Epimerization of the 7-hydroxy group of bile acids by the combination of two kinds of microorganisms with 7α- and 7β-hydroxysteroid dehydrogenase activity, respectively

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Abstract An unidentified gram-positive anaerobic organism capable of dehydrogenating the 7β-hydroxy group of ursodeoxycholic acid was recovered from human feces. By combined action of this organism with the ordinary 7α-dehydrogenating bacteria, chenodeoxycholic acid and cholic acid alike were readily converted into their respective 7β-epimers and the reverse reactions were also carried out. The estimated levels of these 7α- and 7β-dehydrogenating organisms among the intestinal microflora give a satisfactory explanation for the frequent appearance of the 7β-hydroxylated bile acids in vivo.—**Hirano, S., and N. Masuda.** Epimerization of the 7-hydroxy group of bile acids by the combination of two kinds of microorganisms with 7α- and 7β-hydroxysteroid dehydrogenase activity, respectively. *J. Lipid Res.* 1981. **22:** 1060–1068.

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The 7β-hydroxy epimers of chenodeoxycholic acid (CDCA) and cholic acid (CA), the two prominent primary bile acids in human bile, have frequently been detected in both the bile and feces of man (1–4). However, the metabolic sequence of biosynthesis of these 7β-hydroxylated bile acids is not completely understood. Although it has generally been proposed that 7β-hydroxy bile acids may be derived from 7α-analogues by way of an oxone, the exact site of the stereospecific reduction of the 7-oxo group to a β-hydroxy configuration remains unclear. Certainly, there is a prevailing concept, mainly from the results of in vivo studies, that the epimerizing reaction takes place in the liver catalyzed by hepatic enzymes; however, no decisive enzymatic proof has ever been provided (5–10). Recently, Fromm et al. (11) observed that 7-ketolithocholic acid (7KL) administered intravenously to man was largely reduced to a 7α-ol (CDCA) in the liver, only small portions of 7KL being converted to a 7β-ol (ursodeoxycholic acid, UDCA). On the other hand, Fedorowski et al. (12) reported on the conversion of CDCA into its 7β-epimer, UDCA, in in vitro cultures of human feces, and we have also demonstrated an extensive interconversion between CDCA and UDCA by mixed cultures of human fecal flora (13). These results suggest that the epimerization of the 7-hydroxy group can be effected entirely by microbial actions in the intestine, excluding the participation of any liver function. In the course of in vitro testing of a number of microbial strains from human feces for their bile acid-transforming ability, a gram-positive anaerobic organism, which is not infrequently recovered from human feces, was found to be capable of performing reversible dehydrogenation of the 7β-hydroxy group in UDCA. It was then demonstrated that, by combination of this organism with an ordinary 7α-dehydrogenating bacterium, the hydroxy group at C-7 in CDCA and CA was interconverted between the α- and the β-positions, with an oxo product as a prerequisite intermediate, thus providing a satisfactory explanation for the previous finding by mixed fecal cultures (13).

This report describes these experimental data, together with the bacteriological features of the hitherto unreported 7β-dehydrogenating organism.

Abbreviations: HSDH, hydroxysteroid dehydrogenase; PY, peptone-yeast extract; PYG, peptone-yeast extract-glucose; GLC, gas–liquid chromatography; MS, mass spectrometry; m-TMS, methyl ester trimethylsilyl ether; CA, cholic acid (3α,7α,12α-triol-5β-cholanoic acid); CDCA, chenodeoxycholic acid (3α-,7α-diol-5β-cholanoic acid); 7KD, 7-ketodeoxycholic acid (7-oxo-3α,12α-diol-5β-cholanoic acid); 7KL, 7-ketolithocholic acid (7-oxo-3α-ol-5β-cholanoic acid); LCA, lithocholic acid (3α-ol-5β-cholanoic acid); UDCA, ursodeoxycholic acid (3α,7β-diol-5β-cholanoic acid); 7βCA, 7β-epimer of CA (3α,7β,12α-triol-5β-cholanoic acid).

