Mechanism of intestinal formation of deoxycholic acid from cholic acid in humans: evidence for a 3-oxo-Δ^4-steroid intermediate

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Abstract 12α-Hydroxy-3-oxo-4-cholenoic acid coupled to an adenosine nucleotide has been shown to be a metabolite of cholic acid in the intestinal anaerobic bacteria, *Eubacterium* species VPI 12708 (1987. *J. Biol.Chem.* **262:** 4701–4707) and it has been suggested that this may be an intermediate in the conversion of cholic acid into deoxycholic acid. The possibility that the intestinal conversion of cholic acid into deoxycholic acid involves a 3-oxo-Δ^4-steroid as an intermediate has been studied in the present work by use of [3β-^{3}H]- and [5-^{3}H]-labeled cholic acid. Whole cells as well as cell extracts of *Eubacterium* sp. VPI 12708 catalyzed conversion of [3β-^{3}H] + [24-^{14}C]cholic acid into deoxycholic acid with loss of about 50% of ^{3}H label. When unlabeled chenodeoxycholic acid (20 μM) was added along with [3β-^{3}] + [24-^{14}C]cholic acid, then approximately 85% of the [3β-^{3}H]-labeled was lost from deoxycholic acid. After administration of the same mixture to two healthy volunteers, deoxycholic acid could be isolated that had lost 81 and 84%, respectively, of the ^{3}H label. Conversion of a mixture of [5-^{3}H]- and [24-^{14}C]labeled cholic acid by the above intestinal bacteria or cell extracts led to loss of 79-94 of the [5-^{3}H] label. Evidence was obtained that suggests a substantial part of the ^{3}H retained in deoxycholic acid after is formation from [5-^{3}H]- or [3β-^{3}H]cholic acid in vivo and in vitro may be due to the transfer of [5-^{3}H] from the labeled cholic acid to a cofactor in the proposed oxidative step and subsequent utilization of the same cofactor in a reductive step. The results reported herein indicate that at least 80–90% of the overall conversion of cholic acid into deoxycholic acid in man involves the intermediary formation of a 3-oxo-Δ^4-steroid.—**Björkhem, I. K. Einarsson, P. Melone, and P. Hylemon.** Mechanism of intestinal formation of deoxycholic acid from cholic acid in humans: evidence for a 3-oxo-Δ^4-steroid intermediate. *J. Lipid Res.* 1989. **30:** 1033–1039.

Supplementary key words bile acids • 7α-dehydroxylation • *Eubacterium* • 3-oxo-$\Delta^{4,6}$-steroid • intramolecular hydrogen transfer

The primary bile acids, cholic acid and chenodeoxycholic acid, are extensively metabolized by the intestinal microflora in man, and up to 20 different metabolites are known (1). From a quantitative point of view, 7α-dehydroxylation of cholic acid is most important and deoxycholic acid constitutes about 20% of the total bile acid pool.

The mechanism for 7α-dehydroxylation of cholic acid is not known in detail. In an early in vivo study in the rat, Samuelsson (2) showed that the reaction involves a diaxial *trans* elimination of water followed by a *trans* hydrogenation in the 6α- and 7β-positions. No intermediates in the conversion could be demonstrated. In accordance with this mechanism it was later shown that synthetic 3α,12α-dihydroxy-5β-chol-6-enoic acid could be transformed into deoxycholic acid by certain intestinal anaerobic bacteria (3, 4).

In recent work (5) from one of our laboratories, it was shown that an intestinal *Eubacterium* species that contains an inducible 7α-dehydroxylation system could convert cholic acid into 12α-hydroxy-3-oxo-4-cholenoic acid coupled to an adenosine nucleotide. It was suggested that this compound may be an intermediate in the conversion of cholic acid into deoxycholic acid in this bacterium. If a 3-oxo-Δ^4-steroid is an intermediate in this conversion, the 7α-hydroxyl group would be labilized and lost, generating a 3-oxo-$\Delta^{4,6}$-steroid. This proposed intermediate would then be reduced (three-step reduction) to yield deoxycholic acid.

