review

Degradation of steroids in the human gut

I. A. Macdonald, V. D. Bokkenheuser, J. Winter, A. M. McLernon,[1] and E. H. Mosbach

Departments of Medicine and Biochemistry, Dalhousie University, Halifax, Nova Scotia, Canada;[2] Department of Pathology, St. Luke's-Roosevelt Hospital Center, New York, NY 10025;[3] and Department of Surgery, Beth Israel Medical Center, New York, NY 10003[4]

TABLE OF CONTENTS

INTRODUCTION

The intestinal tract of adults contains approximately 1 kg of bacteria, equivalent to 10^{14} organisms representing at least 400 distinct species (1–3). More than 99% of the organisms are obligate anaerobes. The concentration of bacteria increases from jejunum through the ileum and constitutes the bulk of the intestinal

Abbreviations: CCK, cholecystokinin; GLC, gas–liquid chromatography; EHC, enterohepatic circulation; Eh, oxidation–reduction potential; HSDH, hydroxysteroid dehydrogenase; MS, mass spectroscopy; menadione, vitamin K_3 = 2-methyl-1,4-naphthoquinone; TLC, thin-layer chromatography; NDC, nuclear dehydrogenating *Clostridia;* NDH, nuclear dehydrogenase. Sterols are 27 derivatives of cholestane, usually with a 3β-hydroxyl-group. Neutral steroids, in this review, are defined as C-19 and C-21 derivatives of pregnane and androstane. Phenolic steroids are derivatives of estrone in which the A ring is aromatic and hydroxylated.

[1] Present address: Ethicon Inc., Summerville, NJ 08876.
[2] I. A. Macdonald.
[3] V. D. Bokkenheuser, J. Winter, and A. M. McLernon.
[4] E. H. Mosbach.

stream in the colon. Thus both dietary and endogenous substances are destined to come intimately in contact with this population in the lower gut. A wide spectrum of microbial species, both anaerobes and facultatives, are capable of transforming the steroids with the result that the physical and biological properties of the latter can undergo drastic changes. In man and in other vertebrates these steroidal metabolites may often be absorbed and returned to the liver where further metabolism can take place. Recycled metabolites are generally re-excreted in the bile but some are delivered to the blood stream for renal excretion. Thus there is a need to understand the nature of these steroid transformations and their biological significance. Such information may be gained through experiments with humans as well as with appropriate animal models, mixed fecal cultures, pure bacterial cultures, and crude or purified enzyme systems. In this report we will review cholesterol, bile acid, and steroid hormone metabolism by the human intestinal flora including experimental approaches, involved organisms, molecular mechanisms, and medical significance of various steroid transformations. The metabolism of plant and marine steroids will not be addressed in this review.

SYSTEMATIC AND TRIVIAL NAMES

C_{24} and C_{27} steroids

Systematic names of bile acids and cholesterol metabolites referred to in the text by their trivial names are as follows: cholesterol, 5-cholesten-3β-ol; cholestanol, 5α-cholestan-3β-ol; epicholesterol, 5-cholesten-3α-ol; coprostanol (coprosterol), 5β-cholestan-3β-ol; coprostanone, 5β-cholestan-3-one; Δ^4-cholestenone, 4-cholesten-3-one; Δ^5-cholestenone, 5-cholesten-3-one; 7-dehydrocholesterol, 5,7-cholestadien-3β-ol; CA, cholic acid (3α,7α,12α-trihydroxy-5β-cholan-24-oic acid); DC, deoxycholic acid (3α,12α-dihydroxy-5β-cholan-24-oic acid); LC, lithocholic acid (3α-hydroxy-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); UC, ursocholic acid (3α,7β,12α-trihydroxy-5β-cholan-24-oic acid); 7-KLC, 7-keto-lithocholic acid (3α-hydroxy-7-oxo-5β-cholan-24-oic acid); 12-KLC, 12-keto-lithocholic acid (3α-hydroxy-12-oxo-5β-cholan-24-oic acid); β-muricholic acid, 3α,6β,7β-trihydroxy-5β-cholan-24-oic acid; ω-muricholic acid, 3α,6α,7β-trihydroxy-5β-cholan-24-oic acid; hyodeoxycholic acid, 3α,6α-dihydroxy-5β-cholan-24-oic acid.

