ASBMB
JOURNAL OF LIPID RESEARCH

Formation of urso- and ursodeoxy-cholic acids from primary bile acids by a *Clostridium limosum* soil isolate

J. Derek Sutherland,*[,1] Lillian V. Holdeman, C. Noel Williams,*** and Ian A. Macdonald*[,2]**

Departments of Medicine and Biochemistry,* Medicine,*** Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7, and Department of Anaerobic Microbiology,** Virginia Polytechnic Institute and State University, Blacksburg, VA 24061

Abstract A gram-positive, rod-shaped anaerobe (isolate F-14) was isolated from soil. This organism was identified by cellular morphology as well as by fermentative and biochemical data as *Clostridium limosum.* Isolate F-14 formed ursocholic acid (UC) and 7-ketodeoxycholic acid (7-KDC) from cholic acid (CA), and ursodeoxycholic acid (UDC) and 7-ketolithocholic acid (7-KLC) from chenodeoxycholic acid (CDC) in whole cell cultures, but did not transform deoxycholic acid (DC). No hydrolysis or transformation occurred when either taurine- or glycine-conjugated bile acids were incubated with F-14. The type strain of *Clostridium limosum* (American Type Culture Collection 25620) did not transform bile acids. The structures of ursocholic, ursodeoxycholic, 7-ketodeoxycholic, and 7-ketolithocholic acids were verified by mass spectroscopy and by thin-layer chromatography using Komarowsky's spray reagent. The organism transformed cholic and chenodeoxycholic acids at concentrations of 20 mM and 1 mM, respectively; higher concentrations of bile acids inhibited growth. Optimal yields of ursocholic and ursodeoxycholic acids were obtained at 9–24 hr of incubation and depended upon the substrate used. Increasing yields of 7-ketodeoxycholic and 7-ketolithocholic acids, and decreasing yields of ursocholic and ursodeoxycholic acids were observed with longer periods of incubation. Culture pH changed with time and was characterized by a small initial drop (0.2–0.4 pH units) and a subsequent increase to a pH (8.1–8.2) that was above the starting pH (7.4). We propose that the following pathways are associated with the F-14 soil isolate: cholic acid $\rightleftharpoons$ 7-ketodeoxycholic acid $\rightleftharpoons$ ursocholic acid and chenodeoxycholic acid $\rightleftharpoons$ 7-ketolithocholic acid $\rightleftharpoons$ ursodeoxycholic acid, with increasing dominance of the back reaction of the second step on aging (oxygenation) of the culture.—**Sutherland, J. D., L. V. Holdeman, C. N. Williams, and I. A. Macdonald.** Formation of urso- and ursodeoxycholic acids from primary bile acids by a *Clostridium limosum* soil isolate. *J. Lipid Res.* 1984. **25:** 1084–1089.

Supplementary key words 7α-hydroxyl group isomerization • 7β-hydroxyl group formation

Several anaerobes, principally human fecal isolates, have been shown to participate in the epimerization of the 7α-hydroxyl group of primary bile acids. *Clostridium absonum,* isolated from soil (1), readily epimerizes bile acids to their urso derivatives (2, 3) and bile acid-inducible 7α/7β-hydroxysteroid dehydrogenases have been demonstrated and investigated in cell extracts (4–6). Unidentified human intestinal isolates of *Clostridia* that produce neither lecithinase nor lipase have also been shown to epimerize chenodeoxycholic acid to ursodeoxycholic acid (7, 8). Intestinal bacteria which only elaborate a 7β-hydroxysteroid dehydrogenase can also be involved in the epimerization reaction, e.g., *Peptostreptococcus productus* (9, 10) and *Eubacterium aerofaciens* (10, 11). With these types of organisms, co-culturing with a known 7α-hydroxysteroid dehydrogenase elaborating organism, such as *Bacteroides fragilis* (12, 13) or *Escherichia coli* (14, 15), is necessary for complete epimerization. The purpose of the present study was to screen various soil samples for a new organism that readily epimerizes the 7α-hydroxyl group of primary bile acids and to speciate such an organism.

