae; *2)* UDCA is a primary bile acid, being formed by hepatic enzymes, in some if not all of the Ursidae; *3)* UDCA is not present in the Giant panda, Red panda, or many other carnivores; and *4)* biliary bile acids are conjugated solely with taurine in bears, pandas, and carnivores.

UDCA in the Ursidae

Based on the proportion of UDCA in biliary bile acids, the Ursidae can be divided into two groups. In Group I are the closely related Polar, Brown, and Black bears, in which the percent of UDCA in biliary bile acids is high (15–39%); in group II, composed of all other bears, the

TABLE 4. Biliary bile acids (gallbladder bile) of the order Carnivora: HPLC analyses

Family and Common Name	Latin Name	N	Bile Acid Composition, %		
			3α7α	3α7α12α	3α12α
Procyonidae					
Giant panda	*Ailuropoda melanoleuca*	4	62.9 ± 1.7	37.1 ± 1.0	0.0
Red panda	*Ailurus f. styani*	1	5.1	94.9	0.0
Raccoon	*Procyon lotor*	5	17.0 ± 2.6	79.2 ± 2.4	3.8 ± 1.5
Mustelidae					
European ferret	*Mustela furo*	2	5.1 ± 1.4	94.9 ± 1.4	0.0
Striped skunk	*Mephitis mephitis*	2	2.9 ± 0.6	97.1 ± 0.6	0.0
Viverridae					
Large spotted genet	*Genetta tigrina*	1	0.0	100.0	0.0
Suricat	*Suricata suricatta*	2	5.2 ± 0.7	86.5 ± 7.0	8.3 ± 6.0
Hyaenidae					
Brown hyaena	*Hyaena brunnea*	1	14.5	57.2	28.3
Cape aardwolf	*Proteles cristatus*	1	6.5	82.7	10.8
Felidae					
Asian lion	*Panthera leo persica*	1	6.8	91.1	2.1
Sumatran tiger	*Panthera tigris*	2	5.5 ± 0.4	84.6 ± 4.8	9.9 ± 5.2
Canidae					
Chinese dhole	*Nycterelites procyonoides*	1	4.8	83.2	12.0
Bat-eared fox	*Otocyon megalotis*	2	10.7 ± 3.0	84.7 ± 3.7	4.6 ± 0.7

See Table 1 for abbreviations.

percent of UDCA in biliary bile acids is comparatively low (1–8%).

The variability in the percent UDCA in biliary bile acids in the black bear is curious. It is known that bears tend to maintain small localized breeding populations (S. Fain, personal communication), and the absence of UDCA in two bears may have resulted from incomplete genetic expression of the enzymes necessary to form UDCA. UDCA was present in appreciable proportions in both hibernating and nonhibernating bears. For adult bears, no correlation was observed between the age of the bear (15 bears examined, data not shown) and the percent UDCA present in bile. For all bears, UDCA was present in both neonates and cubs; for a given species the percentage in neonates and cubs was lower than in the corresponding adults. In five black bears examined by MacDonald and Williams (22), the two cubs also had a lower proportion of UDCA (9.8%) than was present in biliary bile acids of the adults (21.0%).

UDCA was not present at significant levels in any of the remaining families in the order Carnivora, indicating that only the Ursidae synthesize UDCA as a major primary bile acid. Other carnivore families (Table 4) form chenodeoxycholic acid and cholic acid during bile acid biosynthesis in the liver.

The controversy as to whether UDCA is present in all Ursidae should be resolved by the data presented here. Several workers (22–24) have consistently found UDCA, but others have suggested that it is lacking in at least some species of Ursidae (25, 26).