C_{19} and C_{21} steroids

Androstenedione	= 4-androstene-3,17-dione;
11β-Hydroxyandrostenedione	= 11β-hydroxy-4-androstene-3,17-dione;
Androstadienedione	= 1,4-androstadiene-3,17-dione;
Δ^1-Testosterone	= 17β-hydroxy-1,4-androstadien-3-one;
Dehydroepiandrosterone	= 3β-hydroxy-5-androsten-17-one;
16α-hydroxydehydroepiandrosterone	= 3β,16α-dihydroxy-5-androsten-17-one;
Progesterone	= 4-pregnene-3,20-dione;
Δ^{16}-Progesterone	= 4,16-pregnadiene-3,20-dione;
17α-Progesterone	= 17α-pregn-4-ene-3,20-dione;
16α-Hydroxyprogesterone	= 16α-hydroxy-4-pregnene-3,20-dione;
Pregnanolone	= 3α-hydroxy-5β-pregnan-20-one;
17α-Pregnanolone	= 3α-hydroxy-5β,17α-pregnan-20-one;
Pregnanedione	= 5β-pregnane-3,20-dione;
16α-Hydroxypregnanolone	= 3α,16α-dihydroxy-5β-pregnan-20-one;
Pregnanediol	= 5β-pregnane-3α,20α-diol;
Pregnanetriol	= 5β-pregnane-3α,17α,20α-triol;
Medroxyprogesterone acetate	= 17α-acetoxy-6α-methyl-4-pregnene-3,20-dione;
Megestrol acetate	= 17α-acetoxy-6,methyl-4,6-pregnandiene-3,20-dione;
Deoxycorticosterone (DOC)	= 21-hydroxy-4-pregnene-3,20-dione;
Tetrahydrodeoxycorticosterone (THDOC)	= 3α,21-dihydroxy-5β-pregnan-20-one;
Corticosterone	= 11β,21-dihydroxy-4-pregnene-3,20-dione;
11-Dehydrocorticosterone	= 21-hydroxy-4-pregnene-3,11,20-trione;
Cortisol	= 11β,17α,21-trihydroxy-4-pregnene-3,20-dione;
Tetrahydrocortisol	= 3α,11β,17α,21-tetrahydroxy-5β-pregnan-20-one;
21-Deoxycortisol	= 11β,17α-dihydroxy-4-pregnene-3,20-dione;
5β-Dihydrocortisol	= 11β,17α,21-trihydroxy-5β-pregnane-3,20-dione;

20-Dihydrocortisol = 11β,17α,20,21-tetrahydroxy-4-pregnen-3-one;
Pregnisolone (Δ^1-dehydrocortisol) = 11β,17α,21-trihydroxy-1,4-pregnadiene-3,20-dione.

C_{18} steroids

Estrone = 3-hydroxy-1,3,5,(10)-estratriene-17-one;
Estradiol = 1,3,5,(10)-estratriene-3,17β-diol;
Estriol = 1,3,5(10)-estratriene-3,16α,17β-triol;
16-epiestrol = 1,3,5(10)-estratriene-3,16β,17β-triol;
Ethynyl estradiol = 17α-ethynyl-1,3,5(10)-estratriene-3,17β-diol;
Norgestrel = 13β-ethyl-17α-ethynyl-17β-hydroxy-gon-4-ene-3-one;
Norethisterone = norethindrone = 19-nor-17α-ethynyl-17β-hydroxy-4-androsten-3-one.

ENZYME AND STEROID NOMENCLATURE

Enzymatic reactions can occur at various sites of the ring systems, or at the side chain of the steroid molecule. The following transformations have been observed.

a. Hydrolysis: cleavage of the glycosidic linkage of β-glucuronides, breakage of the ester bond of sulfates and of the amide bond of taurine or glycine conjugates by hydrolases.

b. Oxidation–reduction: conversion of alcohols to ketones and vice versa by oxido-reductases (hydroxysteroid dehydrogenases, HSDH) and introduction of double bonds by nuclear dehydrogenases (NDH).

c. Dehydroxylation: removal of hydroxyl groups by dehydroxylases.

d. Isomerization: shift of a double bond (isomerases) or changes at an asymmetric center (epimerases).

e. Side chain cleavage: breakage of a C-C bond; for example, between C-17 and C-20 by desmolases.

