Biliary bile acids of fruit pigeons and doves (Columbiformes): presence of 1β-hydroxychenodeoxycholic acid and conjugation with glycine as well as taurine

Lee R. Hagey,[1] Claudio D. Schteingart, Huong-Thu Ton-Nu, and Alan F. Hofmann

Division of Gastroenterology, Department of Medicine, University of California, San Diego, La Jolla, CA 93092-0813

Abstract The biliary bile acid composition of 30 species of pigeons and doves belonging to seven genera in the avian order Columbiformes was determined using TLC, HPLC, GLC/MS, LSIMS, and NMR. In 23 of 25 species of fruit pigeons and doves, chenodeoxycholic acid was the major bile acid (>50%). In only 1 species (*Ptilinopus ornatus*) was cholic the major bile acid. A number of species (7 of 15 species in the genus *Ptilinopus,* and 6 of 9 species in the genus *Ducula*) contained 1β,3α,7α-trihydroxy- 5β-cholan-24-oic acid in proportions ranging from 2 to 43%. This 1β-hydroxy derivative of chenodeoxycholic acid has not been previously identified as a major biliary bile acid in vertebrates. Five of 15 species of the genus *Ptilinopus,* 5 of 9 species of the genus *Ducula,* and the only species examined for the genus *Gymnophaps* contained 23*R*-hydroxy chenodeoxycholic acid in detectable proportions, ranging from 1 to 4%. Bile acids were conjugated (in N-acyl linkage) with glycine and taurine in 28 species and with only taurine in 2 species. The fruit pigeons are the first non-mammalian genera identified to date in whom bile acids are conjugated with glycine, as well as with taurine. An incidental finding was that a gallbladder was present in 3 genera (*Ptilinopus, Ducula,* and *Gymnophaps*) and absent in 4 genera (*Gallicolumba, Chalcophaps, Otidiphaps,* and *Treron*).—**Hagey, L. R., C. D. Schteingart, H-T. Ton-Nu, and A. F. Hofmann.** Biliary bile acids of fruit pigeons and doves (Columbiformes): presence of 1β-hydroxychenodeoxycholic acid and conjugation with glycine as well as taurine. *J. Lipid Res.* 1994. **35:** 2041–2048.

Supplementary key words glycine conjugation • phylogeny • gallbladder • *Ptilinopus* • *Ducula*

According to one fable (1), the dove lost its gallbladder after the great flood described in the bible. After the dove was released from the ark by Noah, it burst its gallbladder out of grief when it could not find land; as a result, no dove or pigeon has had one since. Perhaps because of belief in this fable of Lamarckian genetics that all pigeons lacked gallbladders and that it would be difficult to obtain bile from the bile ducts of birds, only one analysis (2) of the biliary bile acids of a Columbiform bird has been reported. In this study, the White Carneau and Show Racer pigeons, two strains of rock dove (*Columba livia*), contained the common primary bile acids, chenodeoxycholic acid and cholic acid; biliary bile acids were shown to be conjugated with glycine as well as with taurine. In previous publications (3, 4), we have shown that avian biliary bile acids vary considerably in type, and have suggested that biliary bile acid composition is a trait that is useful for determining evolutionary relationships. In this paper, we present a survey of the biliary bile acids in 26 of the approximately 100 species of fruit pigeons and doves, as well as of single representatives from 4 other Columbiform genera.

MATERIALS AND METHODS

Samples

Bile samples were obtained from deceased birds housed at the San Diego Zoo, and were provided by the Pathology Laboratory of the San Diego Zoo. While in captivity, all birds had been fed a standard fruit mix diet consisting of papaya, apple, melon, grapes, and cooked rice. Gallbladder samples were obtained by puncture and aspiration; hepatic bile from birds without gallbladders was collected from the common bile duct. Bile samples were diluted in several volumes of reagent grade isopropanol immediately after collection to prevent bacterial degradation and to precipitate biliary proteins.

Abbreviations: TLC, thin-layer chromatography; HPLC, high performance liquid chromatography; GLC-MS, gas-liquid chromatography-mass spectrometry; LSIMS, liquid secondary ion mass spectrometry; NMR, nuclear magnetic resonance; RRT, relative retention time.

[1]To whom correspondence should be addressed.

Analytical procedures

TLC was used to separate and isolate individual classes of bile acids (unconjugated, glycine amidates, sulfates, glucuronides, and taurine amidates) as previously described (4). These classes of bile acids were tentatively identified by group specific spray reagents (5). Bile acids were isolated by scraping selected bands off a TLC plate and eluting with $CHCl_3$–MeOH 2:1 (v/v). Conjugated bile acids were analyzed by HPLC using a modification of the technique of Rossi, Converse, and Hofmann (6). The method uses a octadecylsilane column (RP C-18) with isocratic elution at 0.75 ml/min. The eluting solution is composed of a mixture of methanol (67.4% by volume) and 0.01 M KH_2PO_4, adjusted to an apparent pH 5.4. Bile acids are detected in the column effluent by monitoring absorbance at 205 nm (for the amide bond). The semisystematic nomenclature for bile acids used in this paper is based on recent recommendations of an international committee (7).