UDCA as a primary bile acid in the bear

In the biliary fistula animal, all biliary bile acids are formed in the liver because bile acids are not exposed to bacterial enzymes. The finding that UDCA was a major biliary bile acid in chronic bile fistula bears indicates that UDCA is a primary bile acid in these Ursidae. Additional evidence for UDCA being a primary bile acid in the Polar, Brown, and Black bears was provided by the analyses of the fecal bile acids in six bear species. The fecal bile acid profile of the bears was in contrast to that of the carnivores, shown in Table 4. In these animals, and indeed in the majority of mammals, the fecal bile acid spectrum was dominated by secondary, 7-deoxy bile acids, particularly deoxycholic acid (data not presented). These analyses showed that fecal and biliary bile acid profiles were similar (Tables 1 and 3), and the 7-oxo precursor of UDCA was not detected. Thus, no evidence was found for bacterial formation of UDCA, or its 7-oxo precursor, in bears. Thus, although the data do not exclude some UDCA being formed by microbial biotransformation of chenodeoxycholic acid in the bear intestine, the proportion of UDCA formed by this pathway, if any, is likely to be quite small.

In the chronic biliary fistula bear, cholic acid levels were extremely low; and UDCA was the predominant

biliary bile acid. The reason for this astonishing finding is not known. In the chronic fistula animal, bile acid biosynthesis is likely to be greatly increased because the rate of bile acid biosynthesis is regulated in a negative feedback manner by the return of bile acids from the intestine (27). It may be that the capacity of the hepatic 12α-hydroxylase is extremely limited, or that under conditions of maximal bile acid biosynthesis, the precursor that is thought to undergo 12α-hydroxylation (7α-hydroxy-4-cholesten-3-one) (28) is unavailable to the enzyme.

The small percentage of UDCA in the biliary bile acids of several other vertebrates, including humans, has long been considered to originate at least in part from bacterial rather than hepatic biosynthesis, as biliary bile acids in biliary fistula patients contain predominantly chenodeoxycholic acid and cholic acid (29). Trace proportions of UDCA can arise in the intestine by bacterial interconversion of chenodeoxycholic acid to UDCA via a 7-oxo intermediate (19–21). In addition, the 7-oxo derivative of chenodeoxycholic acid can be formed by bacterial enzymes in the distal intestine and absorbed. In the liver, this compound will be reduced to form chenodeoxycholic acid and UDCA (17, 18). Very recently, the presence of the 7β epimer and the 7-oxo derivative of cholic acid has been reported in the biliary bile acids of a patient with a complete biliary fistula (30). This observation strongly suggests that UDCA can be a primary bile acid in humans.

Because UDCA is a dominant primary bile acid in Ursidae and the coypu, there must be epimerization in the hepatocyte of the 7α-hydroxyl group that is formed by 7α-hydroxylation of cholesterol or of 27-hydroxy-cholesterol, generally accepted steps in bile acid biosynthesis (28). Recently Shoda et al. (31) and his colleagues have found that mitochondria obtained from human liver possess a 7-epimerase that acts on the 7α-hydroxy derivative of 27-hydroxy cholesterol, and it is possible that this pathway is involved.

Biological utility of UDCA: evolutionary considerations

Fig. 1 shows both the phylogenetic relationships (adapted from reference 32) of the family Ursidae (and pandas) and the average percentage of UDCA found in each species. It is apparent that the formation of UDCA by bears is a comparatively recent event in evolution, as UDCA concentrations > 6% are found only in the most recently derived bears.

The biological advantage gained by forming UDCA instead of a trihydroxy bile acid from cholesterol as is common in most vertebrates is unclear. The diet of Group I bears is noteworthy in containing the highest percentage fat and lowest percentage protein in grams/kg body weight of any mammal (33). The taurine conjugate of UDCA has a lower critical micellization concentration than that of taurine-conjugated cholic acid, and should promote triglyceride digestion and absorption at a lower bile acid concentration than cholyltaurine. UDCA appears to have hepatoprotective properties in animal studies and is currently being used in the treatment of cholestatic liver disease (34, 35). UDCA is 7-dehydroxylated to form lithocholic acid by colonic bacteria in animals with a cecum (36), and lithocholic acid is known to be a highly hepatotoxic bile acid. In the bear, a cecum is not present (37) and formation of lithocholic acid by 7-dehydroxylation does not occur in most bear species. In many vertebrates, chenodeoxycholic acid undergoes hydroxylation at the 6 or 12 position during the process of bile acid biosynthesis, precluding the formation of lithocholic acid (10). This step is no advantage to Ursidae as they do not form lithocholic acid in their colon.