In order to test the hypothesis that a 3-oxo-Δ^4-steroid is formed in 7α-dehydroxylation of cholic acid in human intestine and in isolated intestinal bacteria, we synthesized [3β-^{3}H]- and [5β-^{3}H]-labeled cholic acid and measured the loss of ^{3}H during the biosynthesis of deoxycholic acid. Intermediate formation of a 3-oxo-Δ^4-steroid would theoretically be expected to lead to a complete loss of ^{3}H from both compounds, and thus the relative quantitative importance of such mechanism could be evaluated.

Abbreviation: TLC, thin-layer chromatography.

MATERIALS AND METHODS

[5-^{3}H] Cholic acid

This compound was synthesized according to the general scheme shown in **Fig. 1** by transfer of ^{3}H from [4A-^{3}H]NADPH to 7α,12α-dihydroxy-3-oxo-chol-4-enoic acid by a rat liver cytosolic Δ^4-3-oxosteroid-5β-reductase and subsequent conversion of the product into cholic acid.

[4-^{3}H]NADP$^+$ was prepared from 350 mg of NADP$^+$ (Sigma Chemical Co., St. Louis, MO) and 2 Ci of tritiated water (Radiochemical Centre, Amersham, England) according to the procedure of San Pietro (6) as modified by Krakow et al. (7). The purified [4-^{3}H] NADP$^+$ had a specific radioactivity of 1.8 $\times$ 10^6 dpm/ μmol. This material was then converted into [4A-^{3}H] NADPH by reduction with glucose-6-phosphate in the presence of glucose-6-phosphate dehydrogenase as described previously (8). The yield of [4A-^{3}H]NADPH from [4-^{3}H]NADP$^+$ was about 90% as judged by spectrophotometry. The crude mixture containing [4A-^{3}H]NADPH, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase was used directly after heat inactivation for 2 min at 75°C and centrifugation (8). It has been shown previously that the remaining glucose-6-phosphate has no effect on the subsequent 5β-reduction (8).

7α,12α-Dihydroxy-3-oxo-chol-4-enoic acid was prepared from 7α,12α-dihydroxy-3-oxo-5β-cholenoic acid by oxidation with selenium dioxide as described previously (9). The material was purified by preparative thin-layer chromatography using system S10 as solvent (10) before use.

A crude mixture of Δ^4-3-oxosteroid 5β-reductase and 3α-hydroxysteroid dehydrogenase was prepared from rat liver cytosol by ammonium sulfate precipitation and dialysis as described previously (11).

For preparation of [5-^{3}H]cholic acid, the above enzyme preparation (corresponding to about 0.5 g of rat liver) was mixed with about 0.5 μmol of [4A-^{3}H]NADPH and incubated for 1 h at 37°C with 0.25 μmol of 7α,12α-dihydroxy-3-oxo-chol-4-enoic acid dissolved in 100 μl of acetone in a total volume of 3 ml of 0.1 M potassium phosphate buffer, pH 7.4.

The incubations were terminated by addition of ethanol. After acidification, the incubation mixtures were extracted with diethylether. The ether phase was washed with water until neutral, the solvent was evaporated, and the cholic acid formed ([3β,5β-^{3}H$_2$]cholic acid) was isolated by preparative thin-layer chromatography with S10 as solvent. From each incubation the yield of [3β,5β-^{3}H$_2$]cholic acid was about 0.3 $\times$ 10^6 dpm. [3β,5β-^{3}H$_2$] Cholic acid from 10 incubations was pooled and oxidized using 3α-hydroxysteroid dehydrogenase. In the latter oxidation, the cholic acid dissolved in 0.5 ml of ethanol was incubated with about 0.05 I.U. of 3α-hydroxysteroid dehydrogenase (Enzabile Sample Reagent, Nyegaard & Co, Olso, Norway) together with 30 mg NAD$^+$ for 30 min in a total volume of 30 ml of 0.1 M Tris-HCl, pH 9.0, buffer. After extraction of the steroids with ether, the [5-^{3}H] 7α,12α-dihydroxy-3-oxo-5β-cholanoic acid was isolated by preparative thin-layer chromatography, using S10 as solvent system. The isolated 3-oxosteroid was dissolved in 1 ml of methanol; 10 mg of $NaBH_4$ was added and the mixture was allowed to stand for 30 min at room temperature.