MATERIALS AND METHODS

Isolation of bacterial strains from human feces

Viable bacterial counts in three fecal samples collected successively from the same healthy adult (one of our staff members) were determined. Portions (0.1 ml) of serial tenfold dilutions were spread on GAM plates (Gifu anaerobic medium, purchased from Nissui Pharmaceutical Co., Tokyo) (14) and incubated at 37°C for 48 hr in an anaerobic jar under an atmosphere of 90% N_2 and 10% CO_2. The total counts were 5×10^9, 2×10^{10}, and 2×10^{10} cells per mg (wet weight) of feces, respectively. From plates containing discrete colonies, about 60 colonies from each fecal sample (a total of 181 from three samples) were picked and subcultured in tubes of semisolid GAM agar for 48 hr. The resulting cultures were purified by colonial reisolation and maintained in the same medium for the following tests. Before testing, the strains were grown anaerobically overnight in peptone-yeast extract-glucose broth (PYG) (15), and the early stationary phase cultures were used as inocula.

Screening in growing cultures of the isolated strains for bile acid-transforming activity

The 181 isolates were divided into groups of five, and in each group 0.1-ml portions of the five cultures were mixed and inoculated into a tube of buffered 2% peptone-yeast extract broth (PY) containing 200 μM CDCA, the same assay medium as used in the previous report (13). After anaerobic incubation for 4 days, all 36 mixed cultures were analyzed for bile acid metabolites. In cultures in which the expected reaction occurred, the individual constituent strains were tested in a similar way, singly or in combination.

Transformation of bile acids by resting cells

The metabolism of bile acids by active strains was also examined by the use of resting cell suspension. Cells were harvested by centrifugation from a stationary phase culture in GAM broth (with no addition of bile acid) and washed three times with 0.1 M sodium phosphate buffer (pH 7.0) containing 0.1% Na thioglycolate. The reaction mixture contained 20–30 mg (wet weight) of washed whole cells and 500 μg of bile acid in 3.0 ml of the same phosphate buffer at various pH values. After incubation at 37°C for up to 21 hr under anaerobic or aerobic conditions, the reaction was stopped by the addition of HCl, and the acidified samples were submitted to analysis. Anaerobic incubation was carried out in Thunberg tubes under flushing with pure N_2, and aerobic incubation was in L-shaped tubes held aerobically in a shaker incubator.

Assay of bile acid metabolites

The spent culture medium (growing) or the reaction mixture (resting cells) was analyzed for bile acids by the same GLC and MS procedures as described previously (13). Briefly, a 4-ml quantity of sample, acidified to lower than pH 2.0 with 6 N HCl, was thoroughly extracted with ethyl acetate, and the bile acids were converted to methyl esters (m), by an acidic methanol method, for GLC analysis on a 3% QF-1 column. A portion of the methylated sample was further trimethylsilylated (m-TMS) by the method of Makita and Wells (16), and the derivatives were chromatographed on both 3% Hi Eff-8B and 2% OV-17 columns. Individual bile acids were identified by comparative evaluation of the RRT values (retention time relative to that of m- or m-TMS-derivative of deoxycholic acid) from these three GLC procedures. Reference compounds were also treated in a similar way for comparison. Quantities were calculated by measuring peak areas as before (13) and expressed in % composition after it had been confirmed that the total recovery of individual bile acids in each sample was comparable to the substrate bile acid originally added.

The m-TMS derivatives separated on Hi Eff-8B or OV-17 were analyzed by MS, and the mass spectra were compared with those of the corresponding authentic standards, simultaneously recorded and/or previously published by Sjövall, Eneroth, and Ryhage (17).

Examination of bacteriological properties

Biochemical and fermentative reactions of 7β-dehydrogenating strains were examined according to the procedures of Holdeman and Moore (15), using ordinary PY broth as the basal medium. Inoculum was a standard volume of an overnight culture in GAM broth. Final acidity of the carbohydrate broth was determined with a pH meter on the 3rd day of anaerobic incubation. Fatty acids, volatile and nonvolatile, produced in 3-day cultures in PYG were analyzed by GLC on an ethylene glycol adipate column with temperature programming (18). For electron microscopy, cells from a 48-hr anaerobic GAM plate were suspended in a small amount of sterile distilled water, and a drop of the suspension was mounted on a Formvar-carbon coated grid. After negative staining with 3% uranyl acetate, the specimen was examined in a Hitachi H-300 electron microscope.

RESULTS

Conversion of CDCA into UDCA in mixed cultures of fecal isolates and selection of the causative organism

The 181 bacterial strains isolated from the three fecal samples were tested for their ability to transform CDCA in pools of five strains. Three of the 36 mixed cultures, one from each fecal sample, converted large quantities of CDCA into a compound eluting at the same retention time as UDCA, RRT 1.20 (as methyl ester on QF-1) (**Table 1**). The identity of this compound as UDCA was confirmed by the combined data from GLC and MS.