Bile acids were deconjugated chemically (1.0 N NaOH, 130°C, 4 h), and the resulting unconjugated bile acids were isolated by solvent extraction. They were then analyzed by gas–liquid chromatography–mass spectrometry (GLC–MS) as methyl esters, methyl ester acetates, or methyl ester trimethylsilyl derivatives. The instrument was a Hewlett-Packard 5890 Gas Chromatograph-5970 MSD, controlled by HP/UX Chem Station software. The column was a Supelco 30 m 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 was used as the carrier gas with a 6 psi column head pressure. Relative retention times and fragmentation spectra of peaks obtained by GLC–MS were compared with those of known standards for identification. Using this methodology, >95% of all GLC peaks could be assigned to known compounds. Allylic bile acids (Δ^4 or Δ^5) were not present.

Additional mass spectral information was obtained at the Biomedical Mass Spectrometry Facility at the University of California, San Francisco. The negative LSIMS (liquid secondary ion mass spectrometry) data were acquired using a VG 70-SE mass spectrometer (Manchester, UK) equipped with a standard VG LSIMS ion source. The instrument was operated at an accelerating voltage of 8 kv and a mass resolution of 1000 (10% valley definition). The liquid matrix was glycerol. The proton NMR of 1β-hydroxychenodeoxycholyltaurine and 1β-hydroxychenodeoxycholylglycine, isolated and purified as the intact conjugate by TLC, were recorded at 500 MHz in d_4-methanol using a Varian Unity 500 in the University of California, San Diego Department of Chemistry. An authentic sample of 1β-hydroxychenodeoxycholic acid was generously supplied by M. Tohma of Higashi-Nippon-Gakuen University, Japan.

RESULTS

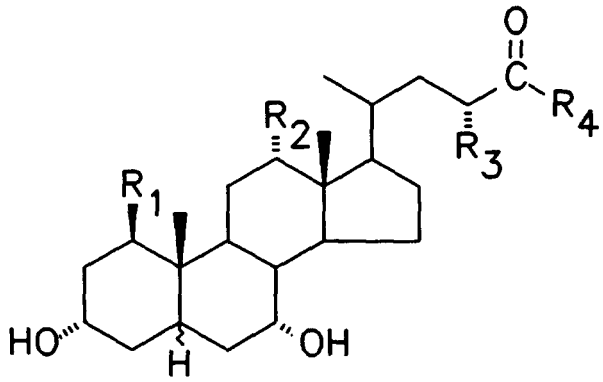
Structure assignments: steroid moiety

The chemical identity and trivial or semisystematic names of the bile acids that were detected are shown in **Fig. 1.** The identification of 1β-hydroxychenodeoxycholic acid was established by LSIMS analysis (*m/z* 514, trihydroxy taurine conjugate; *m/z* 464, trihydroxy glycine conjugate) of material isolated by TLC. After deconjugation and derivatization to trimethylsilyl methyl esters, the resulting GLC–MS spectra exhibited a characteristic base peak at *m/z* 217, indicating the presence of a 1,3-bistrimethylsiloxy structure, as well as fragment ions at *m/z* 316 (formed by the loss of the steroid A-ring) and at *m/z* 255 (ABCD ring fragment ion peak). This spectrum was identical to that reported by Tohma et al. (8); and the GC RRT (1.36 relative to lithocholic acid) was identical to that of the synthetic standard. ^{1}H-NMR (500 MHz in d_4-methanol) showed protons geminal to hydroxy groups at 3.862 bs (H-1α), 3.834 m (H-3β), and 3.787 bs (H-7β). The presence of a 1β-hydroxy group caused a shift in the position of methyl-19 (1.014 ppm) relative to chenodeoxycholyltaurine (0.919 ppm). This spectrum was also identical to that reported by Tohma et al. (9). 23R-Hydroxychenodeoxycholic acid was identified by methods previously reported (4).

Structure assignments: amino acid moiety

Conjugation of bile acids with either taurine or glycine (in N-acyl linkage) was confirmed by a combination of two methods. Initially, bile samples from each species were examined by LSIMS (negative mode) to determine the molecular weight of the anions present. As an example, LSIMS of whole bile (isopropanol extract) from the Ornate fruit dove (*Ptilinopus ornatus*), is shown in **Fig. 2.** The peaks observed at *m/z* 448 and 464 correspond to glycine conjugates of di- and tri-hydroxy bile acids, respectively, which were subsequently shown using HPLC to be primarily chenodeoxycholylglycine (dihydroxy) and cholylglycine (trihydroxy). No other known bile acid-amino acid combination generates signals at *m/z* 448 and 464. The weaker signals seen at *m/z* 470 and 486 are generated by the sodium salts ($M-2H^+ + 23$) of the corresponding glycine conjugate. The peaks observed at *m/z* 498 and 514 correspond to taurine conjugates of di- and trihydroxy bile acids, respectively. By HPLC these peaks were primarily chenodeoxycholyltaurine and cholyltaurine. Minor peaks at *m/z* 520 and 536 correspond in turn to the sodium salts of these two taurine conjugated bile acids.