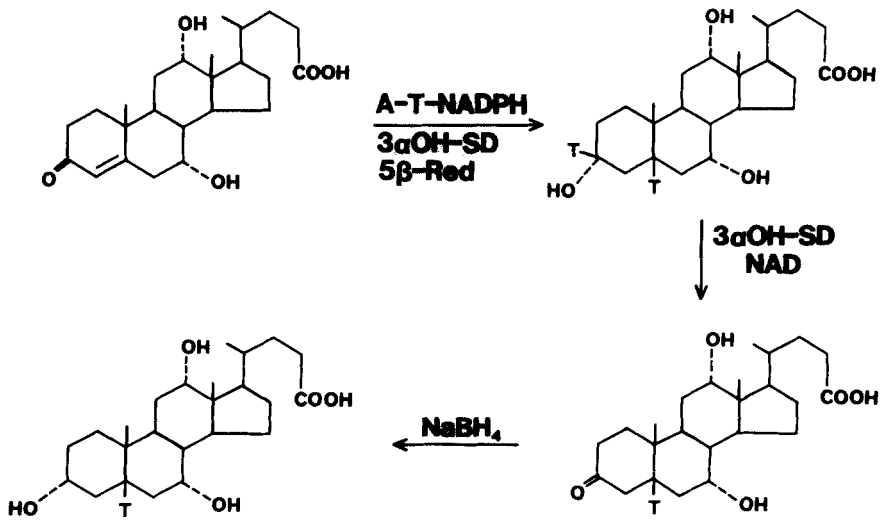

Fig. 1. Scheme of reactions in the synthesis of [5-^{3}H]cholic acid. For further explanation see Materials and Methods.

The cholic acid formed was extracted with ether after addition of water and acidification. The ether phase was washed with water until neutral, the solvent was evaporated, and the cholic acid obtained was purified as above by TLC, using system S10 (10). It should be mentioned that this system is able to separate the 3β-isomer from the 3α-isomer (10). The cholic acid obtained (about 0.1 $\times$ 10^6 dpm from each original incubation with 0.25 μmol of 7α,12α-dihydroxy-3-oxo-chol-4-enoic acid) was pure as judged by TLC and gas–liquid chromatography (as trimethylsilyl ether methyl ester derivative). If the material had contained more than a few percent of the 3β-isomer, it would have been detected by TLC. The identity of the material as cholic acid was further established by combined gas–liquid chromatography–mass spectrometry of the trimethylsilyl ether methyl ester which gave a mass spectrum identical to that of the reference compound. The specific radioactivity of the [5-^{3}H]-labeled cholic acid was estimated to be about 0.6 $\times$ 10^6 dpm/μmol. The fact that the specific radioactivity was lower than that of the [4A-^{3}H]NADPH may be explained by an isotope effect in the $\triangle^4$-3-oxosteroid-5β-reductase-mediated transfer (8, 12).

The radiochemical purity of the [5-^{3}H]cholic acid was tested by mixing part of the material with [24-^{14}C]cholic acid (see below) and oxidation with 3α-hydroxysteroid dehydrogenase and NAD^+ to yield 7α,12α-dihydroxy-3-oxo-5β-cholanoic acid. This oxidation occurred without loss of ^{3}H. Oxidation of the ketone with selenium dioxide to yield 7α,12α-dihydroxy-3-oxo-chol-4-enoic acid (9) resulted in loss of 96% of the label indicating that 96% of the label was in the 5-position.

[24-^{14}C]Cholic acid

This compound was obtained from Radiochemical Centre, Amersham, England and had a specific radioactivity of 30 $\times$ 10^6 dpm/μmol.