MATERIALS AND METHODS

Materials

Cholic acid (CA), parahydroxybenzaldehyde (Komarowsky's reagent), and silica gel plates (250-μm thick,

---

Abbreviations: BHI, brain-heart infusion; TLC, thin-layer chromatography; HSDH, hydroxysteroid dehydrogenase; ATCC, American Type Culture Collection; CA, cholic acid (3α,7α,12α-trihydroxy-5β-cholan-24-oic acid); UC, ursocholic acid (3α,7β,12α-trihydroxy-5β-cholan-24-oic acid); 7-KDC, 7-ketodeoxycholic acid (3α,12α-dihydroxy-7-oxo-5β-cholan-24-oic acid); CDC, chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholan-24-oic acid); UDC, ursodeoxycholic acid (3α,7β-dihydroxy-5β-cholan-24-oic acid); 7-KLC, 7-ketolithocholic acid (3α-hydroxy-7-oxo-5β-cholan-24-oic acid); DC, deoxycholic acid (3α,12α-dihydroxy-5β-cholan-24-oic acid).

[1] To whom reprint requests should be addressed.

[2] This work was initiated in the laboratory of Dr. Ian A. Macdonald at the time of his death, August 5, 1983.

Downloaded from www.jlr.org by guest, on June 18, 2012

medium-hard with organic binder) were from J. T. Baker Chemicals, Phillipsburg, NJ; deoxycholic acid (DC) was from Calbiochemicals, Los Angeles, CA; chenodeoxycholic (CDC) and ursodeoxycholic (UDC) acids were from Sigma Chemicals, St. Louis, MO; 7-ketodeoxycholic (7-KDC) and 7-ketolithocholic (7-KLC) acids were from Steraloids, Wiltshire, NH; ursocholic acid (UC) was kindly donated by Drs. R. A. Di Pietro and A. F. Hofmann, of the Division of Gastroenterology, School of Medicine, University of California, San Diego, CA. All the above bile acids gave one spot with TLC using chloroform–methanol–acetic acid 40:4:2 (v/v/v) for the tri-functional series and chloroform–methanol–acetic acid 40:2:1 (v/v/v) for the di-functional series. Labeled [24-^{14}C]CA and CDC were products of New England Nuclear, Lachine, Quebec. Labeled [24-^{14}C]7-KDC and 7-KLC were made by growing 10-ml cultures of *C. absonum* (3) in the presence of labeled [24-^{14}C]CA or CDC and purifying the respective 7-keto-intermediates by TLC. In all cases TLC revealed that over 99% of the label was associated with the spot corresponding to the radiolabeled bile acid in question.

Diethyl ether, methanol, chloroform, and liquid scintillation fluid (LSC cocktail 19229) were from British Drug House, Montreal, Quebec; brain-heart infusion broth (BHI), agar, and cooked meat were products of Difco Laboratories, Detroit, MI; Gas-Pak systems were from BBL Microbiology Systems, Cockeysville, MD.

Isolation of a 7α-hydroxyl group epimerizing bacterium from soil

Six soil samples (2–3 g each) were collected from urban garden plots and lawned areas in Halifax, Nova Scotia, Canada. About 0.5 g of soil was added to 10 ml of BHI broth containing 0.4 mM CA and the tubes were incubated at 37°C for approximately 18 hr. A 3-ml sample from each culture was then transferred to a glass-stoppered test tube and acidified to approximately pH 3 with 0.5 ml of 1 M HCl. Each sample was extracted twice with an equal volume of diethyl ether. The two ether extracts from each sample were pooled and evaporated to dryness. Samples were reconstituted in 50 μl of methanol–water 4:1 (v/v), and 20 μl of each was spotted along with pure standards of CA, UC, and 7-KDC on Baker TLC plates (20 by 20 cm). Plated samples were chromatographed in chloroform–methanol–acetic acid 40:4:2 (v/v/v). The plates were sprayed with Komarowsky's reagent (16), gently heated with a heat gun, and inspected under visible and UV light. The culture whose extract most actively produced a spot corresponding to UC was streaked onto blood agar plates for isolated colonies and incubated in a Gas-Pak jar for 24 hr at 37°C. Isolated colonies of different morphology were picked and restreaked several times. These restreaked bacteria were assessed for UC formation and a small anaerobic colony type (subsequently called F-14) was thus isolated.

Characterization of strain F-14

The biochemical reactions and volatile acids produced were determined with methods and prereduced media described previously (17).

Growth of F-14 isolate

Stock cultures were grown in freshly boiled cooked meat broth at 37°C for 18 hr and were stored at 4°C. Freshly boiled or freshly autoclaved BHI broth was used for subsequent growth of the organism. A 10% (v/v) inoculum was used throughout the entire study and, unless otherwise specified, cultures were incubated in an aerobic atmosphere at 37°C. Brain-heart infusion broth cultures incubated for 18 hr were used to inoculate media containing labeled bile acids.