The five strains from each positive mixed culture were all unable to exhibit the same activity against CDCA, when tested in individual pure cultures under the same cultural conditions, but invariably included one or two strains capable of performing 7α-dehydrogenation of CDCA to give a 7-oxo acid, 7KL (Table 1). Strains a-16, b-52, or c-15 (one of the five strains from each fecal sample) effected an extensive converison of CDCA into UDCA when cocultured with any one of the 7α-dehydrogenating organisms; in the table, data for b-52 and c-15 are omitted. As noted in the table, all three of these strains were gram-positive anaerobic cocci, and all the 7α-dehydrogenating strains were gram-negative anaerobic bacilli. Similar epimerizing conversion was also caused with *Escherichia coli* C or *Bacteroides fragilis* 2536 as a 7α-dehydrogenating companion (Table 1). These results clearly suggest that the gram-positive cocci may act on the 7-oxo acid which had been produced from CDCA through the action of the 7α-dehydrogenating organism and may convert the oxo group into a 7β-hydroxy group leading to the formation of UDCA. The latter reaction represents the reductive process by a reversible 7β-hydroxysteroid dehydrogenase (HSDH).

TABLE 1. Transformation of CDCA by intestinal isolates in mixed cultures of five strains, individual pure cultures, and a combination of two selected strains

Culture	Bile Acid Metabolites[a] 1.12[b] CDCA	1.20[b] UDCA	1.68[b] 7KL
		%	
Mixed culture[c] of a-16 to a-20		57	43
Pure culture[d] of a-16 (+C)	100		
a-17 (+C)	100		
a-18 (−R)	19		81
a-19 (−R)	86		14
a-20 (−R)	100		
Combination[e] of a-16 + a-18		100	
a-16 + a-19		100	
a-16 + C		100	
a-16 + 2536		100	
Mixed culture of b-51 to b-55	77	23	
Pure culture of b-51 (−R)	100		
b-52 (+C)	100		
b-53 (−R)	100		
b-54 (−R)	34		66
b-55 (−R)	69		31
Combination of b-52 + 2536		99	1
Mixed culture of c-11 to c-15	62	36	2
Pure culture of c-11 (+R)	100		
c-12 (−R)	95		5
c-13 (−R)	100		
c-14 (−R)	100		
c-15 (+C)	100		
Combination of c-15 + 2536		99	1
Pure culture of C	9		91
2536	44		56

[a] The strains were grown in buffered PY broth containing 200 μM CDCA under anaerobic conditions for 4 days. Bile acids extracted from the spent culture medium were separated as methyl esters on QF-1.

[b] Relative retention times (RRT values).

[c] Three mixed cultures consisting of a-16 to a-20, b-51 to b-55, and c-11 to c-15 were positive for the conversion of CDCA into UDCA.

[d] The individual strains from the positive mixed cultures were tested in pure cultures. Their morphological appearance is given in parentheses: +R, gram-positive rod; +C, gram-positive coccus; −R, gram-negative rod.

[e] Selected strains a-16, b-52, and c-15 were tested for the transformation of CDCA in cocultures with a 7α-dehydrogenating member of the mixed culture (a-18 or a-19) or a 7α-dehydrogenating stock culture of *E. coli*, strain C, or *Bacteroides fragilis*, strain 2536.

Reversible dehydrogenation between UDCA and 7KL

To confirm the above assumption, one of these strains, b-52, was grown anaerobically in broth in the presence of either UDCA or 7KL (**Fig. 1**). 7KL was rapidly reduced to and almost quantitatively converted into UDCA during the early phase of incubation, while the substrate UDCA was scarcely oxidized to 7KL and more than 90% of UDCA remained unchanged until the 8th day, presumably because of the reductive environment elaborated by anaerobic bacterial growth. The identity of the respective conversion products was established by means of GLC and MS.

Oxidative conversion of UDCA to give 7KL was tested by the use of resting cells, which can be incubated under either anaerobic or aerobic conditions. The conversion took place under both conditions,

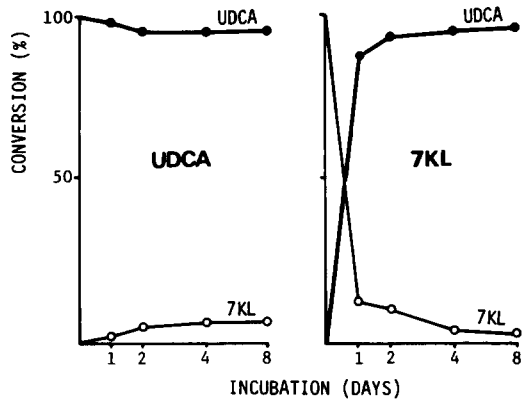

Fig. 1. Mutual conversion of UDCA and 7KL by anaerobic cultures of strain b-52. The strain was grown for 8 days in buffered PY broth containing UDCA or 7KL; samples were taken at 1-day intervals for assay.