[3β-^{3}H]Cholic acid

This compound was prepared as described previously (13) and had a specific radioactivity of about 1 $\times$ 10^9 dpm/μmol. It has been shown that about 96% of the ^{3}H in this compound is located in the 3β-position (13).

Subjects and ethical aspects

Two healthy females (A, age 50 years and B, age 62 years) and one healthy male (C, age 36 years) participated in the study. The male subject participated both in the [5-^{3}H]cholic acid and in the [3β-^{3}H]cholic acid experiments, with an interval of about 3 months between the two experiments. The ethical aspects of the present study were approved by the Ethical Committee of the Karolinska Institute at Huddinge Hospital.

Administration of the ^{3}H and ^{14}C-labeled bile acids and collection of bile

Each ^{3}H-labeled bile acid was mixed with [24-^{14}C] cholic acid to give a ratio between 1 and 5 (see Tables 1 and 2). The amount of [5-^{3}H]cholic acid administered was about 1.2 $\times$ 10^6 dpm and the amount of [3β-^{3}H]cholic acid was about 5 $\times$ 10^6 dpm. The labeled bile acid mixture was given dissolved in 10 ml of 40% aqueous ethanol in the afternoon (4 PM). Two samples of fasting duodenal bile were collected after about 16 and 40 h. In order to obtain a sufficient flow, cholecystokinin (40 Ivy-units) was administered intravenously, and about 20 ml of concentrated duodenal bile was obtained through a thin polyvinyl tube with the collecting orifice positioned fluoroscopically at the ampulla of Vater.

Isolation of bile and assay of radioactivity

The bile samples were hydrolyzed with 1 M KOH at 110°C for 12 h. After acidification, the deconjugated bile acids were extracted with diethyl ether and methylated with diazomethane. The methyl esters of cholic acid deoxycholic acids were isolated from the remaining material by TLC using system S10 (10). In this chromatography each chromatoplate (precoated with silica gel, Merck AG, Darmstadt, West Germany) was loaded with about 5 mg of the methylated material. The different chromatographic zones were visualized by exposure to iodine vapor. After evaporation of the iodine, at room temperature, the methylated bile acids were eluted from the silic acid with methanol. Significant amounts of radioactivity were detected only in the cholic acid and deoxycholic acid fractions. In some cases the deoxycholic acid fraction was rechromatographed in system S11 (10). Radioactivity was measured with an LKB Wallac liquid scintillation counter (1217 Rackbeta) using Luma gel (LUMAC, BV, The Netherlands) as scintillation fluid. Under the conditions used with a high discrimination between ^{3}H and ^{14}C the counting efficiency was 53% for ^{14}C and 34% for ^{3}H. Internal standards were added to correct for quenching. In general the quenching was low and insignificant. In one experiment with [5-^{3}H]cholic acid, the product, deoxycholic acid, was diluted with methyl deoxycholic and crystallized twice from methanol–water mixtures. As expected, this crystallization did not change the ^{3}H/^{14}C ratio.

Experiments with isolated bacteria and bacterial extracts

Characteristics, growth conditions, and medium for the culturing of *Eubacterium* sp. VPI 13708 and *Clostridium leptum* VPI 10900 have been described previously (14). Mixed fecal flora were prepared as previously described (15).

Bacteria were cultured under anaerobic conditions and were induced to synthesize bile acid 7-dehydroxylase activity by the addition of 0.1 mM sodium cholate at hourly intervals during logarithmic growth. Preparation of soluble cell extracts has been described previously (15). Cell extracts were dialyzed anaerobically against 50 mM sodium phosphate buffer, pH 6.8, containing 10 mM 2- mercaptoethanol for 18 h (5).

Enzymatic 7α-dehydroxylation of [3β-^{3}H]- + [24-^{14}C] cholic acid and [5β-^{3}H]- + [24-^{14}C]cholic acid was performed by incubating either cell extracts (1.5 mg protein) or whole cells (1 mg protein) for 2 and 10 min, respectively. Unlabeled cholic acid was added to cell extracts (50 μM) and whole cells (20 μM) in reaction mixtures described previously (3, 14). Reactions were initiated by addition of protein and stopped by addition of 0.1 ml of HCl (0.5 M). After extraction with ethyl acetate, the product was separated from the substrate by TLC using solvent system 6 (10). Treatment of deoxycholic acid with 3α-hydroxysteroid dehydrogenase was performed as described previously (16).