To determine the effect of bile acid concentration on the F-14 isolate, cultures were grown in 10 ml of BHI broth containing various concentrations of [24-^{14}C]CA (1 through 30 mM) or [24-^{14}C]CDC (0.1 through 1.5 mM). Three-ml samples were removed at 10.5, 24, and 48 hr. The absorbance at 660 nm of each culture sample was measured in an LKB Biochrom Ultrospec 4050. Samples were frozen at −20°C until extraction.

To determine the effect of time, cultures were grown in 50 ml of BHI broth in either 125-ml Erlenmeyer flasks or 50-ml graduated cylinders. The broth medium contained either 0.2 mM 24-^{14}C-labeled primary or 7-keto bile acid. Approximately 0.01 μCi of label was used per study. Sampling volumes of 3 ml were removed at various times, the absorbance at 660 nm was measured, and samples were frozen until extraction.

To determine pH changes during the course of the incubation, 3-ml samples were periodically removed from model flask and cylinder cultures and the pH was measured using a Metrohm 632 pH meter.

Extraction, TLC, and counting procedure

Extraction and TLC were performed as described above except that extracts from BHI-CDC cultures were chromatographed in chloroform–methanol–acetic acid 40:2:1 (v/v/v). After the TLC plates had been sprayed wih Komarowsky's reagent, spots corresponding to the starting material and the 7β- and 7-keto-transformation products were scraped. The scrapings were transferred to Pasteur pipettes plugged with cotton wool and eluted with 1–2 ml of ether–methanol 50:50 (v/v) into counting vials. Ten ml of liquid scintillation fluid was added and the vials were counted for 10 min in a Nuclear Chicago Mark II liquid scintillation counter. The extent of

ASBMB JOURNAL OF LIPID RESEARCH

Downloaded from www.jlr.org by guest, on June 18, 2012

primary bile acid transformation was determined by calculating the % distribution of ^{14}C in primary, urso, and 7-keto bile acids.

Mass spectroscopy identification of F-14 products

Mass spectra of the F-14 transformation products were obtained as described previously for *C. absonum* (2).

Investigation of the type strain of *C. limosum* for 7α-hydroxyl group epimerizing activity

A lyophilized preparation of *C. limosum,* originally obtained from the ATCC (#25620), was grown in freshly boiled cooked meat broth for 18 hr at 37°C. The purity of the preparation was confirmed by streaking the culture onto blood agar plates that were incubated in a Gas-Pak jar for 24 hr at 37°C. Ten-ml BHI broth cultures containing 0.2 mM CA, 7-KDC, CDC, and 7-KLC, respectively, were grown and 3-ml samples were removed at 48 and 72 hr. Samples were frozen, then subjected to extraction and TLC as described above.

RESULTS

Of the six soil samples studied in the initial phase of this study, three demonstrated what appeared to be a similar 7α-hydroxyl group epimerizing activity. The F-14 isolate from one of these samples was a spore-forming, gram-positive, anaerobic, motile rod that was identified as *C. limosum.* The strain produced lecithinase; digested gelatin, milk and meat; and produced principally acetic acid in peptone–yeast extract broth or cooked meat (17) cultures. Tests were negative for production of indole and lipase, reduction of nitrate, hydrolysis of starch or esculin, and fermentation of amygdalin, arabinose, cellobiose, erythritol, esculin, fructose, glucose, glycogen, inositol, lactose, maltose, mannitol, mannose, melezitose, raffinose, rhamnose, ribose, salicin, sorbitol, starch, sucrose, trehalose, and xylose. No hydrogen was detected in the headspace of peptone–yeast extract–glucose cultures.

The most significant ions in the mass spectra of the methyl esters of F-14 putative 7-KLC, UDC, 7-KDC, and UC are summarized in **Table 1.** For comparison the fragment patterns of standard samples are also shown. The major fragment ions are those resulting from the loss of water molecules from the molecular ion and those resulting from the combination of a loss of water and the side chain. These data revealed that within experimental error the mass spectra of the methyl esters of the F-14 transformation products that were thought to be urso and 7-keto bile acids were identical to those of the commercially available bile acids. This information, along with the ability of the F-14 products

TABLE 1. Significant fragment ions in the mass spectra of the methyl esters of 7β-hydroxylated and 7-keto bile acids