To provide additional structural information to that given by LSIMS, the samples were analyzed by HPLC (reverse phase). **Figure 3** shows the HPLC bile acid profile of a Pinon Imperial pigeon (*Ducula pinon*), a species containing glycine and taurine conjugates in about equal proportions. Samples from each of the major peaks were



Conjugated Biliary Bile Acids of the Columbidae

Bile Acid	H-5	R_1	R_2	R_3	R_4	HPLC RRT	Semisystematic Name
A	β	βOH	H	H	taurine	0.13	1β-hydroxychenodeoxycholyltaurine
B	β	βOH	H	H	glycine	0.16	1β-hydroxychenodeoxycholylglycine
C	β	H	H	*R*OH	taurine	0.34	23*R*-hydroxychenodeoxycholyltaurine
D	β	H	αOH	H	taurine	0.38	cholyltaurine
E	β	H	H	*R*OH	glycine	0.46	23*R*-hydroxychenodeoxycholylglycine
F	B	H	αOH	H	glycine	0.55	cholylglycine
G	β	H	H	H	taurine	0.67	chenodeoxycholyltaurine
H	α	H	H	H	taurine	0.70	allochenodeoxycholyltaurine
J	β	H	H	H	glycine	1.00	chenodeoxycholylglycine
K	α	H	H	H	glycine	1.11	allochenodeoxycholylglycine

Fig. 1. Structural identity, HPLC retention times (reverse phase C_{18}), and semisystematic names of the conjugated bile acids observed in Columbiform bile. Semisystematic names are based on recently published guidelines (7).

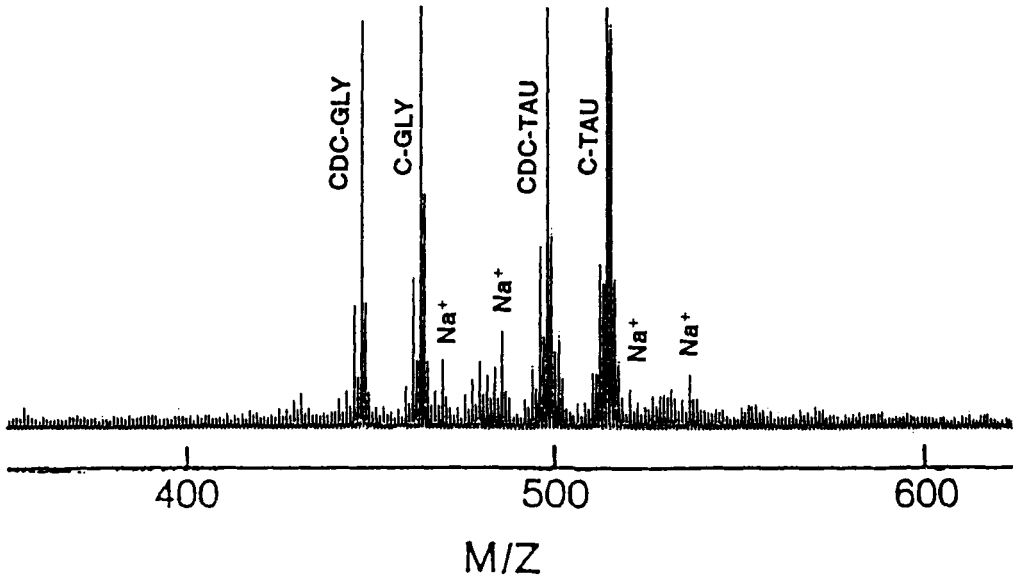


Fig. 2. Negative mode secondary ionization mass spectra of gallbladder bile of the Ornate Fruit Dove (*Ptilinopus ornatus*). Peak identification: CDC-gly (*m/z* 448), chenodeoxycholylglycine; C-gly (*m/z* 464), cholylglycine; CDC-tau (*m/z* 498), chenodeoxycholyltaurine; C-tau (*m/z* 514), cholyltaurine. Peaks labelled Na^+ are the corresponding sodium salts (*m/z* M-2H + 23).

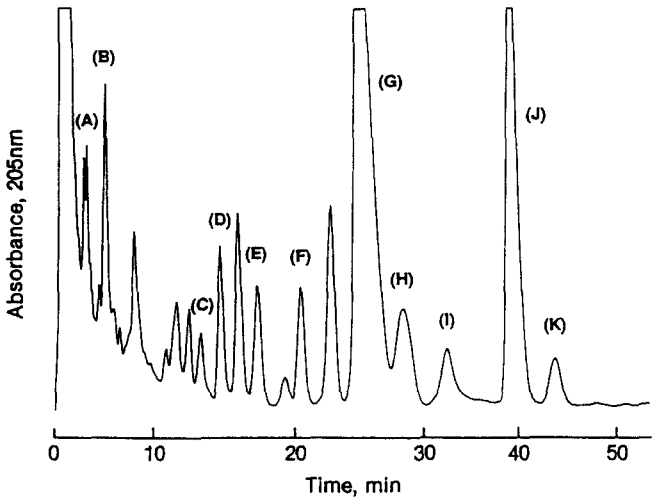


Fig. 3. Reversed phase (RP C-18) HPLC bile acid profile of the Pinon Imperial Pigeon. The relative retention times are given in Fig. 1. The percent composition follows the semisystematic name of the compound. (A) 1β-hydroxychenodeoxycholyltaurine 2.3% (the second peak); (B) 1β-hydroxychenodeoxycholylglycine 4.8%; (C) 23*R*-hydroxychenodeoxycholyltaurine 3.0%; (D) cholyltaurine 3.6%; (E) 23*R*-hydroxychenodeoxycholylglycine 3.2%; (F) cholylglycine 3.6%; (G) chenodeoxycholyltaurine 40.3%; (H) allochenodeoxycholyltaurine 6.1%; (I) unidentified bile pigment; (J) chenodeoxycholylglycine 29.9%; (K) allochenodeoxycholylglycine 3.2%.