RESULTS

Experiments with [3β-^{3}H]cholic acid

Mixtures of [3β-^{3}H]- and [24-^{14}C]-labeled cholic acid were incubated with whole cells and extracts of *Eubacterium* sp. VPI 12708 (cf. Experimental Procedure). Reisolated cholic acid had a ^{3}H/^{14}C ratio almost identical to that of the incubated material; whereas the isolated product, deoxycholic acid, had lost about 50% of its ^{3}H label (**Table 1**). Similar results were also obtained after incubation with whole cells of *Clostridium leptum*, another intestinal bacterium that has 7α-dehydroxylation activity (Table 1). It is evident that at least 50% of the conversion of cholic acid into deoxycholic acid by the isolated intestinal bacteria involves intermediary formation of a 3-oxo-bile acid. In order to study whether this occurs in vivo in humans, a mixture of [3β-^{3}H]- and [24-^{14}C]cholic acid was given to two healthy volunteers. The cholate reisolated from bile samples collected after 16 and 40 h had ^{3}H/^{14}C ratios that were about 90% of that of the administered material. The deoxycholate isolated, however, had lost more than 80% of the ^{3}H label in both subjects (Table 1). There were no significant differences between the relative loss of ^{3}H in the samples collected after 16 and 40 h (the results shown in Table 1 are those obtained with the 40-h sample).

The small loss of ^{3}H from the reisolated [3β-^{3}H] cholic acid (about (10%) is in accordance with the results of a previous investigation in which a small oxidoreduction of C-3 in cholic acid was demonstrated during enterohepatic circulation in man (13).

The relatively high retention of ^{3}H in deoxycholic acid isolated from incubations of [3β-^{3}H]cholic acid with bacteria and bacterial extracts may be due to transfer of ^{3}H

TABLE 1. 3β-^{3}H from [3β-^{3}H]- + [24-^{14}C]cholic acid during 7α-dehydroxylation[a]

Bile Acid	^{3}H DPM	^{14}C DPM	^{3}H/^{14}C Ratio	^{3}H Loss (%)	Source
Cholate reisolated[b] (n = 3)			4.57 ± 0.39		*Eubacterium* 12708,
Deoxycholate isolated[b]			2.78 ± 0.64	38 ± 15	whole cells
Cholate reisolated[c] (n = 4)			5.09 ± 0.72		*Eubacterium* 12708,
Deoxycholate isolated[b]			0.74 ± 0.17	85 ± 6	whole cells
Lithocholate isolated	7823	258[d]			
Cholate reisolated[b] (n = 5)			4.14 ± 0.30		*Eubacterium* 12708,
Deoxycholate isolated[b]			2.2 ± 0.16	47 ± 8	extract
Cholate reisolated[b] (n = 3)			3.79 ± 0.04		*C. leptum* 10900,
Deoxycholate isolated[e]			2.12 ± 0.76	44 ± 19	whole cells
Cholate reisolated	19453	6062	3.21		Subject A
Deoxycholate isolated	203	333	0.61	81	
Cholate reisolated	15468	5592	2.76		Subject B
Deoxycholate isolated	1259	2910	0.43	84	

[a]The results given from experiments with isolated bacteria and bacterial extracts are the mean of several independent experiments. The results of the in vivo experiments are those obtained in the analysis of the 40-h bile sample. Essentially identical results were obtained with the 16-h bile sample.
[b]^{14}C Radioactivity was more than 5000 dpm in each experiment.
[c]^{14}C Radioactivity was more than 1000 dpm in each experiment.
[d]^{14}C Radioactivity would not be expected to be present in the lithocholic acid fraction. We believe that this very small amount of radioactivity (about 1% of ^{14}C radioactivity) may be due to an impurity in the material incubated or due to an incomplete correction for the overlapping in the ^{14}C channel.
[e]^{14}C Radioactivity was more than 500 dpm in each experiment.