Methyl Ester of Bile Acid	Significant Fragment Ions (m/e)	% Relative Intensity[a]: Standard Sample	F-14 Product
7-KLC	$404(M)^+$	100	100
	$386(M - H_2O)^+$	77	79
	$368(M - 2H_2O)^+$	29	30
	$271\ (M - H_2O - 115)^+$	48	46
	$253(M - 2H_2O - 115)^+$	39	36
UDC	$406(M)^+$	16	15
	$388(M - H_2O)^+$	89	88
	$370(M - 2H_2O)^+$	100	100
	$273(M - H_2O - 115)^+$	32	30
	$255(M - 2H_2O - 115)^+$	68	69
7-KDC	$420(M)^+$	9	10
	$402\ (M - H_2O)^+$	23	23
	$384(M - 2H_2O)^+$	21	23
	$366(M - 3H_2O)^+$	13	15
	$287(M - H_2O - 115)^+$	39	47
	$269(M - 2H_2O - 115)^+$	100	100
	$251(M - 3H_2O - 115)^+$	24	32
UC	$422(M)^+$	<1	<1
	$404(M - H_2O)^+$	4	4
	$386(M - 2H_2O)^+$	30	25
	$368(M - 3H_2O)^+$	13	13
	$289(M - H_2O - 115)^+$	100	100
	$271(M - 2H_2O - 115)^+$	76	72
	$253(M - 3H_2O - 115)^+$	71	65

[a] Intensities of fragment ions were normalized against the base peak.

to co-chromatograph with known bile acids, confirmed that urso and 7-keto bile acids (di- and tri-functional series) were indeed the products of primary bile acid transformation.

As shown in **Fig. 1A–D,** strain F-14 was capable of growth and significant transformation at a CDC concentration of 1 mM and a CA concentration of 20 mM, although there was partial growth inhibition. Higher bile acid concentrations inhibited growth substantially, yet a measurable amount of transformation was shown to occur at 48 hr (Figs. 1B and D). Throughout this study yields of UC (75–80%) were substantially higher than UDC (55–60%).

Time course curves (**Fig. 2** and **Fig. 3**) showed that for cultures containing CA and CDC substrates, respectively (Figs. 2A and C, 3A and C), optimal levels of UC were substantially higher than those of UDC (verifying a similar observation for Fig. 1). This observation was not true for cultures containing 7-keto substrates. When 7-KDC was substrate (Fig. 2B), 66% UC could be recovered, while as much as 80% UDC could be recovered when 7-KLC was substrate (Fig. 3B). Growth of the organism in a cylinder instead of a conventional Erlenmeyer flask was effective in sustaining a higher level of urso-product (Fig. 2A compared to 2C, Fig. 3A compared to 3C). When the organisms were grown in a flask, most of the initially made urso-product was rapidly

