Degradation of bile salts by human intestinal bacteria

VIVIENNE ARIES, J. S. CROWTHER, B. S. DRASAR, AND M. J. HILL

From the Bacteriology Department, Wright-Fleming Institute, St Mary's Hospital Medical School, London

We have previously reported that many strains of enterococci and the strictly anaerobic bacteria of the intestine (*Bacteroides*, *Bifidobacteria*, *Clostridia*, and *Veillonella*) hydrolyse bile acid conjugates (Drasar, Hill, and Shiner, 1966; Hill and Drasar, 1968). More detailed studies on the deconjugating enzyme, cholanylglycine hydrolase, obtained from *Bacteroides* spp, *Bifidobacterium* spp, *Clostridium* spp, and *Strep. faecalis* (two strains of each) are reported elsewhere (Aries and Hill, 1969). Here we report our studies on the metabolism of cholate and deoxycholate, and further studies on deconjugation.

METHODS

Samples of faeces from normal persons living on a mixed diet in England, and from normal persons living on a vegetarian diet in Uganda were examined bacteriologically using the methods described previously (Drasar, 1967), modified to facilitate the isolation and identification of *Bacillus* spp, *Pseudomonas* spp and anaerobic *Sarcina*. The differences between the faecal flora of Ugandans and English people are discussed elsewhere (Aries, Crowther, Drasar, Hill and Williams, 1969).

The bacteria were grown at 37°C under the conditions listed in Table I. Bacteria from 50 ml of broth culture were harvested by centrifugation and resuspended in 0.5 ml of supernatant. This suspension was mixed with 0.5 ml of a 0.1% solution of substrate (taurocholate, cholic acid, or deoxycholic acid) in 0.02 M phosphate buffer solution pH 7 and incubated at 37°C for 48 hours. Degradation products were separated by thin layer chromatography on silica gel G (Merck) using the solvents described by Eneroth (1963). Standard solutions were included on all plates; these contained sodium taurocholate (Maybridge Research Chemicals Ltd, Launceston, Cornwall), cholic acid, deoxycholic acid, chenodeoxycholic acid, lithocholic acid (all from Koch-Light Laboratories), cholanolic acid (Steraloid Ltd), 7 ketodeoxycholic acid, 12 keto lithocholic acid, and 7,12 diketo lithocholic acid, all prepared by oxidation methods from the parent hydroxyl compound (Fieser and Rajagopalan, 1949; Bergstrom and Haslewood, 1939).

TABLE I

GROWTH MEDIA AND CONDITIONS OF THE VARIOUS ORGANISMS STUDIED

Bacteria	Medium	Period of Growth (hr)	Atmosphere	Other Conditions
Enterobacteria	Glucose broth	24	Air	
Enterococci	Glucose broth	24	Air	
<i>Str. salivarius</i>	Reinforced clostridial medium	48	Air	
Lactobacilli	Reinforced clostridial medium	48	90% CO ₂ + 10% air	
Clostridia	Reinforced clostridial medium	48	10% CO ₂ in H ₂	
Veillonella	Reinforced clostridial medium	72	10% CO ₂ in H ₂	
Bacteroides	Reinforced clostridial medium	72	10% CO ₂ in H ₂	
Bifidobacteria	Reinforced clostridial medium	72	10% CO ₂ in H ₂	
<i>Bacillus</i> spp	Glucose broth	24	Air	Shaking
Anaerobic <i>Sarcina</i>	Reinforced clostridial medium	24	10% CO ₂ in H ₂	

RESULTS

Groups of organisms unable to deconjugate sodium taurocholate in the more concentrated solutions used in our previous studies were unable to do so in the dilute solutions used here.

Many strains of *Bacteroides* spp, *Bifidobacterium* spp, *Clostridium* spp, *Veillonella* spp and enterococci were able to degrade cholate and deoxycholate yielding one or more products (Table II). Strains of these genera also deconjugated bile salts. In addition, many enterobacteria, none of which were able to deconjugate bile salts, were able to metabolize the bile acids. The reaction was subject to substrate inhibition and very little reaction with 0.5% cholate was detected (Hill and Drasar, 1968).

TABLE II

ORGANISMS ABLE TO DEGRADE TAUROCHOLATE, CHOLATE, AND DEOXYCHOLATE

Organism	Source of Faeces	No. Tested	Percentage Able to Degrade Bile		
			Taurocholate	Cholate	Deoxycholate
Enterobacteria	Uganda	180	0	53	32
	England	87	0	78	39
Enterococci	Uganda	162	90	68	18
	England	90	93	81	29
<i>Str. salivarius</i>	Uganda	37	0	0	0
	England	45	0	0	0
Lactobacilli	Uganda				
	England	48	0	0	0
Clostridia	Uganda	60	96	91	43
	England	70	94	87	47
Bifidobacteria	Uganda	137	86	47	18
	England	57	74	56	21
Bacteroides	Uganda	17	71	48	18
	England	37	82	79	30
Bacilli	Uganda	36	11	25	0
	England	45	49	9	0
Veillonella	Uganda	72	36	19	1
	England	20	95	90	30
Anaerobic Sarcina	Uganda	12	67	58	0

(It is possible that a higher proportion of strains would prove to be active at still lower substrate concentrations.)

A number of strains of each of the active genera are under more extensive investigation. In general the enterobacteria produce only a single degradation product from cholic acid, usually 7 ketodeoxycholate. The Bacteroides and Clostridia are much more active, producing a number of products, some of which have been identified as deoxycholic acid, lithocholic acid, and possibly cholanolic acid together with 7 ketodeoxycholate. In addition, a number of products in which the α hydroxyl groups have been inverted to the β form (presumably by way of keto intermediates) have been tentatively identified.

DISCUSSION

The conversion of cholate to deoxycholate (Midtvedt, 1967; Coccucci, and Ferrari, 1963) and other products by bacteria is now well documented. Midtvedt and Norman (1967) have screened a

number of anaerobic bacteria and identified the products of cholate metabolism as 3 α 12 α dihydroxy 7 keto cholanate, 3 α hydroxy 7,12 diketo cholanate, and 3, 7, 12 triketo cholanate. We have not detected the two latter metabolites but this may be a result of the reaction conditions; Midtvedt used a much lower substrate concentration and incorporated it in the growth medium. The concentration used in our work (0.05%) is the lowest that can conveniently be used with our detection methods.

In conclusion, our results demonstrate that, in the metabolism of bile salts and acids by intestinal bacteria, the strictly anaerobic bacteria are of major importance. Our investigations on human faecal steroids indicate that this is probably true *in vivo* as well as in the situation *in vitro* described here.

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