from cholic acid to a cofactor in an oxidative step and subsequent utilization of the same cofactor in a reductive step (13). Oxidation of deoxycholic acid isolated from incubations of [3β-^{3}H]cholic acid with 3α-hydroxysteroid dehydrogenase led to loss of 92% of the original label. Thus it is evident that the major part of the ^{3}H retained in deoxycholic acid is still in the 3β-position. In order to further study the possibilities of a transfer of ^{3}H from cholic acid to a cofactor and then back to the steroid, a mixture of [3β-^{3}H]cholic acid, [24-^{14}C]cholic acid, and unlabeled chenodeoxycholic acid (20 μM) was incubated with whole cells of *Eubacterium* sp. VPI 12708. In addition to ^{3}H-labeled deoxycholic acid, significant amounts of ^{3}H-labeled lithocholic acid could be isolated as a product (Table 1). It is evident that the same labeled pool of cofactor(s) may be utilized in the parallel 7α-dehydroxylation of cholic acid to deoxycholic acid and chenodeoxycholic acid to lithocholic acid. The exact amount of transfer of ^{3}H from [3β-^{3}H]cholic acid to the cofactor and then back to the steroid could not be calculated from this experiment. Most probably, however, the major part of the ^{3}H retained in deoxycholic acid formed from [3β-^{3}H]cholic acid may have originated from a labeled cofactor.

Experiments with [5-^{3}H]cholic acid

Mixtures of [5-^{3}H]- and [24-^{14}C]-labeled cholic acid were incubated with whole cells and cell extracts of *Eubacterium* sp. VPI 12708, with whole cells of *Clostridium leptum*, and with mixed fecal cells. The conversion of the material into deoxycholic acid led to loss of most of the ^{3}H label (79–94%) (**Table 2**). To study whether the conversion of cholic acid into deoxycholic acid involves loss of the hydrogen in 5-position also in vivo in humans, a mixture of [5-^{3}H]- and [24-^{14}C]cholic acid was given to two healthy volunteers. The cholic acid reisolated in the 16-h samples had a ^{3}H/^{14}C ratio essentially identical to that of the administered material. The deoxycholate isolated, however, had lost more than 77% of the ^{3}H in one of the experiments and 95% in the other (Table 2).

The relatively higher retention of ^{3}H in one of the in vivo experiments may also be due to transfer of ^{3}H from the labeled bile acid to a cofactor in an oxidative step and a subsequent utilization of the same labeled cofactor in a reductive step. If so, there may be a small transfer of ^{3}H from the 5β-position back to the 5β-position or the 3β-position. To study the latter possibility, the deoxycholate isolated that had retained 23% of the ^{3}H was oxidized with NAD$^+$ and 3α-hydroxysteroid dehydrogenase to yield 12α-hydroxy-3-oxo-5β-cholanoic acid. The latter compound had lost an additional 12% of the ^{3}H (Table 2). It is evident that some transfer of hydrogen from the 5β-position to the 3β-position may occur during the biosynthesis of deoxycholic acid from cholic acid. Oxidation of deoxycholic acid from incubations of [5-^{3}H]- and [24-^{14}C]-labeled cholic acid with bacterial preparations also led to removal of most of the remaining ^{3}H (results not shown).