ASBMB JOURNAL OF LIPID RESEARCH

Downloaded from www.jlr.org by guest, on June 18, 2012

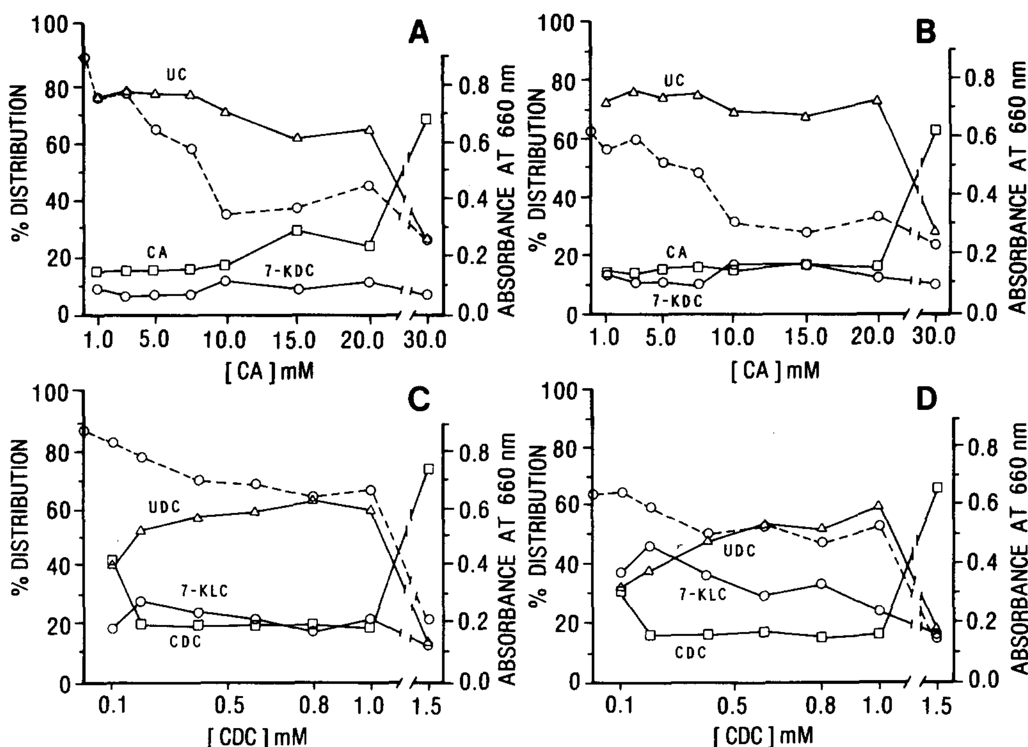

Fig. 1. Effect of initial bile acid concentration on the formation of UC and 7-KDC from [24-^{14}C]CA by F-14 isolate harvested at (A) t = 24 hr, (B) t = 48 hr; and UDC and 7-KLC from [24-^{14}C]CDC by F-14 isolate harvested at (C) t = 24 hr, (D) t = 48 hr. Symbols: (□——□), primary bile acid substrate; (△——△), 7β-transformation product; (○——○), 7-keto transformation product; and (○ - - - ○), absorbance of culture at 660 nm.

converted to the 7-keto compound and could not thereafter be recovered.

Studies of the pH of F-14 incubation mixtures indicated that a small initial drop from pH 7.4 to pH 7.0–7.2 occurred within the first 3 hr and was followed by a gradual increase to pH 8.1–8.2. In a flask this entire sequence of events occurred within a 24-hr period; however, in a cylinder, near neutral pH was maintained for a longer period of time and 72 hr was required before the pH rose above 8.0.

Incubation of primary and 7-keto bile acids with the ATCC strain of *C. limosum* revealed no 7α-hydroxyl group epimerizing activity. In fact, TLC analysis showed no transformation of bile acids at all.

DISCUSSION

Except for *C. absonum,* all of the organisms known to participate in the epimerization of the 7α-hydroxyl group of bile acids have been isolated from human feces. All eight active strains of *C. absonum* (4) were originally isolated from soil by Hayase and co-workers (1). It might be reasonable to assume that the soil from which these strains of *C. absonum* were isolated may have been contaminated with feces. To date, however, neither Hayase (1) nor ourselves (J. D. Sutherland and I. A. Macdonald, unpublished observation) have been able to isolate *C. absonum* from human or animal feces.

Clostridium limosum is phenotypically distinct from the two other clostridia (*C. absonum* (2–6) and the unidentified species described by Edenharder et al. (7, 8)) known to epimerize the 7α-hydroxyl group of bile acids. The type strain of *C. limosum* was isolated from African mud; most isolates have been from soil or animal infections (18). Because the type strain of *C. limosum* did not epimerize the 7α-hydroxyl group of bile acids, it appears that there is strain-to-strain variation within this species.

The studies reported here are consistent with an oxidative–reductive mechanism in which a 7-keto intermediate is involved, i.e., primary (7α-OH) bile acid ⇌ 7-keto bile acid ⇌ urso (7β-OH) bile acid. When labeled 7-keto intermediates are incubated with F-14, label is transformed both to 7α- and 7β-hydroxylated bile acids (Figs. 2B and 3B). Recent studies by Fromm, Sarva, and Bazzoli (19) support a reaction pathway in which a 7-keto intermediate is involved. Through isotope dilution studies, 7-KLC was shown to be the major intermediate in the intestinal bacterial conversion of CDC to UDC. To date, all organisms that can participate in 7α-hydroxyl group epimerization appear do so via the above-mentioned mechanism. A second mechanism of formation