Specific enzymes or enzyme systems catalyzing these reactions and specific cofactors are described in the text and listed in the tables. The structures and the numbering systems of the various steroids discussed in this review are shown in **Fig. 1.**

CLASSES OF STEROIDS IN THE HUMAN GUT

Three major classes of steroids pass through the gastrointestinal tract, interact with the gut flora, and sub-

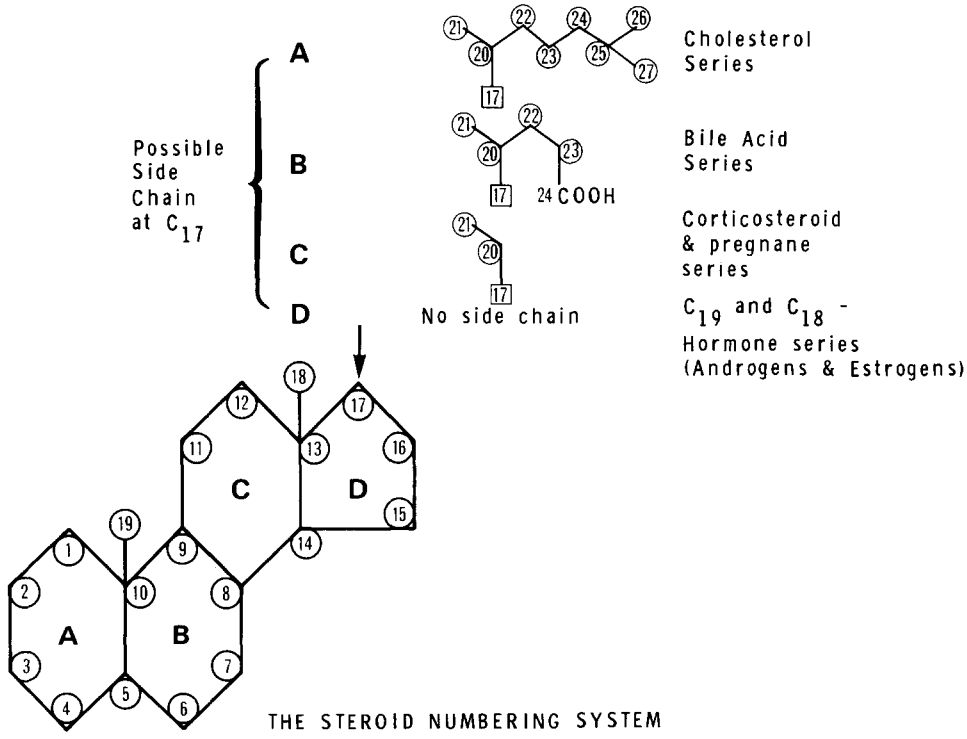

Fig. 1. Structure of steroids found in the human gut.

sequently can undergo microbial transformations. These classes (see Fig. 1) are *i*) cholesterol originating from the diet, liver, intestinal epithelium, and other tissues; *ii*) bile acids synthesized from cholesterol in the liver and excreted via the biliary tract, and *iii*) steroid hormones synthesized from cholesterol in the adrenal cortex and gonads, cleared by the liver, and excreted via the biliary tract.

Roughly, 400–1000 mg of cholesterol, 100–500 mg of bile acids, and less than 2 mg of steroid hormones pass daily through the colon in a healthy nonpregnant human subject on a "mixed western" diet. Both the cholesterol and the steroid hormones are generally water-insoluble. In bile they form mixed micelles (polymolecular aggregates) with the highly soluble bile acid conjugates and other polar lipids such as phospholipids. Bile acids can occur both in the monomeric and micellar forms and are generally ionized. During the passage through the lower gastrointestinal tract, all three types of steroids interact with the intestinal flora. This review highlights the various established transformations and, when known, the properties of the organism(s) and enzyme system(s) responsible.

