ω-Muricholate: A Tertiary Bile Acid of the Wistar Rat

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Abstract

Hyodeoxycholate (HDC) and *w*-muricholate (*w*-muri) are two bile acids found in gut contents of conventional but not of germfree rats. Both disappear upon intensive oral treatment with antibiotics. It was known that HDC is formed under bacterial influence, and that this bile acid may then be a precursor of *w*-muri. After feeding HDC to germfree rats we found *w*-muri in intestinal contents and feces. This indicates that HDC, and not a bacterial metabolite of this bile acid, is transformed by the liver to *w*-muri.

Introduction

The liver of vertebrates forms bile acids (BAs) de novo directly from cholesterol. These primary BAs may be transformed by intestinal bacteria to secondary BAs. Both types are for the most part reabsorbed in the ileum and large intestine, with a small fraction being excreted with the feces. Secondary BAs, after returning to the liver via the portal vein, may be changed back to their original, primary type. However, in certain cases the liver will remodel secondary BAs to those which differ qualitatively from both their primary and secondary precursors. One such "tertiary" BA is hyodeoxycholate (3α, 6α-dihydroxy-5β-cholanic acid) which has been shown to be derived ultimately from chenodeoxycholate (3α, 7α-dihydroxy-5β-cholanic acid) (1) or from β-muricholate (3α, 6β, 7β-dihydroxy-5β-cholanic acid) (2).

The determination of the origin of BAs in conventional animals often involves complicated procedures, including surgery (bile duct cannulation) or antibiotic treatment. Results can nevertheless be equivocal, especially since bile duct cannulation affects bile acid homeostasis irreversibly by removing material from the enterohepatic circulation.

By contrast, BAs which are found in the enterohepatic circulation of germfree rats are *a priori* defined as primary. In the germfree Lobund/Wistar rat, cholate and β-muri together comprise about 97% of all intestinal and fecal BAs, the remainder being chenodeoxycholate and traces of keto BAs (Table 1). In conventional rats many additional BAs occur, and these are presumably secondary. Hyodeoxycholate (HDC) and ω-muricholate (ω-muri) comprise about 30% and 20%, respectively, of total fecal BAs (Table 1) of the conventional Lobund rat.

ω-Muricholate (3α, 6α, 7β-trihydroxy-5β-cholanic acid) has only rarely been reported as a constituent of the enterohepatic circulation of the conventional rat. For this reason we attempted to prove the identity of the presumed ω-muri beyond reasonable doubt. Since the absence of ω-muri from germfree rats suggested that it is a secondary or possibly a "tertiary" BA, ω-muri would be unique in being the only known trihydroxy BA not primary in origin. We thus conducted experiments designed to elucidate the origin of ω-muri.

TABLE 1. *Fecal bile acids of germfree and conventional rats.*

Status	Bile Acids; % of Total (Mean ± S.E.)							
	Cholic	β-Muri	HDC	ω-Muri	Deoxy.	Cheno.	Litho.	Keto.
Germfree (8)[a]	40.5 ±3.1	56.9 ±3.3	—	—	—	1.4 ±0.7	0	tr[b]
Conventional (16)	3.9 ±0.4	2.2 ±0.4	34.0 ±2.4	18.7 ±1.6	15.6 ±2.5	—	1.2 ±0.4	24.2 ±2.5

[a] ( ) = number of samples analyzed
[b] trace = less than 0.5%

Materials and Methods

Germfree rats (LOB:(WI)h) were maintained in flexible plastic isolators by generally accepted procedures (5). The genetically closely related conventional animals were housed in wire bottom cages in an open animal room. All rats were males, 3-6 months of age. During the experimental periods they were fed steam sterilized semisynthetic diet L-488, based on the casein/rice starch formula L-474 E_{29} (6) with some slight modifications.