but to a larger extent in an aerobic than an anaerobic environment (**Fig. 2A**). Aerobic oxo formation as a function of pH was then tested (Fig. 2B). The reaction proceeded significantly over a broad pH range above 6.0, with an optimum of 8.0. Similar findings were obtained with the other strains, a-16 and c-15. From these observations, it may be concluded that the strains of the b-52 group constitutively synthesize 7β-HSDH which mediates the reversible oxidation-reduction reactions between the 7β-hydroxy and 7-oxo groups.

Mutual conversion between CDCA and UDCA by collaboration of 7α- and 7β-dehydrogenating organisms

The 7β-dehydrogenating strains were cocultured anaerobically with *E. coli* strain C (7α-dehydrogenating) in the presence of CDCA or UDCA (**Table 2**). The same metabolites were obtained from the two bile acids, indicating interconversion between these two epimeric bile acids via 7KL as an intermediate.

The reaction sequence of the interconversion was examined with resting cells of a-16 (7β-dehydrogenating) and *E. coli* C (7α-dehydrogenating) (**Table 3**). Cells of a-16 reduced 7KL to UDCA under anaerobic conditions and oxidized UDCA to 7KL under aerobic incubation, thus mediating the reversible dehydrogenation between the 7β-hydroxy and 7-oxo groups. The 7KL produced through the oxidation of UDCA by a-16 was reduced to CDCA when incubated anaerobically with *E. coli* cells, and the 7KL derived from CDCA by aerobic action of *E. coli* was also converted into UDCA by anaerobic incubation with a-16 cells.

Conversion of CA into its 7β-epimer by combined action of 7α- and 7β-dehydrogenating organisms

The metabolic bile acids produced from CA in cocultures of the 7β-dehydrogenating strains with *E. coli* C are shown in Table 2. Two compounds eluting at RRTs of 0.97 and 3.4 (as silyl ethers on Hi Eff-8B) were found as principal metabolites. The same products were also observed with resting cells (**Table 4**). In the light of their GLC behavior and MS features, the 0.97 compound was identified as 7βCA, the 7-epimerized product of CA, and the 3.4 compound as 7KD, an oxidation metabolite of CA. The results may be explained by assuming that CA was oxidized by *E. coli* to 7KD (7α-dehydrogenation), which, in turn, was reduced to the 7β-hydroxy acid by the 7β-dehydrogenating organism. When 7KD was exposed as a substrate to the latter organism, the keto acid was converted into 7βCA but not into CA. It should be

TABLE 2. Transformation of CDCA, UDCA, and CA in cultures of a 7β-dehydrogenating organism (a-16, b-52, or c-15) combined with 7α-dehydrogenating *E. coli* C

	Bile Acid Metabolites[a] from								
	CDCA			UDCA			CA		
Culture	1.09 CDCA	1.68 UDCA	5.2 7KL	1.09 CDCA	1.68 UDCA	5.2 7KL	0.65 CA	0.97 7βCA	3.4 7KD
	%								
a-16 + C	41	30	29	Not determined			43	18	39
b-52 + C	52[b]	9	39	3	95	2	49	3	48
	47	38	15	7	74	19	79	11	10
	13	84	3	12	84	4	32	53	15
c-15 + C	26	55	19	Not determined			45	23	32

[a] Each pair of strains was cocultured anaerobically for 4 days in buffered PY broth with the desired bile acid at 200 μmol. Bile acids extracted from the spent culture medium were identified by GLC of their m-TMS derivatives on Hi Eff-8B. The RRT values of the bile acids are indicated.

[b] Tested in triplicate.