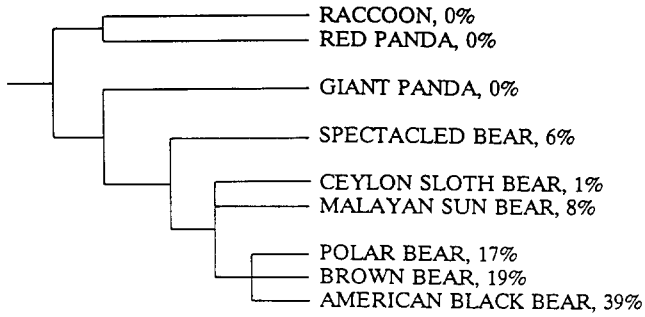

Fig. 1. A comparison of the percent UDCA in biliary bile acids with a phylogenetic tree of the family Ursidae and related procyonids (adapted from reference 31). UDCA is present as a major biliary bile acid only in Ursidae. Concentrations of UDCA > 8% are found only in the most recently derived Ursidae.

Medicinal use of UDCA and bear bile

Chinese traditional medicine has taught for many centuries that the bile of various animals (mammals, turtles, snakes, birds, and fishes) is useful in the treatment of biliary stone disease (J. Needham, personal communication 1975 and 1979; to be published as Part III, Volume 6, *Science and Civilization in China*, Cambridge University Press, New York). A historical survey of the therapeutic use of bile in Chinese medicine is available in the Chinese literature (38), as well as summaries of the Japanese use of bear bile (39, 40).

Throughout the Oriental literature on the analysis of "Yûtan" (Japanese for "bear's bile") or bear gallbladders, persistent references to the presence of glycine amidates are encountered (23, 41). In the capsules of "bear bile" purchased in traditional Chinese pharmacies that were available to us for analysis, the major constituent was pig bile, based on the biliary bile acid composition which showed the presence of hyocholic and hyodeoxycholic acids (data not presented). In addition, only 3% of crude dried "bear bile" samples originating from Asia and seized as criminal evidence contained UDCA; the remainder

contained dried pig bile (42). Pig bile differs from the bile of all carnivores not only in being rich in hyocholic and hyodeoxycholic acids, but also because its bile acids are amidated mostly with glycine, facilitating its identification by HPLC. This frequent substitution of pig bile for that of bear bile, coupled with the complete absence of glycine amidation in this study and in that of any other carnivore (10), makes the literature reporting glycine amidation in bears highly suspect.