collected from the HPLC effluent, spotted on TLC, and analyzed for molecular weight by LSIMS. After subsequent deconjugation and derivatization, they were analyzed by GLC-MS. The following bile acids (conjugated with taurine and glycine, respectively) were identified: 1β-hydroxychenodeoxycholic acid, peaks A and B; 23*R*-hydroxychenodeoxycholic acid, peaks C and E; cholic acid, peaks D and F; chenodeoxycholic acid, peaks G and J; and allochenodeoxycholic acid, peaks H and K. Peak I was an unidentified pale blue-grey biliary pigment.

Biliary bile acid composition of individual Columbid species

The complete biliary bile acid composition of 15 species of fruit doves in the genus *Ptilinopus* is summarized in **Table 1.** In 11 of 15 species, chenodeoxycholic acid was the predominant bile acid present (range 69–94%). Allochenodeoxycholic acid, the 5α-isomer of chenodeoxycholic acid, was present in small proportions in 14 of 15 birds. In the 4 remaining species, appreciable amounts of cholic acid were present (range 25–55%). In these 4 species, detectable proportions (up to 5%) of allocholic acid were observed (data not shown in Table 1). A substantial proportion of 1β-hydroxychenodeoxycholic acid was detected in 7 of 15 species, with the greatest proportion (20%) being present in Merrill's fruit pigeon (*Ptilinopus merrilli*). Low proportions of the side-chain hydroxy bile acid, 23*R*-hydroxychenodeoxycholic acid, were also detected in many species. The common secondary bile acids, lithocholic acid and deoxycholic acid (bacterial 7-dehydroxylation products of chenodeoxycholic acid and cholic acid, respectively) were not present (concentrations $<0.5\%$ of total bile acids).

TABLE 1. HPLC analysis of the biliary bile acids present in gallbladder bile from the Columbiform genus *Ptilinopus* (fruit pigeons)

			Bile Acid Composition, %[a]					
				Steroid Moiety				
Common Name	Latin Name	n	Allo 3α7α[b]	3α7α	23R3α7α	1β3α7α	3α7α12α	Amino Acid Moiety % Gly[c]
Merrill's fruit dove	*P. merrilli*	2	7	69 (27)	0	20	4	53 (19)
Marche's fruit dove[d]	*P. marchei*	1	5	82	2	10	1	46
Wompoo fruit dove	*P. magnificus*	2	5	87 (4.6)	0	8	0	29 (14)
Superb fruit dove	*P. superbus*	2	4	88 (3.4)	1	5	2	67 (15)
Black-chinned fruit dove	*P. leclancheri*	2	5	86 (6.6)	0	7	2	57 (1.2)
Jambu fruit dove	*P. jambu*	7	3	89 ± 4.8	1	5	2	59 ± 5.4
Black-naped fruit dove	*P. melanospila*	1	6	90	0	2	2	74
Vogelkop coronated fruit dove	*P. c. trigeminus*[e]	1	6	92	0	0	2	74
Beautiful fruit dove	*P. pulchellus*	2	3	94 (1.7)	1	0	2	74 (0.3)
Yellow-breasted fruit dove	*P. occipitalis*	1	5	95	0	0	0	87
Black-backed fruit dove	*P. c. albocinctus*	1	5	93	0	0	2	65
Pink-neck fruit dove	*P. porphyrea*	1	3	72	0	0	25	55
Pink-spotted fruit dove	*P. perlatus*	2	1	63 (2.2)	0	0	36	93 (2.3)
Orange-bellied fruit dove	*P. iozonus*	2	0	58 (7.8)	1	0	41	96 (0.5)
Ornate fruit dove	*P. ornatus*	1	tr	45	0	0	55	82

[a]Gallbladder bile acid composition has been normalized to 100%. Bile acids reported usually comprise >95% of total bile acids.
[b]Abbreviations: allo3α7α, allochenodeoxycholic acid; 3α7α, chenodeoxycholic acid; 23R3α7α, 23R-hydroxychenodeoxycholic acid; 1β3α7α, 1β-hydroxychenodeoxycholic acid; 3α7α12α, cholic acid; tr, trace.
[c]Bile acids were amidated with either glycine or taurine. The percentage of amidation with glycine is indicated.
[d]Values for three or more samples are expressed as the mean ± standard deviation. When two samples were analyzed, the percent difference is indicated in parentheses.
[e]Subspecies are designated by three Latin names with the middle initial shown.