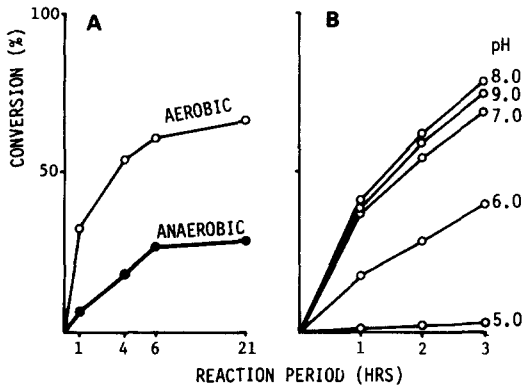

Fig. 2. 7β-Dehydrogenating conversion of UDCA into 7KL by resting cells of strain b-52, compared between anaerobic and aerobic conditions (A) and as a function of pH (B). A: a resting cell suspension was incubated with UDCA in phosphate buffer at pH 7.0 in a Thunberg tube under N_2 (anaerobic) or in an L-shaped tube with continuous shaking in air (aerobic). B: the same reaction mixtures were incubated under aerobic conditions using phosphate buffer at different pH values.

noted in this connection that the two 7-epimeric trihydroxy bile acids, CA and 7βCA, were quite indistinguishable by the chromatography of their methyl esters on QF-1 because of the similarity of elution times (RRT 2.0 for the two). The 7-epimerization of CA had been overlooked until the silylation procedure was undertaken.

Lastly, preliminary studies have shown that 7β-HSDH activity is extractable and NADP-dependent. A washed cell suspension of strain b-52 was disrupted with a Branson Sonifier B-12 (Branson Sonic Power Co., Stamford, CT) at 80 W for 4 min and centrifuged at 6,000 *g* for 30 min to remove cell debris. A 0.5-ml portion of the supernatant (equivalent to 10 ml of the original GAM broth culture) was incubated with 1 μmol of UDCA in 3.0 ml of glycine-NaOH buffer at pH 9.5, with the addition of 2.5 μmol of NAD or NADP (Sigma Chemical Co., St Louis, MO). After 30 min at 20°C, about 80% of UDCA was converted into 7KL in the presence of NADP but the conversion was negligible in the presence of NAD.

TABLE 3. Conversion between CDCA and UDCA via 7KL by resting cells of strain a-16 in collaboration with resting cells of *E. coli* C

Cells	Substrate Bile Acid	Incubation	Bile Acid Metabolites		
			CDCA	UDCA	7KL
				%	
a-16 + C[a]	CDCA	21 hr, N_2	17	68	15
a-16 + C[a]	UDCA	21 hr, N_2	18	66	16
a-16[b]	7KL	21 hr, N_2	0	78	22
a-16	UDCA	5 hr, O_2	0	24	76
+ C[c]		16 hr, N_2	14	63	23
C	CDCA	5 hr, O_2	70	0	30
+ a-16[c]		16 hr, N_2	17	62	21

[a] Washed cells of a-16 and C were incubated with CDCA (or UDCA) under an atmosphere of N_2 for 21 hr.
[b] Cells of a-16 alone were incubated with 7KL under N_2 for 21 hr.
[c] Cells of a-16 (or C) were incubated with UDCA (or CDCA) under aerobic shaking (O_2) for 5 hr. The atmosphere in the reaction tube was made anaerobic by flushing with N_2, and C (or a-16) cells were added to react under N_2 for 16 hr.

TABLE 4. Conversion of CA into its 7β-epimer by collaboration of resting cells of strains a-16 and *E. coli* C

Cells	Incubation	Bile Acid Metabolites		
		CA	7βCA	7KD
			%	
a-16 + C	21 hr, N_2	17	67	16
C	5 hr, O_2	85	0	15
+ a-16	16 hr, N_2	27	55	22

For procedures, see Table 3.

Bacteriological characterization of 7β-dehydrogenating strains

The three strains are identical in cellular and colonial appearance and also in the biochemical reactions so far tested. The cells are anaerobic, gram-positive cocci arranged in pairs and having an ellipsoidal form of 0.7–1.0 × 1.6–1.9 μm as a whole (**Fig. 3**). The growth was poor in PY but was stimulated in PYG and GAM broth possibly because of the presence of fermentable carbohydrate. Colonies on GAM agar plates were about 2 mm in diameter, circular, slightly raised, smooth, and gray (**Fig. 4**). The biochemical and fermentative reactions by these strains are listed in **Table 5.** The organisms produced acid from a variety of carbohydrates including glucose and lactose but failed to produce indol or hydrogen sulfide or to liquefy gelatin. They shared many bacteriological features with *Peptostreptococcus productus,* although their exact taxonomical position still remains to be decided.