Downloaded from www.jlr.org by guest, on June 18, 2012

ASBMB JOURNAL OF LIPID RESEARCH

ASBMB
JOURNAL OF LIPID RESEARCH

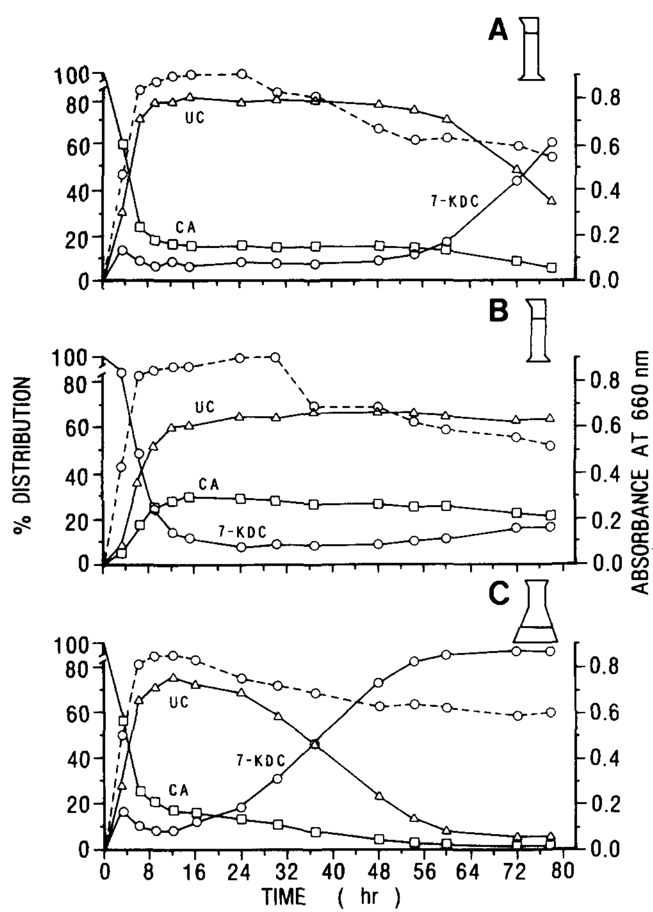

Fig. 2. Time course curves for the degradation of (A) 0.2 mM [24-^{14}C]CA by a 50-ml culture of F-14 isolate grown in a cylinder; (B) 0.2 mM [24-^{14}C]7-KDC by a 50-ml culture of F-14 isolate grown in a cylinder; and (C) 0.2 mM [24-^{14}C]CA by a 50-ml culture of F-14 isolate grown in a flask. Symbols: (□ —— □), CA; (△ —— △), UC; (○ —— ○), 7-KDC; and (○ - - - ○), absorbance of culture at 660 nm.

of urso bile acid from primary bile acid in which an unsaturated intermediate is involved has been proposed by Fedorowski et al. (20) and has been discussed in the work of Fromm et al. (19) and Sarva et al. (21). Although this alternate mechanism is possible, it appears to be a less likely explanation for the F-14 isolate and other related bacterial organisms. The total reversibility of the F-14 reaction pathway has not been firmly established here; however, preliminary work (J. D. Sutherland, unpublished results) has shown that incubation of urso (7β-OH) bile acids with F-14 does result in the production of small amounts of primary (7α-OH) and 7-keto bile acids. This would indicate that the reaction mechanism is totally reversible to some degree.

It appears that with time and oxygen exposure, cultures of *C. limosum* F-14, as well as those of *C. absonum*, tend to oxidize urso bile acids back to 7-keto bile acids. The explanation proposed for *C. absonum*, which may be applicable here, is that oxygen exposure (increasing Eh) gradually reduces the intracellular NADPH/NADP ratio and thereby shifts the reaction in the oxidative direction. A cylinder, as compared to a flask, restricts oxygen diffusion and thereby delays the formation of 7-keto bile acid (3).

Changes in the pH of F-14 cultures are similar to those of *C. absonum* (3) in that the pH initially drops (regardless of whether the culture was in a flask or a cylinder) and then recovers. With F-14, however, the initial drop was small (0.2–0.4 pH units) and the final pH (8.1–8.2) was consistently higher than the starting pH (7.4). The pH increase was rapid in a flask and slow in a cylinder and in general it paralleled the observed metabolic shift that resulted in the accumulation of 7-keto bile acid later in the F-14 incubation period. The rise in pH may influence this 7-keto formation but, in our opinion, the primary controlling factors are oxygen exposure and rate of oxygen diffusion into the culture. Our observations are consistent with studies by Fromm et al. (19) which showed that for fecal incubations, formation of 7-KLC from CDC was favored by alkaline fecal pH and aerobic conditions.