TABLE 2. HPLC analysis of biliary bile acids present in gallbladder bile from the Columbiform genera *Ducula* and *Gymnophaps*

Common Name	Latin Name	n	Bile Acid Composition, %[a]					
			Steroid Moiety					Amino Acid Moiety
			Allo 3α7α	3α7α	23R3α7α	1β3α7α	3α7α12α	% Gly
Spotted imperial pigeon	*D. carola*	1	8	44	2	43	3	58
Celebes imperial pigeon	*D. forsteni*	1	5	56	3	35	1	51
Pinon imperial pigeon	*D. pinon*	4	5	82 ± 7.9	4	5	4	48 ± 11.4
Purple-tail pigeon	*D. rufigaster*	3	3	87 ± 3.7	1	4	5	56 ± 1.5
Zoe imperial pigeon	*D. zoeae*	1	4	88	2	3	3	72
Grey imperial pigeon	*D. pickeringii*	1	4	71	0	2	23	76
Green imperial pigeon	*D. aenea*	3	3	96 ± 0.9	0	0	1	89 ± 4.3
Nutmeg pigeon	*D. spilorrhoa*	2	3	93 (0.8)	0	0	4	68 (0.4)
Flores dark-back pigeon	*D. l. sasakensis*	1	3	97	0	0	0	63
Papuan mountain pigeon	*G. albertisii*	2	0	90 (0.5)	1	0	9	32 (0.3)

[a]See Table 1 for abbreviations.

Glycine conjugation (range 29–95%) was observed in all 15 species. The mode of conjugation was essentially identical for all bile acids in a given species.

Table 2 shows the bile acid composition of 9 species of fruit pigeons in the genus *Ducula* as well as that of the Papuan Mountain pigeon (*Gymnophaps albertisii*), a bird sometimes classified as a fruit pigeon. Only one species, the Grey Imperial pigeon (*Ducula pickeringii*), had more than 6% cholic acid. Two birds had high proportions of 1β-hydroxychenodeoxycholic acid: the Celebes Imperial (*Ducula forsteni,* 35%) and the Spotted Imperial pigeon (*Ducula carola,* 43%).

The relative proportions of the three major bile acids found in fruit pigeons are shown using triangular coordinates in **Fig. 4.** This method of graphics was applicable because these three bile acids made up >90% of the total bile acids. The three different genera from Tables 1 and 2 are represented in the figure using different symbols. Chenodeoxycholic acid was the predominant biliary bile acid in most species (as depicted by the clustering at

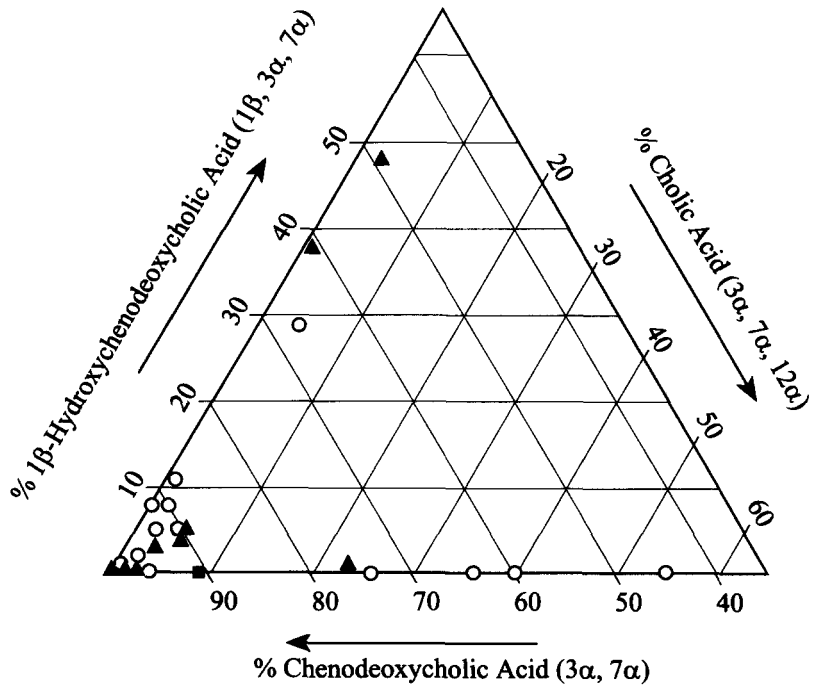


Fig. 4. Relative proportions of the three major bile acids of the genera *Ptilinopus* (○), *Ducula* (▲), and *Gymnophaps* (■) shown using triangular coordinates. Data have been normalized to 100% as these three bile acids composed 90% of the total bile acids in each species.

TABLE 3. HPLC analysis of biliary bile acids present in common duct bile from four genera of pigeons and doves that lack gallbladders (Columbiformes)

Common Name	Latin Name	n	Bile Acid Composition, %[a]					
			Steroid Moiety					Amino Acid Moiety
			Allo 3α7α	3α7α	23R3α7α	1β3α7α	3α7α12α	% Gly
Celebes quail dove	*Gallicolumba t. tristigmata*	1	0	100	0	0	0	0
Luzon bleeding heart dove	*Gallicolumba luzonica*	1	7	91	0	0	2	0
Green wing dove	*Chalcophaps i. indica*	1	0	89	0	0	11	14
Green napes pheasant pigeon	*Otidiphaps n. nobilis*	1	4	96	0	0	0	77
W. African green pigeon	*Treron calva*	1	0	0	0	0	100	86

[a]See Table 1 for abbreviations.

the chenodeoxycholic acid apex). The absence of species in the center of the graph indicates that only a single mode of additional hydroxylation to form a trihydroxy bile acid occurs within most species.