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REFERENCES

1. Vahl, M., G. C. Amdrup, and L. Bobe. 1928–1929. Greenland. Volume 1. Commission for the Direction of the Geological and Geographical Investigations in Greenland, Ad. S. Jensen, Copenhagen.
2. Hammarsten, O. 1901. Untersuchungen über die Gallen einiger Polarthiere. *Hoppe-Seyler's Z. Physiol. Chem.* **32:** 435–466.
3. Hammarsten, O. 1902. Untersuchungen über die Gallen einiger Polarthiere. *Hoppe-Seyler's Z. Physiol. Chem.* **36:** 525–555.
4. Shoda, M. 1927. Über die Ursodesoxycholsäure aus Barengalle und ihre Physiologische Wirking. *J. Biochem.* **7:** 505–517.
5. Iwasaki, T. 1936. Über die Konstitution der Ursodeoxycholsäure. *Z. Physiol. Chem.* **244:** 181–193.
6. Kanazawa, T., A. Shimazake, T. Sato, and T. Hoshino. 1954. Synthesis of ursodeoxycholic acid and its conjugated bile acid. *Proc. Jpn. Acad.* **30:** 391–394.
7. Sjövall, J. 1959. The occurrence of 7β-hydroxylated bile acids in human bile. *Acta Chem. Scand.* **13:** 711–716.
8. Tammar, A. R. 1970. A comparative study of steroids with special reference to bile salts. Ph.D. Thesis. University of London, London. 1–218.
9. Haslewood, G. A. D. 1978. The Biological Utility of Bile Salts. North Holland Publishing Co., Amsterdam.
10. Hagey, L. R. 1992. Bile acid biodiversity in vertebrates: chemistry and evolutionary implications. Ph.D. thesis. University of California, San Diego, San Diego, CA. 1–205.
11. Haslewood, G. A. D. 1954. Comparative studies of "bile salts". 7. Bile acids of the coypu, *Myocastor coypus. Biochem. J.* **56:** 581–587.
12. Tint, G. S., J. Bullock, A. K. Batta, S. Shefer, and G. Salen. 1986. Ursodeoxycholic acid, 7-ketolithocholic acid, and chenodeoxycholic acid are primary bile acids of the nutria *Myocastor coypus. Gastroenterology.* **90:** 702–709.
13. Hofmann, A. F. 1989. Enterohepatic circulation of bile acids. *In* Handbook of Physiology. Section on the Gastrointestinal System. S. G. Schultz, editor. American Physiological Society, Bethesda. 567–596.
14. Mills, J. 1992. Milking the bear trade. *Int. Wildl.* **May/June:** 38–45.
15. Rossi, S. S., J. L. Converse, and A. F. Hofmann. 1987. High pressure liquid chromatographic analysis of conjugated bile acids in human bile: simultaneous resolution of sulfated and unsulfated lithocholyl amidates and the common conjugated bile acids. *J. Lipid Res.* **28:** 589–595.
16. Roovers, J., E. Evrard, and H. Vanderhaeghe. 1968. An improved method for measuring human blood bile acids. *Clin. Chim. Acta.* **19:** 449–457.
17. Fromm, H., G. L. Carlson, A. F. Hofmann, S. Farivar, and P. Amin. 1980. Metabolism in man of 7-ketolithocholic acid: precursor of cheno- and ursodeoxycholic acid. *Am. J. Physiol.* **239:** G161–G166.
18. Nakagaki, M., R. G. Danzinger, A. F. Hofmann, and A. Roda. 1983. Hepatic biotransformation of two hydroxy-7-oxotaurine-conjugated bile acids in the dog. *Am. J. Physiol.* **245:** G411–G417.
19. Hirano, S., N. Masuda, and H. Oda. 1981. In vitro transformation of chenodeoxycholic acid and ursodeoxycholic acid by human intestinal flora, with particular reference to the mutual conversion between the two bile acids. *J. Lipid Res.* **22:** 735–743.
20. MacDonald, I. A., D. Hutchinson, and T. Forrest. 1981. Formation of urso- and ursodeoxy-cholic acids from primary bile acids by *Clostridium absonum. J. Lipid Res.* **22:** 458–466.
21. Higashi, H., T. Setoguchi, and T. Katsuki. 1979. Conversion of 7-ketolithocholic acid to ursodeoxycholic acid by human intestinal anaerobic microorganisms: interchangeability of chenodeoxycholic acid and ursodeoxycholic acid. *Gastroenterol. Jpn.* **14:** 417–424.
22. MacDonald, A. C., and C. N. Williams. 1985. Studies of bile lipids and bile acids of wild North American black bears in Nova Scotia, showing a high content of ursodeoxycholic acid. *J. Surg. Res.* **38:** 173–179.
23. Kurozumi, K., T. Harano, K. Yamasaki, and Y. Ayaki. 1973. Studies on bile acids in bear bile. *J. Biochem.* **74:** 489–495.
24. Jirsa, M., J. Klinot, E. Klinotova, K. Ubik, and K. Kucera. 1989. Classical bile acids in animals, β-phocaecholic acid in ducks. *Comp. Biochem. Physiol.* **92B:** 357–360.
25. Takuma, T. 1949. The composition of bile of Polar bear, *Ursus maritimus,* and Japanese bear, *Ursus thibetanus japonicus. J. Jpn. Biochem. Soc. (Seikagaku)* **21:** 26–27.
26. Haslewood, G. A. D., and V. Wootton. 1950. Comparative studies of 'bile salts'. 1. Preliminary survey. *Biochem. J.* **47:** 584.
27. Vlahcevic, Z. R., D. M. Heuman, and P. B. Hylemon. 1991. Regulation of bile acid synthesis. *Hepatology.* **13:** 590–600.
28. Russell, D. W., and K. D. R. Setchell. 1992. Bile acid biosynthesis. *Biochemistry.* **31:** 4737–4749.
29. Ekdahl, P. H., and J. Sjövall. 1957. On the conjugation and formation of bile acids in the human liver. I. On the excretion of bile acids by patients with postoperative choledochostomy drainage. *Acta Chir. Scand.* **114:** 439–452.
30. Férézou, J., P. Beau, M. Parquet, G. Champarnaud, C. Litton, and C. Matuchansky. 1993. Cholesterol and bile acid biodynamics after total small bowel resection and bile diversion in humans. *Gastroenterology.* **104:** 1786–1795.