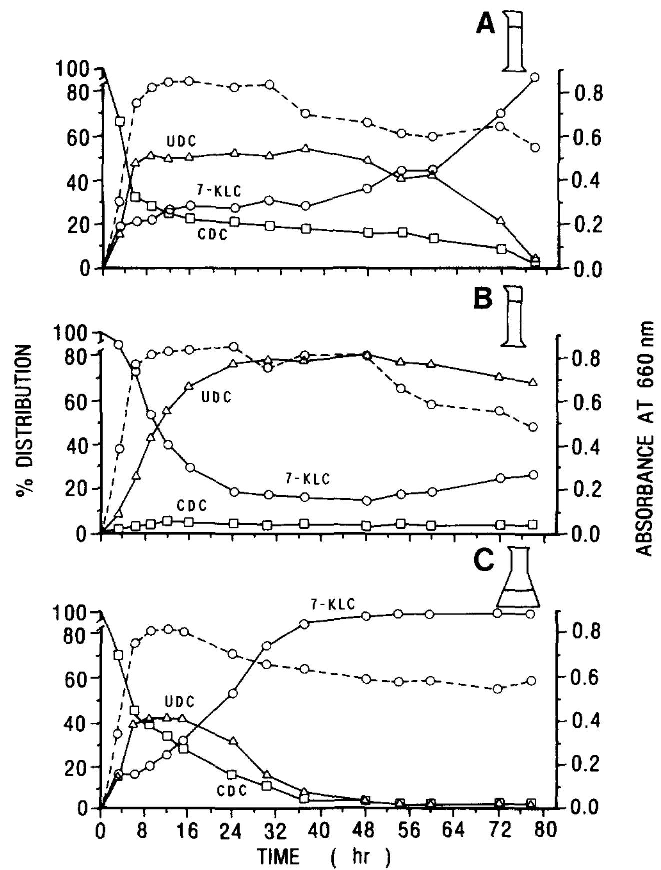

Fig. 3. Time course curves for the degradation of (A) 0.2 mM [24-^{14}C]CDC by a 50-ml culture of F-14 isolate grown in a cylinder; (B) 0.2 mM [24-^{14}C]7-KLC by a 50-ml culture of F-14 isolate grown in a cylinder; and (C) 0.2 mM [24-^{14}C]CDC by a 50-ml culture of F-14 isolate grown in a flask. Symbols: (□ —— □), CDC; (△ —— △), UDC; (○ —— ○), 7-KLC; and (○ - - - ○), absorbance of culture at 660 nm.

Downloaded from www.jlr.org by guest, on June 18, 2012

Clostridium limosum F-14 can grow at rather high bile acid concentrations (20 mM CA and 1 mM CDC). These values are higher than the 1.5 mM CA and 0.5 mM CDC levels found for *C. absonum*. This discovery may have important implications in the commercial microbiological synthesis of urso bile acids.

Clostridium limosum F-14 and *C. absonum* are two distinct species, both of which are capable of epimerizing the 7α-hydroxyl group of bile acids independent of other organisms. This fact implies that both organisms must contain a 7α- and 7β-HSDH. In the case of *C. absonum*, a bile acid-inducible 7α/7β-HSDH enzyme system has been clearly shown and thoroughly investigated (4–6). A similar enzyme system for *C. limosum* F-14 has been demonstrated (J. D. Sutherland, unpublished results). Current work focuses on investigating the properties of this system, carrying out further metabolic studies, and screening additional strains of *C. limosum* for 7α-hydroxyl group epimerizing activity.

We are grateful to Dr. D. E. Mahony (Department of Microbiology, Dalhousie University) and Drs. T. P. Forrest and J. H. Kim (Department of Chemistry, Dalhousie University) for consultation in various aspects of this study. This work was supported by the Medical Research Council of Canada, grant MA-5075, and by project 2022820 from the Commonwealth of Virginia.

Manuscript received 1 February 1984.