DISCUSSION

This is the first report of an organism biotransforming the 7β-hydroxy group of bile acids. The reaction concerned is reversible dehydrogenation, which, in combination with an ordinary 7α-HSDH, establishes an equilibrium between the 7β- and 7α-hydroxy groups via a 7-oxo group: 7β-OH ⇌ 7-

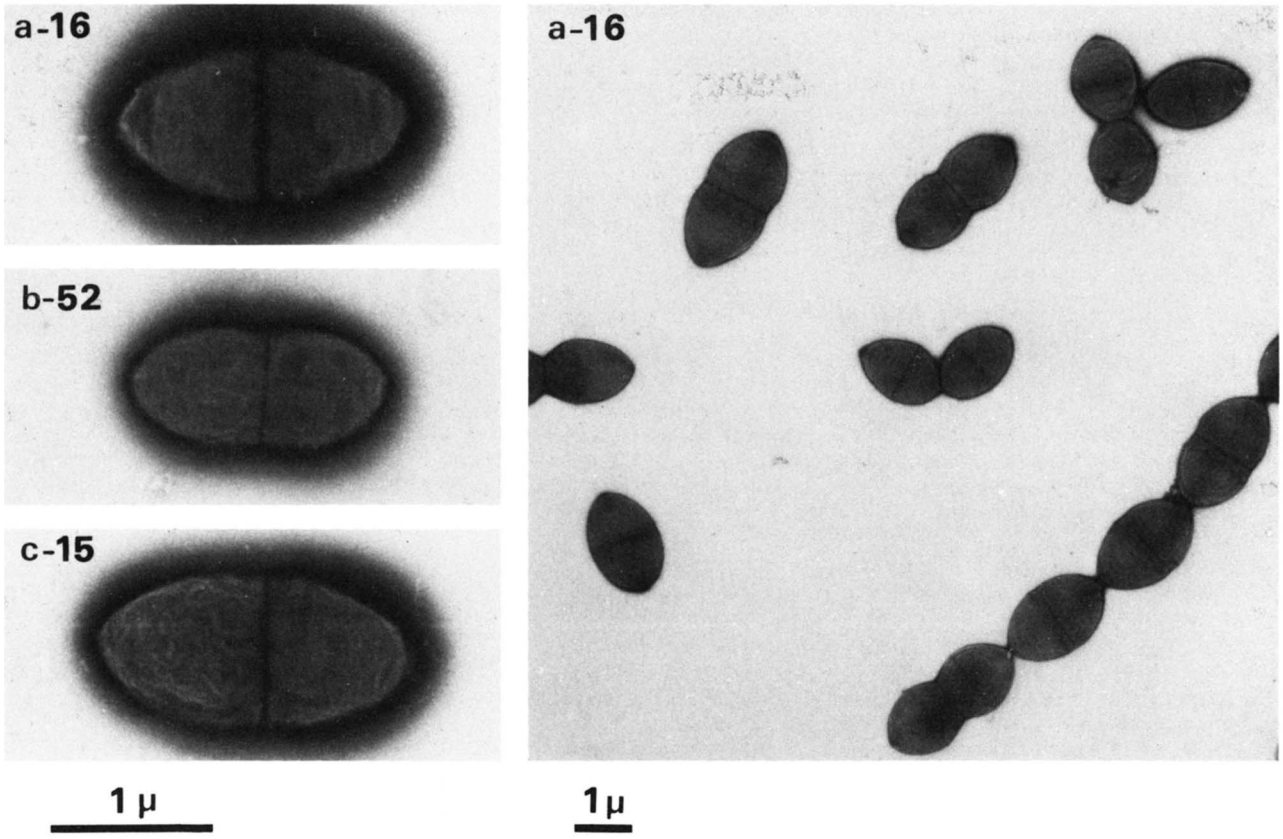

Fig. 3. Electron micrographs of 7β-dehydrogenating organisms.