TABLE 2. Loss of 5-^{3}H from [5-^{3}H]- + [24-^{14}C]cholic acid during 7α-dehydroxylation

Bile Acid	^{3}H DPM	^{14}C DPM	^{3}H/^{14}C Ratio	^{3}H Loss (%)	Source
Cholate reisolated[b] (n = 8)			1.92 ± 0.16		*Eubacterium* 12708,
Deoxycholate isolated[c]			0.14 ± 0.06	93 ± 3	whole cells
Cholate reisolated[b] (n = 4)			1.18 ± 0.03		*Eubacterium* 12708,
Deoxycholate isolated[b]			0.07 ± 0.03	94 ± 3	extract
Cholate reisolated[c] (n = 4)			1.21 ± 0.01		*C. leptum* 10900
Deoxycholate isolated[d]			0.08 ± 0.09	93 ± 8	whole cells
Cholate reisolated[e] (n = 2)			1.44[f]		Mixed fecal cells
Deoxycholate isolated[d]			0.29[f]	79[f]	
Cholate reisolated	2375	1858	1.28		Subject C
Deoxycholate isolated	64	1053	0.06	95	
Cholate reisolated	3604	2243	1.61		Subject B
Deoxycholate isolated	334	899	0.37	77	
Deoxycholate after oxidation in 3-position	180	1005	0.18	89	

[a]The results given from experiments with isolated bacteria and bacterial extracts are the mean of several independent experiments. The results of the in vivo experiments are those obtained in the analysis of the 40-h bile sample. Essentially identical results were obtained with the 16-h bile sample.
[b]^{14}C Radioactivity was more than 1000 dpm in each experiment.
[c]^{14}C Radioactivity was more than 5000 dpm in each experiment.
[d]^{14}C Radioactivity was more than 400 dpm in each experiment.
[e]^{14}C Radioactivity was more than 50 dpm in each experiment.
[f]Mean of the two experiments.

DISCUSSION

The results of the present work give strong support to the hypothesis that the intestinal conversion of cholic acid into deoxycholic acid involves a 3-oxo-Δ^4-intermediate. As could be expected if such a mechanism is dominating, the major part of both a [3β-^{3}H] and a [5-^{3}H] label in cholic acid should be lost during 7α-dehydroxylation in the in vivo experiments. In the in vitro experiment with [3β-^{3}H]cholic acid, however, a substantial part of the ^{3}H was retained in deoxycholic acid, unless unlabeled chenodeoxycholic acid was added to reaction mixtures.

The fact that the ^{3}H label was not completely lost in any of the experiments may have two explanations. The first explanation is that there is a minor mechanism for 7α-dehydroxylation of cholic acid that does not involve a 3-oxo-Δ^4-intermediate. The second and most likely explanation is that there is a transfer of ^{3}H from the labeled bile acid to the cofactor of a steroid dehydrogenase in an oxidative step and a subsequent utilization of the same cofactor in a reductive step (cf. ref. 13). This would lead to a retention of ^{3}H in a 3β-position and possibly also in the 5β-position. Clear evidence for such a mechanism was obtained by incubating a mixture of [3β-^{3}H]cholic acid and unlabeled chenodeoxycholic acid with bacteria, which led to transfer of ^{3}H to the 7α-dehydroxylated product of chenodeoxycholic acid, lithocholic acid. It was also shown that deoxycholic acid isolated from bile of a subject to which [5-^{3}H]cholic acid had been administered had some ^{3}H in 3β- position (about 12% of the original label). It is possible that the ^{3}H remaining in this deoxycholic acid after removal of the label in the 3β-position (about 11% of the original label) may be located in the 5β-position. If so, the major part of this label may have been reintroduced during saturation of the Δ^4-double bond by a ^{3}H-labeled cofactor. A mechanism similar to that above has been shown previously in the conversion of cholesterol into coprostanol by fecal microorganisms (17). Thus [3α-^{3}H]cholesterol was converted into coprostanol in this system with retention of some of the ^{3}H in spite of a demonstrated intermediate oxidoreduction of the 3β-hydroxy group. Shefer and collaborators (18) and Batta, Salen, and Shefer (19) have shown that 3β-isomers of bile acids with a ^{3}H in the 3α-position were converted into the corresponding 3α-hydroxy bile acids in the liver with retention of most of the label. A possible explanation may be that there is a transfer of ^{3}H from the 3β-hydroxysteroid to the cofactor of the 3β-hydroxysteroid dehydrogenase and a subsequent utilization of the same labeled cofactor by a 3α-hydroxysteroid dehydrogenase.