The biliary bile acid composition of 5 additional Columbiform species belonging to 4 genera that lacked gallbladders is shown in **Table 3.** In the two species of the genus Gallicolumba (Celebes Quail Dove *Gallicolumba t. tristigmata,* Luzon Bleeding-Heart dove *Gallicolumba luzonica*), bile acids were conjugated with taurine only. None of these four genera contained 1β-hydroxychenodeoxycholic acid or 23*R*-hydroxychenodeoxycholic acid. The West African Green Pigeon (*Treron calva*) appears to have a unique biliary bile acid composition among Columbids, in consisting solely of cholyl conjugates.

DISCUSSION

The major findings in the analyses reported in this paper may be summarized as follows. *1)* The predominant bile acid for a representative sample of fruit pigeons and doves is the simplest C_{24} primary bile acid, chenodeoxycholic acid; only one species contained cholic acid as its major bile acid; *2)* the biliary bile acids of some species of fruit pigeons contain appreciable proportions of the novel bile acid, 1β-hydroxychenodeoxycholic acid as well as low levels of the uncommon bile acid 23R-hydroxychenodeoxycholic acid; *3)* glycine conjugation as well as taurine conjugation of bile acids is present in most species of fruit pigeons; *4)* one Columbid, (West African Green Pigeon, *Treron calva*) had solely cholic acid in its biliary bile acids; and *5)* the common bacterial metabolites, lithocholic acid and deoxycholic acid, are absent in bile. An incidental observation was that all birds examined from the genera *Ptilinopus, Ducula,* and *Gymnophaps* were found to possess gallbladders, whereas birds from the genera *Gallicolumba, Chalcophaps, Otidiphaps,* and *Treron* lacked gallbladders.

Presence of 1β-hydroxychenodeoxycholic acid

1β-Hydroxy bile acids have not been previously reported in vertebrates other than as trace constituents in the urine of neonatal humans (10), in human fetal bile (11), in meconium, amniotic fluid, and cord blood of infants (9, 12), and in urine of patients with cholestatic liver disease (12). Hydroxylation of the bile acid ursodeoxycholic acid at the 1β position has been observed following its administration to patients with cholestatic liver disease (13). In the present study, 1β-hydroxychenodeoxycholic acid was a major bile acid in two species (*Ducula carola,* 43%; *Ducula forsteni,* 35%) and a minor bile acid (2–20%) in 11 of the 23 species of fruit pigeons and doves described in this paper.

23*R*-Hydroxychenodeoxycholic acid in fruit pigeons

Many of the species examined in this study contained small proportions (1–4%) of glycine and taurine conjugated 23*R*-hydroxychenodeoxycholic acid. The taurine conjugate of this bile acid is present in Anseriforms (ducks and geese) and in flamingos (*Phoenicopteridae*) (4), as well as in marine mammals (14). Until now, however, the glycine conjugate of 23*R*-hydroxychenodeoxycholic acid had not been previously observed in vertebrate bile.

Lack of secondary bile acids in Columbid bile

Deoxycholic acid, a 7-deoxy bile acid commonly observed in many animals as the major intestinal bacterial biotransformation product of cholic acid, was not detected in the fruit pigeon species that had appreciable proportions of cholic acid. The bacteria mediating 7-dehydroxylation are known to be strict anaerobes (15). Hoshita and Ohigashi (16) reported that secondary bile acids (like deoxycholic acid) are absent in the fecal bile acids of the domestic pigeon. In general, seed-eating birds such as the Columbids have relatively short large intestines (approximately 3% of total intestinal length) and the cecum is often only a pair of tiny blind pouches (17). In

a study of Columbiform intestinal anatomy performed by Garrod in 1874 (18), the cecum was found to be absent from the genera *Ptilinopus, Treron,* and *Chalcophaps;* a very short cecum was present in only 7 of 26 genera examined and was lacking in the remainder. Therefore, we interpret our finding of an absence of deoxycholic acid in the biliary bile acids of those Columbids with cholic acid in bile to indicate that without the anaerobic environment of a cecum, the 7-dehydroxylation of cholic acid to form deoxycholic acid cannot occur. The absence of lithocholic acid in all Columbids is likely to have the same explanation.

Glycine conjugation in birds

Among vertebrates, glycine conjugation is relatively rare, having been found previously only in placental mammals (14). The present work extends the previous work from this laboratory which reported the mode of bile acid conjugation in over 150 species of birds (3). With the sole exception of some Columbidae, all avian species were found to conjugate bile acids entirely with taurine.