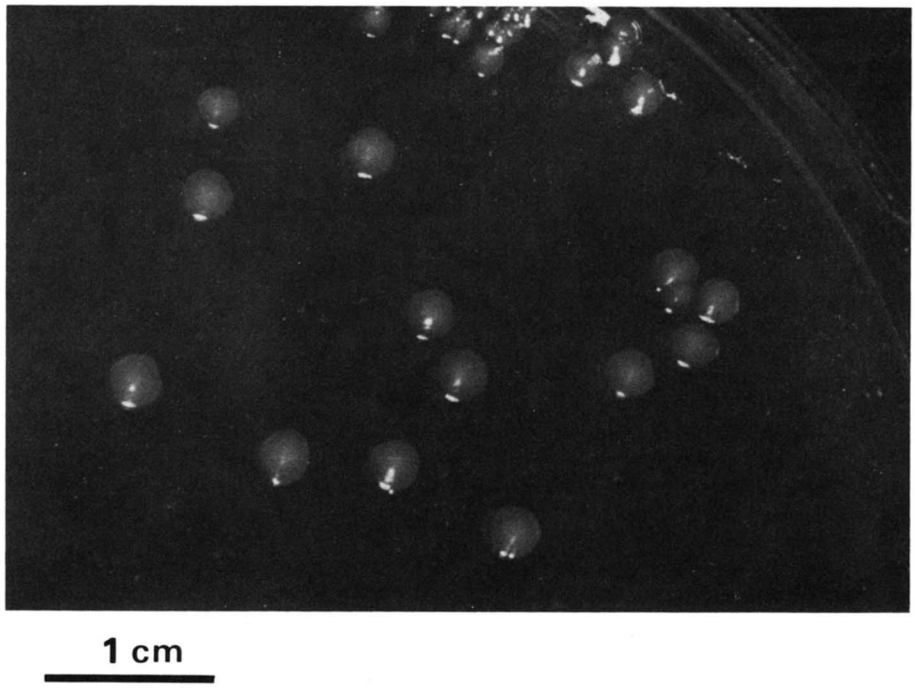

Fig. 4. Colonies of 7β-dehydrogenating strain a-16, on GAM agar plate incubated anaerobically for 48 hr.

oxo ⇌ 7α-OH. The 7β-dehydrogenating organism showed no activity against the 7α-hydroxy groups of the prevailing bile acids. Therefore, the detection of the organism in this study in which a 7α-hydroxy bile acid (CDCA) was employed as a substrate, is due to the use of mixed cultures for screening and the coexistence of 7α-dehydrogenating organisms in the mixed cultures, i.e., the prevalence of both organisms among the fecal isolates under test.

The occurrence of two such separate dehydrogenases that act on the respective epimeric bile acids is consistent with the high degree of stereospecificity for the substrate of steroidalcohol dehydrogenases in general. Samuelsson (19) postulated two similar enzyme systems involved in the conversion of CDCA (7α-OH) into Acid I (7β-OH) in the rat, but he considered the enzymes to be of hepatic origin.

7α-HSDH activity is known to be widely distributed among the intestinal microorganisms. In this study, 22 of 36 mixed cultures covering 181 fecal isolates produced detectable amounts of 7KL from CDCA, and 5 of the 15 individual strains (Table 1) were capable of performing this reaction. In our previous investigations, 14 of 15 stock cultures of *E. coli* (20) and 30 of 61 intestinal strains of *B. fragilis* (21) were found to elaborate 7α-HSDH which acts on both CA and CDCA, giving rise to the formation of the respective 7-oxo acids. These bacterial 7α-HSDH were reversible but the reduction of the oxo group in this case yielded only a 7α-hydroxy group.

In this study, the stereospecific reduction of the 7-oxo group to a β-hydroxy group was found to be carried out by a distinct organism that was recovered at a frequency of 1 per 60 colony-forming units from every fecal sample, suggesting the considerable prevalence of this property among the intestinal microflora. Such prevalence of both 7α- and 7β-dehydrogenating organisms may facilitate the epimerizing conversion of the 7α-hydroxy groups of CA as well as of CDCA in the intestine, and the conversion products may appear in the bile also after absorption through the intestinal wall, as frequently reported previously (1, 4). The equilibria of the epimerizing conversions in the intestine may vary depending upon the relative number of these two kinds of dehydrogenating organisms, and, in the normal human gut, the 7α-configuration seems to be favored owing to the predominance in number of 7α-dehydrogenating bacteria over the 7β-dehydrogenating organisms.

Concerning the biosynthesis of 7β-epimers, there have been proposed two opposite opinions from in vivo studies. Using rats with bile fistulas, Norman and Sjövall (8) attributed the epimerization of CA into its 7β-epimer to the action of the intestinal microorganisms of the rat, while Samuelsson (6) and Gustafsson, Norman, and Sjövall (10) emphasized that the stereospecific reduction of the 7-oxo group of bile acids is one of the metabolic events in the liver due