31. Shoda, J., A. Toll, M. Axelson, F. Pieper, K. Wikvall, and J. Sjövall. 1993. Formation of 7α- and 7β-hydroxylated bile acid precursors from 26-hydroxycholesterol in human liver microsomes and mitochondria. *Hepatology.* **17:** 395–403.
32. O'Brien, S. J., W. G. Nash, D. E. Wildt, M. Bush, and R. E. Benveniste. 1985. A molecular solution to the riddle of the Giant Panda's phylogeny. *Nature.* **317:** 140–144.
33. Nelson, R. A. 1987. Black bears and Polar bears—still metabolic marvels. *Mayo Clin. Proc.* **62:** 850–853.
34. Poupon, R. E., B. Balkau, E. Eschwege, R. Poupon, and the UDCA-PBC Study Group. 1991. A multicenter, controlled trial of ursodiol for the treatment of primary biliary cirrhosis. *N. Engl. J. Med.* **324:** 1548–1554.
35. Heuman, D. M. 1993. Hepatoprotective properties of ursodeoxycholic acid. *Gastroenterology.* **104:** 1865–1870.
36. Yahiro, K., T. Setoguchi, and T. Katsuki. 1980. Effect of cecum and appendix on 7α-dehydroxylation and 7β-epimerization of chenodeoxycholic acid in the rabbit. *J. Lipid Res.* **21:** 215–222.
37. Stevens, C. E. 1988. Comparative Physiology of the Vertebrate Digestive System. Cambridge University Press, Cambridge.
38. Thang, T-H. 1959. Than Tan Chih ti Liao Hsiao Yung. *Shanghai J. Trad. Chinese Med.* **11:** 42.
39. Makino, I., and K. Takebe. 1982. Bear bile and ursodeoxycholic acid. *Sogo Rinsho.* **31:** 2385–2387.
40. Lindley, P. F., and M. C. Carey. 1987. Molecular packing of bile acids: structure of ursodeoxycholic acid. *J. Crystallogr. Spectrosc. Res.* **17:** 231–249.
41. Miyazi, S. 1937. Über Glyko-ursodeoxycholsäure aus Baerengalle. *Hoppe-Seyler's Z. Physiol. Chem.* **250:** 34–36.
42. Espinoza, E. O., J. A. Shafer, and L. R. Hagey. 1993. International trade in bear gallbladders: forensic source inference. *J. Forensic Sci.* In press.