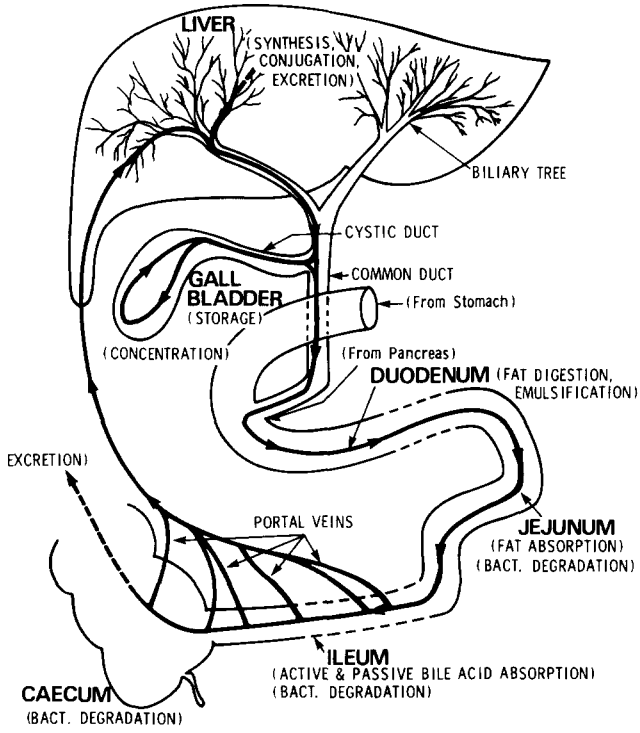

Fig. 2. The enterohepatic circulation.

THE ENTEROHEPATIC CIRCULATION

Several reviews dealing with different aspects of the enterohepatic circulation (EHC) are available including historic (4) and current (5–8) perspectives. However, for the sake of clarity and completion, a brief summary of the role of the EHC in the metabolism of cholesterol, bile acids, and steroid hormones is outlined below.

Cholesterol

Strictly speaking, cholesterol does not undergo a clearly defined EHC. After absorption, it enters the lymphatic system in chylomicrons that are partially metabolized by lipoprotein lipases in the walls of the blood vessels. The ensuing secondary particles, containing the cholesterol, are further metabolized in the liver to lipoproteins.

Bile acids

The EHC of the bile acids in man and presumably most vertebrates (**Fig. 2**), is a system by which bile acids are recycled and conserved. In a healthy fasting man, virtually the entire bile acid pool, about 2 g is found within the EHC (7, 8). Thus, the EHC is of central importance in bile acid physiology. Bile acids are synthesized and conjugated exclusively in the liver and excreted through the canaliculi into the biliary system. In addition to conjugated bile acids, the bile contains cholesterol, phospholipids (primarily lecithin), conjugated steroids, proteins, bile pigments (primarily bilirubin), and inorganic salts. The bile is concentrated 5- to 10-fold in the gallbladder where it is stored until required.

On the appearance of food, chiefly fats and protein, in the duodenum, specialized cells of the duodenum secrete a hormone cholecystokinin (CCK) into the blood; this simultaneously causes contraction of the gallbladder and relaxation of the sphincter of Oddi. Thus, bile flows from the gallbladder into the duodenum and jejunum where the bile acids play a major physiological role in the digestion and absorption of lipids.

More than 97% of the bile acids are actively reabsorbed in the distal ileum and returned to the liver via the portal veins. The remaining bile acids pass into the large intestine where they undergo extensive bacterial degradation. The degradation products and some unchanged bile acids are largely excreted in the feces but a certain proportion is absorbed from the colon and returned to the liver. Serum contains a low concentration (3–4 μmol/l) of bile acids predominantly CDC, DC, and CA (9). Small amounts (mg) of bile acids are also present in the urine, primarily as 3-sulfates.

In human adults without liver disease, urinary bile acid excretion averages about 10 μmol per 24-hr period. Levels ranging from 1.2 μmol/24 hr in a 2-year-old child with recurrent benign intrahepatic cholestasis, to 308 μmol/24 hr in a patient with portal cirrhosis have been reported in liver disease. Almé et al. (10) studied 43 patients with various liver diseases and found that

CA and CDC accounted for 49–78%, and 3,12-disubstituted and monohydroxylated bile acids for 8–27% of total urinary bile acids. Bile acids hydroxylated at C-1 or C-6 (e.g., 1, 3, 6, 7, 12; 1, 3, 7, 12 or 3, 6, 7, 12) constitute 5–15% of the total. The occurrence of relatively high proportions of such bile acids in urine clearly distinguishes hepatic disease from the normal state (10–12). In cirrhotic patients, norcholic acid (3α,7α,12α-trihydroxy-24-nor-5β-cholanoic acid) was considerably more abundant, accounting for 6% of total bile acid, than in noncirrhotic subjects (average 1%). Otherwise it has not been possible so far to distinguish between different liver diseases by comparing urinary bile acid profiles.