In view of the above considerations, the presence of a major pathway for 7α-dehydroxylation of cholic acid that does not involve a 3-oxo-Δ^4-intermediate seems less likely. It is evident that if such a pathway exists, it must be of little or no quantitative importance.

The previous demonstration that the conversion of cholic acid into deoxycholic acid involves intermediary dehydration from carbons 6 and 7 (2) and the recent isolation of adenosine nucleotide-coupled 12α-dihydroxy-3-oxo-4-cholenoic acid as a metabolite of cholic acid in *Eubacterium* sp. VPI 12708 (5) has led to the suggested hypothetical mechanism shown in **Fig. 2** (20). The results of the pre-

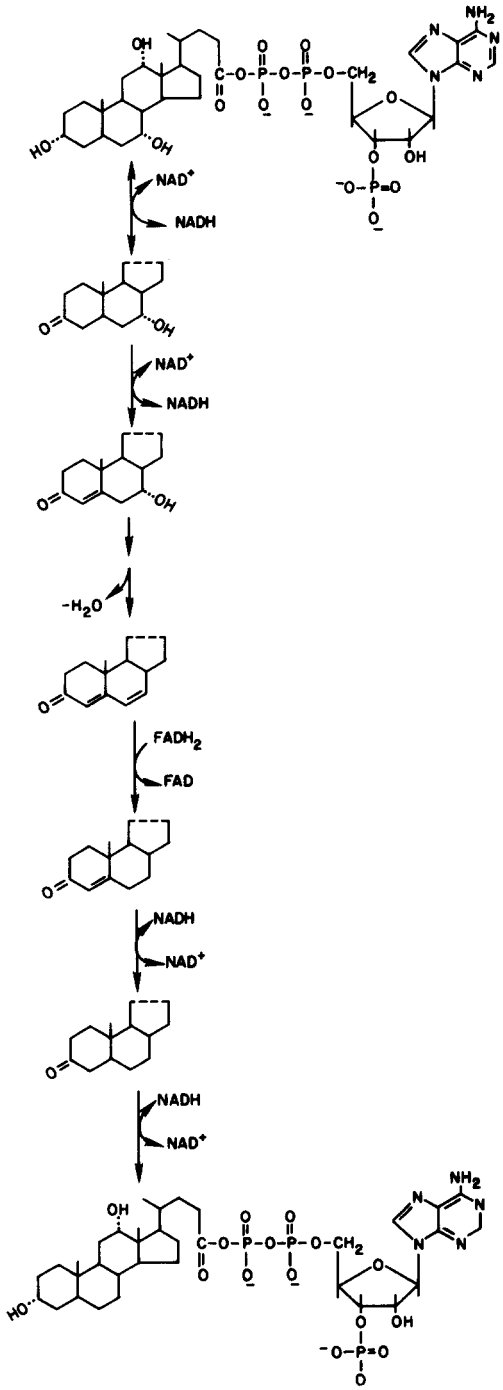

Fig. 2. Proposed mechanism of bile acid 7α-dehydroxylation in the intestinal *Eubacterium* sp. VPI 12708. For further explanation see Discussion.

sent work are in accord with this mechanism. In the proposed mechanism, nucleotide-bound cholic acid is oxidized in two steps to yield the corresponding nucleotide of 7α,12α-dihydroxy-3-one-4-cholenoic acid. It is well established that a 7α-hydroxylated 3-oxo-Δ^4-steroid is unstable and easily loses the 7α-hydroxyl group (21). Loss of the 7α-hydroxyl group would then yield 12α-hydroxy-3-oxo-4,6-cholenoic acid. The latter intermediate would be subsequently reduced in three steps to yield the nucleotide of deoxycholic acid. It may be mentioned in this connection that 7α,12α-dihydroxy-3-oxo-4-cholenoic acid as well as 12α-hydroxy-3-oxo-4,6-cholenoic acid have been isolated from *Corynebacterium simplex* (22, 23). Work is now in progress to define the detailed mechanism of conversion of cholic acid into deoxycholic acid.

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