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Microbial Metabolism of Deoxycholate in the Mammalian Gastrointestinal Tract

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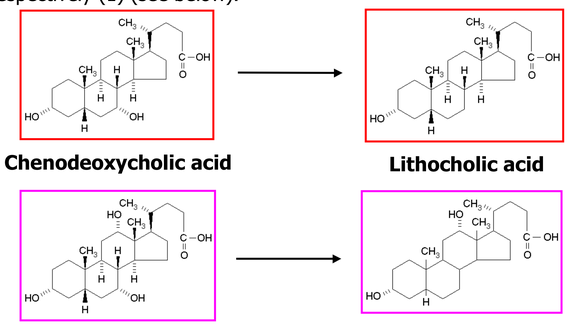
Introduction

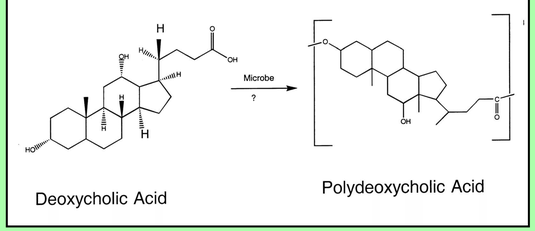
Although cholesterol is used by the body for a variety of functions, increased levels of cholesterol can have adverse affects such as heart attacks, heart disease, and strokes. One of the functions of cholesterol is the production of bile acids that aid in digestion of fats. Most of these bile acids are usually reaborbed by the body and recycled so cholesterol is not used up in the synthesis of bile acids.

In the intestinal tract, bile acids encounter numerous bacteria. Some of these bacteria convert the primary bile acids chenodeoxycholic acid and cholic acid into lithocholic acid and deoxycholic acid, respectively (1) (see below).



Recently, polydeoxycholic acid (a long-chain polymer of deoxycholic acid) was shown to be formed in the feces of humans and hamsters (2). It is believed that gut bacteria are able to convert deoxycholic acid to polydeoxycholic acid (**Figure 1**). If deoxycholic acid is indeed microbially converted to polydeoxycholic acid then it can not be reabsorbed and the body is forced to use more cholesterol to synthesize bile acids. However, experimental evidence which documents this microbial conversion in the mammalian gut is lacking.

Figure 1: Pathway for the formation of polydeoxycholic acid from deoxycholic acid.



Objectives

- To develop an enzyme assay to monitor the decrease of deoxycholic acid in fecal cultures
- To determine if microbes in the gastrointestinal tract are capable of converting deoxycholic acid to polydeoxycholic acid and other metabolites

Methods

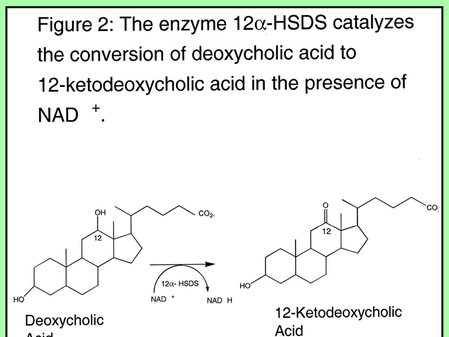
Collection and Processing of Hamster Feces

Hamsters (Syrian Golden) were used as a human model. Hamsters were fed a diet containing dog food, grain, and squirrel food. Feces (less than 24 h old) were collected and added to a 125-ml serum bottles (0.9 grams of feces per bottle) containing 50 ml of anaerobic Brain Heart Infusion Broth (DIFCO). Bottles were placed on a shaker and mixed for the contents mixed for 30 min at 500 rpm. After mixing, sterile anoxic sodium deoxycholate was added to achieve a final concentration of 0.1 or 1 mM, and fecal cultures were incubated at 37°C. At indicated time intervals, 1-ml samples were removed and frozen for further analysis.

Enzyme Assay for the Detection of Deoxycholic Acid

Fecal samples were clarified by microcentrifugation. After clarification, 100 µl of supernatant fluid was transferred to TAPS buffer (55 mM, pH 8) containing NAD^+ (1 mM) and 12a-hydroxysteroid dehydrogenase (12a-HSDS; 0.1 units). The enzyme 12a-HSDS is specific for deoxycholic acid and catalyzes the conversion of deoxycholic acid to 12-ketodeoxycholic acid in the presence of NAD^+ (**Figure 2**). The reaction was monitored by detecting the appearance of NADH at 340 nm. Cultures that showed a decrease in the amount of deoxycholic acid were subjected to thin-layer chromatography (TLC) to determine if polydeoxycholic acid or other deoxycholic acid-derived products were formed.

Figure 2: The enzyme 12α-HSDS catalyzes the conversion of deoxycholic acid to 12-ketodeoxycholic acid in the presence of NAD^+.