In animals that conjugate bile acids with either taurine or glycine, the proportion of these two classes of conjugates in bile is the result of a combination of genetic and environmental factors. A steady state balance exists between hepatic conjugation of bile acids with glycine (versus taurine), bacterial deconjugation of glycine-conjugated bile acids (versus taurine-conjugated bile acids) in the distal intestine, and the intestinal absorption of glycine-conjugated bile acids (versus taurine-conjugated bile acids) during enterohepatic cycling (19). The use of glycine for bile acid conjugation by the fruit pigeons and doves indicates expression of either a new bile acid amino acid transferase or modification of an existing taurine amino acid transferase (or both) in the hepatocyte. The human enzyme has recently been cloned (20) and shown to mediate conjugation with either glycine or taurine.

Gallbladders in the Columbidae

Although early anatomists claimed that the gallbladder is absent within the Columbidae, in 1874 Garrod (18) observed that a gallbladder was present in all the species of *Ptilinopus, Lopholaimus,* and *Carpophaga.* The work of Garrod has been extended in the present work by the finding that a gallbladder is also present in the genera *Ducula* and *Gymnophaps.*

Evolutionary considerations

Fruit pigeons have generally been described as being distinct from other Columbids. The fact that all fruit pigeons possess a gallbladder suggests that they are related to an early ancestor of the family Columbidae. Garrod (18) believed that the presence of a gallbladder should be considered an important trait in Columbid systematics.

Little is known of the three closely related mountain pigeons of the genus *Gymnophaps.* Although they are often placed in the genus *Columba,* Goodwin (21) concluded that they are an offshoot of the fruit pigeons. The presence of a gallbladder and the close similarity of their bile acid composition to other members of the genus *Ducula* offer support for Goodwin's conclusion.

The geographical distribution of those species containing the highest proportions of 1β- and 12α-hydroxy bile acids (see Fig. 4) is not random. Three of four species of the genus *Ptilinopus* with the highest percentage of 1β-hydroxy biliary bile acids (*P. merrilli, P. marchei, P. leclancheri*) and both species from the genus *Ducula* (*D. carola, D. forsteni*) are from the Philippine islands; three of four species of the genus *Ptilinopus* with the highest percentage of 12α-hydroxylation (*P. perlatus, P. iozonus, P. ornatus*) are found in the region of New Guinea. Chang et al. (22) have shown that the substrate specificity of at least one bile acid hydroxylase (lithocholic acid 6β-hydroxylase) is not restricted to bile acids alone. It may be that the appearance of new hydroxylation sites in the bile acids of these frugivorous birds is the result of evolution of hydroxylases that initially served to detoxify dietary xenobiotics.

It is well established that conjugates of cholic acid, containing three hydroxy groups on the steroid nucleus, are intrinsically less cytotoxic than conjugates of chenodeoxycholic acid, a dihydroxy bile acid, when studied in vitro (23, 24). We have suggested (3) that the addition of a third hydroxy group has evolved as a means of detoxifying chenodeoxycholic acid. Work from our group and others has previously identified five types of nuclear hydroxylation pathways used by vertebrates to add an additional hydroxy group to chenodeoxycholic acid: 1α- (the Australian marsupial *Trichosurus vulpecula*) (25), 6α- (rodents and pigs) (14), 6β- (rodents), 12α- (most vertebrates), and 16α- (birds and snakes) (3). The data presented in this paper indicate that 1β-hydroxylation is an additional site of hydroxylation of chenodeoxycholic acid in vertebrates. It will be of interest to compare the cytotoxic properties of trihydroxy bile acids other than cholic acid with those of chenodeoxycholic acid.

We express our thanks to M. P. Anderson, Kent Osborn, and Anne Sawyers of the Pathology Department, San Diego Zoological Society, for collecting these samples. We also acknowledge Dazhi Pang and the Bio-Organic, Biomedical Mass Spectrometry Resource (A. L. Burlingame, Director), supported by the NIH Division of Research Resources, Grant RR0161H. Dr. M. Tohma kindly provided us with synthesized standards of 1β-hydroxychenodeoxycholic acid, 1β-hydroxycholic acid, and 1β-hydroxydeoxycholic acid. We also thank Vicky Huebner for typing the manuscript, and Joseph Steinbach for useful discussions. Financial support for this work was provided in part by NIH grants DK21506, DK32130, and DK07202, as well as grants in aid from Burroughs Wellcome and the Falk Foundation e.v. Germany. L.R.H. was a Postdoctoral Fellow supported by NRSA Grant DK07202 and the recipient of the Frank M. Chapman award of the American Museum of Natural History.

Manuscript received 16 March 1994 and in revised form 8 June 1994.