BAs were purified and analyzed by thin-layer-(TLC) and gas-liquid-chromatographic (GLC) techniques described elsewhere (3). The material presumed to be ω-muricholic acid was isolated from conventional rat feces by the above procedure combined with additional TLC purifications; purity was monitored by GLC. Nuclear (proton) magnetic resonance spectra and Mass spectra of authentic[1] and putative ω-muri were determined by Dr. D. Pasto of the Department of Chemistry, University of Notre Dame.

The HDC (free acid) was purchased from Applied Science, Inc. No contaminants were detected by TLC or by GLC.

Results

Identity of ω-Muricholate

It was found that the authentic sample of ω-muri and our purified isolate had identical R_f values in three different TLC solvent systems (3). The two compounds also had identical retention times on two GLC packings—1% SE-30 and 3% QF-1. Finally, proton magnetic resonance and mass spectra were identical. Dr. Pasto advises that all of this evidence indicates very strongly that the BA in question is actually ω-muri.

Metabolism of ω-Muricholate

In a study to be published elsewhere we fed an antibiotic cocktail (consisting of Streptomycin, Neomycin, Bacitracin, and Fungizone (4)) in drinking water to conventional rats. This resulted in the disappearance of HDC, ω-muri, and deoxycholate from the feces, thus strengthening the idea that ω-muri is a secondary or a "tertiary" BA.

There was a reason to expect that HDC might be a precursor of ω-muri (see discussion). In order to elucidate the possible role of bacteria in the formation of ω-muri from HDC, we fed HDC to germfree rats with the diet. ω-Muri was subsequently detected in the intestines and feces of these germfree rats (Table 2).

Discussion

Einarsson (1966) showed that HDC is formed in the rat by a complex sequence, as follows:

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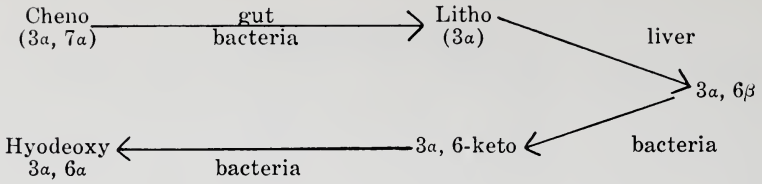
[1] Authentic *w*-muri was a generous gift of Dr. William Elliott, Department of Biochemistry, St. Louis University, St. Louis Mo.

TABLE 2. *Bile acids of the germfree rat: Result of feeding hyodeoxycholate.*[a]

Sample	Bile Acids: % of Total					
	Cholic	β-Muri	Hyodeoxy	ω-Muri	Chenodeoxy	Ketones
Before feeding HDC						
Feces	38.1	59.5	—	—	—	2.4
After feeding HDC						
Intestine (3)[b]	43.2	34.8	16.4	2.9	1.4	0.8
Cecum (3)	31.9	32.5	25.7	8.4	0.7	0.7
Feces	36.0	37.5	19.6	4.7	0.6	1.6

[a] HDC (free acid) was included in the diet (11 mg/rat/day) for 6 days.
[b] number of rats.



Sacquet, *et al.* (1974) presented evidence that β-muricholate (3α, 6β, 7β) could also be a precursor of HDC in rats, although they did not attempt to elucidate the pathway involved.

The total absence of HDC in the germfree rat, which produces β-muricholate as a major primary BA, indicates that microbial action is essential for the eventual formation of HDC in the rat. By the simple expedient of feeding HDC to germfree rats, we have shown that HDC (3α6α) itself can be absorbed from the gut and converted by the rat liver to ω-muri (3α,6α,7β). The latter is now secreted into the gut and accumulates in the feces. Thus, both HDC and ω-muri are "tertiary" BAs, i.e., they are formed from secondary BAs in one or more steps including the action of the liver.

It is worth noting that this experiment does not preclude the possibility that any bacterial metabolite(s) of HDC could *also* be absorbed and converted to ω-muri. While this possibility is amenable to experimentation, we have not yet addressed ourselves to it.

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