TLC Analysis

Samples (1 ml) of fecal cultures were removed and extracted with 2 ml of chloroform. The lower organic phase was removed and dried under Argon gas at room temperature. Once dry, 100 µl of chloroform was added to resuspend the sample, and 50 µl was spotted onto a TLC plate (silica gel). The spotted plate was placed in a developing tank containing only chloroform. After the solvent had migrated nearly to the top of the plate, the plate was removed, allowed to dry, and placed in a developing tank containing ethyl acetate, cyclohexane, glacial acetic acid, and chloroform (9:9:2:9). After the solvent had migrated nearly to the top of the plate, the plate was removed, sprayed with a charring agent (water, methanol, sulfuric acid, and $MnCl_2$), and charred at 110°C for 15 minutes so bile acids could be viewed under UV light.

Results

Enzyme and TLC Development

- Deoxycholic acid was detected to a concentration of 0.01 mM.
- Without the enzyme (12a-HSDS) or NAD^+, no reaction was observed.
- The bile acids 5ß-cholanic acid-3ß, 12a-diol and 5ß-cholanic acid- 3,12-dione also reacted in the enzyme assay.
- By first running the TLC plates in chloroform, the separation of polydeoxycholic acid and other bile acids (as standards prepared from stock solutions) was greatly improved (**Figure 3**).

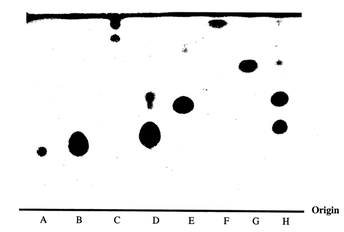
Fecal Cultures

- A decrease in the amount of deoxycholic acid was detected in fecal cultures incubated for 2 and 4 days (**Table 1**).
- Bile acids other than deoxycholic acid were also detected in fecal cultures (**Figure 4**).
- Polydeoxycholic acid was not detected in any of the fecal cultures examined (**Figure 4; data not shown**).

Table 1: The disappearance of deoxycholic acid in hamster fecal cultures.

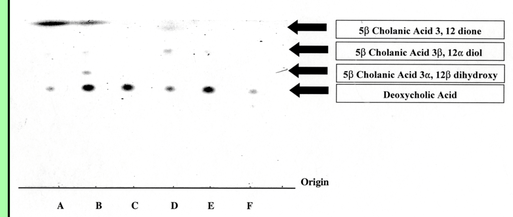
Incubation (days)	To	Tx	Tf	DCA Concentration	DCA Decrease	% DCA Decrease
0	0.100	0.492	0.392	0.63		
1	0.057	0.305	0.248	0.40	0.23	37%
6	0.058	0.262	0.204	0.33	0.33	48%
8	0.036	0.189	0.153	0.25	0.38	60%
13	0.046	0.191	0.145	0.24	0.39	62%
20	0.058	0.203	0.145	0.24	0.39	62%

Figure 3. Thin layer chromatography of bile acid standards.



A= BHI alone
B= BHI and deoxycholic acid
C= BHI and polydeoxycholic acid
D= BHI and 5β cholanic acid 3α, 12 β dihydroxy
E= BHI and 5β cholanic acid 3β, 12α diol
F= BHI and 5β cholanic acid 3, 12 dione
G= BHI and 5β cholanic acid 3α ol, 12 one
H= Mixture of D, E, F, and G

Figure 4. Thin layer chromatography of fecal cultures supplemented with deoxycholic acid (1 mM).



A, B, C = BHI cultures analyzed at 2 days of incubation.

D, E, F = BHI cultures analyzed at 4 days of incubation.

Summary

- Deoxycholic acid-metabolizing bacteria were present in hamster feces.
- Based on TLC analysis, the bile acids 5ß-cholanic acid-3ß, 12a-diol and 5ß-cholanic acid-3a-ol, 12-one were produced by fecal bacteria via the metabolism of deoxycholic acid.
- Further research needs to be done to discover more sensitive methods for detecting small amounts of polydeoxycholic acid along with the exact pathway for the conversion of deoxycholic acid to polydeoxycholic acid and other bile acid derivatives.
- The role of gastrointestinal microbiota on the host's health is very important in digestion and could be of further importance if it is found that fecal bacteria are capable of converting bile acids into forms that cannot be reabsorbed by the body, thereby lowering serum cholesterol levels.

References

- Baron, S. F., and P.B. Hylemon. 1997. Biotransformation of bile acids, cholesterol, and steroid hormones. In: Gastrointestinal Microbiology (R. I. Mackie, B. A. White, eds.), p. 470-510. Chapman & Hall, New York.
- Benson, G.M., N.J. Haskins, C. Eckers, P.J. Moore, D.G. Reid, R.C. Mitchell, S. Waghmare, and K.E. Suckling. 1993. Polydeoxycholate in human and hamster feces: a major product of cholate metabolism. J. Lipid Res. 34:2121-2134.