REFERENCES

1. Hayase, M., N. Mitsui, K. Tamai, S. Nakamura, and S. Nishida. 1974. Isolation of *Clostridium absonum* and its cultural and biochemical properties. *Infect. Immun.* **9:** 15–19.
2. Macdonald, I. A., D. M. Hutchison, and T. P. Forrest. 1981. Formation of urso- and ursodeoxy-cholic acids from primary bile acids by *Clostridium absonum. J. Lipid Res.* **22:** 458–466.
3. Sutherland, J. D., and I. A. Macdonald. 1982. The metabolism of primary, 7-oxo, and 7β-hydroxy bile acids by *Clostridium absonum. J. Lipid Res.* **23:** 726–732.
4. Macdonald, I. A., and P. D. Roach. 1981. Bile salt induction of 7α- and 7β-hydroxysteroid dehydrogenases in *Clostridium absonum. Biochim. Biophys. Acta.* **665:** 262–269.
5. Macdonald, I. A., and J. D. Sutherland. 1983. Further studies on the bile salt induction of 7α- and 7β-hydroxysteroid dehydrogenases in *Clostridium absonum. Biochim. Biophys. Acta.* **750:** 397–403.
6. Macdonald, I. A., B. A. White, and P. B. Hylemon. 1983. Separation of 7α- and 7β-hydroxysteroid dehydrogenase activities from *Clostridium absonum* ATCC# 27555 and cellular response of this organism to bile acid inducers. *J. Lipid Res.* **24:** 1119–1126.
7. Edenharder, R., and T. Knaflic. 1981. Epimerization of chendeoxycholic acid to ursodeoxycholic acid by human intestinal lecithinase-lipase-negative *Clostridia. J. Lipid Res.* **22:** 652–658.
8. Edenharder, R., and H. J. Deser. 1981. The significance of the bacterial steroid degradation for the etiology of large bowel cancer. VIII. Transformation of cholic, chenodeoxycholic and deoxycholic acid by lecithinase-lipase-negative *Clostridia. Zbl. Bakt. Hyg. I. Abt. Org.* **174:** 91–104.
9. Hirano, S., and N. Masuda. 1981. 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.* **22:** 1060–1068.
10. Hirano, S., and N. Masuda. 1982. Characterization of NADP-dependent 7β-hydroxysteroid dehydrogenases from *Peptostreptococcus productus* and *Eubacterium aerofaciens. Appl. Environ. Microbiol.* **43:** 1057–1063.
11. Macdonald, I. A., Y. P. Rochon, D. M. Hutchison, and L. V. Holdeman. 1982. Formation of ursodeoxycholic acid from chenodeoxycholic acid by a 7β-hydroxysteroid dehydrogenase-elaborating *Eubacterium aerofaciens* strain when cocultured with 7α-hydroxysteroid dehydrogenase-elaborating organisms. *Appl. Environ. Microbiol.* **44:** 1187–1195.
12. Hylemon, P. B., and J. A. Sherrod. 1975. Multiple forms of 7α-hydroxysteroid dehydrogenase in selected strains of *Bacteroides fragilis. J. Bacteriol.* **122:** 418–424.
13. Macdonald, I. A., C. N. Williams, D. E. Mahony, and W. M. Christie. 1975. NAD- and NADP-dependent 7α-hydroxysteroid dehydrogenases from *Bacteroides fragilis. Biochim. Biophys. Acta.* **384:** 12–24.
14. Haselwood, E. S., and G. A. D. Haselwood. 1976. The specificity of a 7α-hydroxysteroid dehydrogenase from *Escherichia coli. Biochem. J.* **157:** 207–210.
15. Macdonald, I. A., C. N. Williams, and D. E. Mahony. 1973. 7α-Hydroxysteroid dehydrogenase from *Escherichia coli B:* preliminary studies. *Biochim. Biophys. Acta.* **309:** 243–253.
16. Macdonald, I. A. 1977. Detection of bile acids with Komarowsky's reagent and group specific dehydrogenases. *J. Chromatogr.* **136:** 348–352.
17. Holdeman, L. V., E. P. Catto, and W. E. C. Moore. 1977. Anaerobe Laboratory Manual. 3rd ed. Virginia Polytechnic Institute Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg, VA. 108–110.
18. Smith, L. D. S., and G. Hobbs. 1975. *Clostridium* Prazmowski, 1880, 23. *In* Bergey's Manual of Determinative Bacteriology. R. E. Buchanan and N. E. Gibbons, editors. Williams and Wilkins, Baltimore, MD. 551–572.
19. Fromm, H., R. P. Sarva, and F. Bazzoli. 1983. Formation of ursodeoxycholic acid from chenodeoxycholic acid in the colon: studies of the role of 7-ketolithocholic acid as an intermediate. *J. Lipid Res.* **24:** 841–853.
20. Fedorowski, T., G. Salen, G. S. Tint, and E. H. Mosbach. 1979. Transformation of chenodeoxycholic acid and ursodeoxycholic acid by human intestinal bacteria. *Gastroenterology.* **77:** 1068–1073.
21. Sarva, R. P., H. Fromm, G. L. Carlson, L. Mendelow, and S. Ceryak. 1980. Intracolonic conversion in man of chenodeoxycholic acid (CDC) to ursodeoxycholic acid (UDC) with and without formation of 7-ketolithocholic acid (KLC) as an intermediate. *Gastroenterology.* **78:** 1252 (Abstract).

ASBMB JOURNAL OF LIPID RESEARCH

Downloaded from www.jlr.org by guest, on June 18, 2012