TABLE 5. Bacteriological characteristics of 7β-dehydrogenating strains

Characteristics[a]	Strains[b] a-16	b-52	c-15	f-75	PB6K
Amygdalin[c]	—	—	—	—	—
Arabinose	w	a	a	a	—
Cellobiose	a	a	a	—	—
Erythritol	—	—	—	—	—
Esculin, acid	w	—	—	w	—
Esculin, hydrolysis	+	+	+	+	—
Fructose	a	a	w	a	a
Glucose	a	a	w	a	a
Glycogen	—	—	—	—	a
Inositol	—	—	—	—	a
Lactose	a	a	a	a	a
Maltose	a	a	w	w	a
Mannitol	—	—	—	—	—
Mannose	w	a	a	—	a
Melezitose	—	—	—	—	—
Melibiose	a	a	a	w	—
Raffinose	a	a	a	w	w
Rhamnose	w	a	a	a	—
Ribose	a	a	w	a	a
Salicin	—	a	a	a	—
Sorbitol	—	—	—	—	—
Starch, acid	—	—	—	—	a
Starch, hydrolysis	—	—	—	—	+
Sucrose	a	a	a	a	a
Trehalose	—	—	—	—	a
Xylose	a	a	a	a	—
Adonitol	—	—	—	—	—
Dulcitol	—	—	—	—	—
Galactose	a	a	a	a	a
Glycerol	—	—	—	—	w
Inulin	—	w	—	w	—
Sorbose	—	—	—	—	—
Fermentation products[d]					
Acetic acid	0.62	0.42	0.69	0.53	2.29
Butyric acid	—	—	—	—	1.91
Lactic acid	0.20	0.13	0.10	0.09	0.48
Succinic acid	0.01	0.01	0.02	0.02	—
Gelatin liquefaction	—	—	—	—	+
Milk, curd formation	+	+	+	+	+
Indol production	—	—	—	—	—
Nitrate reduction	—	—	—	—	—
Hydrogen sulfide prod.	—	—	—	—	+
Motility	—	—	—	—	—
Catalase activity	—	—	—	—	—
Lecithinase activity	—	—	—	—	+
Lipase activity	—	—	—	—	—

[a] All the tests, except GLC of fermentation products (for which, see text), were performed according to the descriptions of Holdeman and Moore (15).

[b] Strain f-75 is another 7β-dehydrogenating strain, recently recovered from human feces by the same procedure as in this study. Strain PB6K is a stock culture of *Clostridium perfringens*, included for comparison.

[c] Acidity in carbohydrate broth: a, strong acid (pH below 5.5); w, weak acid (pH 5.5–6.0); —, no acid (pH 6.0 or more).

[d] Fatty acids produced in PYG cultures: quantified in meq/100 ml.

to hepatic enzymes. It seems difficult to draw any decisive conclusion from these in vivo studies for the hepatointestinal relationship of the epimerizing reaction.

Samuelsson (22) observed the conversion of 3α,7β,-12α-trihydroxycholanoate into deoxycholate in the rat cecum and suggested that the microbial removal of the 7β-hydroxy group is analogous with 7α-dehydroxylation. A similar idea was presented by Fedorowski et al. (23) as to the formation of LCA from UDCA in man. However, no microorganism has so far been shown to be capable of performing the direct removal of these 7β-hydroxy groups, and it seems more likely that the 7β-hydroxy groups were transformed into an α-orientation by the conversion system described here before the ordinary 7α-dehydroxylation.

The concept that two kinds of microorganisms take part in the epimeric conversion of the hydroxy group at C-7 contrasts with the idea of a similar reaction at C-3 being carried out by one and the same organism. Hayaishi et al. (24) extracted from *Escherichia (Citrobacter) freundii* an NAD-dependent dehydrogenase preparation that was capable of oxidizing LCA to a 3-oxo acid and also of reducing the oxo group to both α- and β-hydroxy groups. On the other hand, Marcus and Talalay (25) and Talalay and Marcus (26) demonstrated that *Pseudomonas testosteroni* elaborates two distinct oxidoreductases specifically affecting 3α- and 3β-hydroxysteroids, respectively, to perform the epimerizing conversion of the 3-hydroxy group. Similar conversion at C-3 has also been reported in certain strains of *Clostridium perfringens* (27), *Bacillus cereus* (27), *Eubacterium lentum* (28), and anaerobic streptococci (29), although it is undecided at present whether these organisms synthesize an ambivalent enzyme, as suggested by the work of Hayaishi et al. (24), or two specific dehydrogenases, as was the case with *P. testosteroni*. However, whether the reaction is caused by one enzyme or two separate enzymes, all of these 3-hydroxy epimerizations are carried out by a single bacterium. The involvement of two different organisms has never been reported until the present study of 7-hydroxy epimerization.

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