REFERENCES

1. Brewer, E. 1927. Brewer's Dictionary of Phrase and Fable. Lippincott Co., London.
2. Subbiah, M. T. R., B. A. Kottke, I. A. Carlo, and K. M. Weaver. 1971. Bile salts of spontaneously atherosclerosis-susceptible white carneau pigeon. *Steroids.* **17:** 509–519.
3. Hagey, L. R. 1992. Bile acid biodiversity in vertebrates: chemistry and evolutionary implication. Ph.D. Thesis. University of California, San Diego, San Diego, CA. 1–205.
4. Hagey, L. R., C. D. Schteingart, H-T. Ton-Nu, S. S. Rossi, D. Odell, and A. F. Hofmann. 1990. β-Phocacholic acid in bile: biochemical evidence that the flamingo is related to an ancient goose. *The Condor.* **92:** 593–597.
5. Oude-Elferink, R., J. Haan, K. J. Lambert, L. R. Hagey, A. F. Hofmann, and P. L. M. Jansen. 1989. Selective hepatobiliary transport of nordeoxycholate side chain conjugates in mutant rats with a canalicular transport defect. *Hepatology.* **9:** 861–865.
6. 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.
7. Hofmann, A. F., J. Sjövall, G. Kurz, A. Radominska, C. D. Schteingart, G. S. Tint, Z. R. Vlahcevic, and K. D. R. Setchell. 1992. A proposed nomenclature for bile acids. *J. Lipid Res.* **33:** 599–604.
8. Tohma, M., R. Mahara, H. Takeshita, T. Kurosawa, S. Ikegawa, and H. Nittono. 1985. Synthesis of the 1β-hydroxylated bile acids and identification of 1β,3α,7α-trihydroxy- and 1β,3α,7α,12α-tetrahydroxy-5β-cholan-24-oic acids in human meconium. *Chem. Pharm. Bull.* **33:** 3071–3073.
9. Tohma, M., R. Mahara, H. Takeshita, T. Kurosawa, and S. Ikegawa. 1986. Synthesis of the 1β-hydroxylated bile acids; unusual bile acids in human biological fluids. *Chem. Pharm. Bull.* **34:** 2890–2899.
10. Obinata, K., H. Nittano, K. Yabuta, R. Mahara, and M. Tohma. 1992. 1β-Hydroxylated bile acids in the urine of healthy neonates. *J. Pediatr. Gastroenterol. Nutr.* **15:** 1–5.
11. Setchell, K. D. R., R. Dumaswala, C. Colombo, and M. Ronchi. 1988. Hepatic bile acid metabolism during early development revealed from the analysis of human fetal gallbladder bile. *J. Biol. Chem.* **263:** 16637–16644.
12. Shoda, J., R. Mahara, T. Osuga, M. Tohma, S. Ohnishi, H. Miyazaki, N. Tanaka, and Y. Matsuzaki. 1988. Similarity of unusual bile acids in human umbilical cord blood and amniotic fluid from newborns and in sera and urine from adult patients with cholestatic liver diseases. *J. Lipid Res.* **29:** 847–858.
13. Fischer, S., M. Neubrand, and G. Paumgartner. 1993. Biotransformation of orally administered ursodeoxycholic acid in man as observed in gallbladder bile, serum and urine. *Eur. J. Clin. Invest.* **23:** 28–36.
14. Haslewood, G. A. D. 1978. The Biological Importance of Bile Salts. North Holland Publishing Co., Amsterdam.
15. Macdonald, I. A., V. D. Bokkenheuser, J. Winter, A. M. McLernon, and E. H. Mosbach. 1983. Degradation of steroids in the human gut. *J. Lipid Res.* **24:** 675–700.
16. Hoshita, T., and T. Ohigashi. 1960. The metabolism of bile acids (VII). On the metabolism of bile acids in the pigeon intestine. *Hiroshima J. Med. Sci.* **9:** 111–116.
17. Levi, W. 1963. The Pigeon. Levi Publishing Co., Sumter, SC.
18. Garrod, A. H. 1874. On some points on the anatomy of the Columbae. *Proc. Zool. Soc. London.* **17:** 249–259.
19. Hoffman, N. E., and A. F. Hofmann. 1977. Metabolism of steroid and amino acid moieties of conjugated bile acids in man. V. Equations for the perturbed enterohepatic circulation and their application. *Gastroenterology.* **72:** 144–148.
20. Johnson, M., C. N. Falany, S. Barnes, and R. B. Diasio. 1994. Isolation, sequencing and bacterial expression of a human liver cDNA encoding a bile acid CoA:amino acid N-acyltransferase which catalyzes both glycine and taurine amidation of bile acids. *J. Biol. Chem.* **269:** 19375–19379.
21. Goodwin, D. 1983. Pigeons and Doves of the World. Comstock Publishing Associates, Ithaca, NY.
22. Chang, T. K. H., J. Teixeira, G. Gil, and D. J. Waxman. 1993. The lithocholic acid 6β-hydroxylase cytochrome P-450 CYP 3α10, is an active catalyst of steroid hormone 6β-hydroxylation. *Biochem. J.* **291:** 429–434.
23. Heuman, D. M., W. M. Pandak, P. B. Hylemon, and Z. R. Vlahcevic. 1991. Conjugates of ursodeoxycholic acid protect against cytotoxicity of more hydrophobic bile salts: in vitro studies in rat hepatocytes and human erythrocytes. *Hepatology.* **14:** 920–926.
24. Quist, R. G., H-T. Ton-Nu, J. Lillienau, A. F. Hofmann, and K. E. Barrett. 1991. Activation of mast cells by bile acids. *Gastroenterology.* **101:** 446–456.
25. Lee, S. P., R. Lester, and J. S. Pyrek. 1987. Vulpecholic acid (1α,3α,7α-trihydroxy-5β-cholan-24-oic acid): a novel bile acid of a marsupial, *Trichosurus vulpecula. J. Lipid Res.* **28:** 19–31.