The origin of polyhydroxylated bile acids in urine is not known; they are probably of hepatic origin. We have not encountered hydroxylation in experiments with intestinal microorganisms.

The quantitatively most significant secondary bile acids occurring in the gut are DC derived from CA, and LC derived from CDC by microbial 7-dehydroxylation. The secondary bile acids, like the primary bile acids, are conjugated with either glycine or taurine during hepatic passage. DC may comprise up to 20–30% of the human bile acid pool, while LC accounts for only 1–3% of the total. A large proportion of conjugated LC is sulfated at the 3-position in the human liver but this detoxification mechanism appears to be largely unavailable in other species, such as the baboon (13) or rhesus monkey (14). The conjugate is poorly absorbed. In the colon, tauro-LC and glyco-LC sulfates are presumably first hydrolyzed to LC sulfate, which is also poorly absorbed, and hydrolyzed further by bacterial sulfatases so that LC is finally excreted mostly in the unconjugated form.

Neutral, phenolic, and synthetic steroids

Like bile acids, neutral and phenolic steroids can undergo EHC. Differences in chemical structure such as an extra hydroxyl group, or the shape of the molecule can influence the excretion route of the steroids (15). Otherwise, little is known about the factors determining whether a particular molecule leaves the liver cell via the biliary canaliculi or through the sinusoids of the circulatory system. It is believed that a molecular weight of 300–600 is required for EHC, precisely the range of weights of the conjugated compounds. The steroids are conjugated in the liver with sulfuric or glucuronic acid or both. The type of conjugation of neutral steroids is determined by the chemical structure of the steroid. For example, $C_{19}O_2$ and $C_{21}O_2$ steroids with 3α-hydroxy-5β-structures are usually conjugated with glucuronic acid (ethereal glucuronides) while 3α-hydroxy-5α and 3β-hydroxy-Δ^5 steroids generally form sulfates (esters). The conjugation increases the polarity of the molecule which, at the prevailing pH, leads to ionization and therefore increased solubility. Quantification of biliary steroid hormones is difficult. Measurements in fistula bile are obviously erroneous since the EHC is interrupted. Analyses in intact individuals indicate that males secrete about 13 mg and females 6.5 mg of neutral steroids in the bile per 24 hr (16), but less than 2 mg arrives in the colon.

Some of the phenolic steroids are conjugated with both sulfuric and glucuronic acids, e.g., estriol is secreted in the bile as the 3-sulfate, 16α-glucuronide, while others are conjugated with sulfuric acid only (estrone) or glucuronic acid only (estradiol) (16–20). It appears that quantification has only been attempted in fistula patients. Aldercreutz and Luukkainen (21) found that nonpregnant and pregnant women excrete 16 mg and 3750 mg estriol, respectively, per 24 hr. Estrone and estradiol, both biologically less active than estriol, constitute less than 15% of the biliary phenolic steroids. Aldercreutz and Martin (18) noted that the bulk of estrogens participating in the EHC are hydroxylated at the 15 or 16 position in the liver.

Synthetic steroids also undergo EHC. After absorption these compounds are transported to the liver. Little is known about their immediate fate but it seems safe to assume that the bulk of the progestins escapes ring-A reduction in the liver and enters the circulation via the sinusoids. A small proportion of the molecules is reduced in the liver, despite the protective ethynyl group, conjugated, and excreted in the bile. These molecules are devoid of hormonal activity. Most of the synthetic estrogens probably also pass unaltered through the liver, but sooner or later circulating estrogens are taken up by the liver cells, conjugated, and excreted in the bile.

Most of the conjugated biliary steroids, normal or synthetic, are deconjugated in the gut, and reabsorbed before or after bacterial alterations. It is noteworthy that synthetic steroids have a higher rate of fecal excretion than the natural compounds. For example, following intravenous administration, 7% of estrone and estradiol (22), but 30% of ethynyl estradiol is excreted in the feces (23). The fecal excretion of norgestrel and norethisterone is even higher (24, 25).

The hepatic uptake and conjugation of steroids appear to be abnormal in certain diseases (17). For example, in Gilbert's disease, a genetic disorder associated with glucuronyl transferase deficiency, the hepatic uptake and conjugation of steroids are decreased. In Dubin-Johnson syndrome (an inherited liver transport defect of conjugated bilirubin) the excretion of conjugated anions is reduced. Cholestasis decreases biliary excretion of estrogen glucuronides.

EXPERIMENTAL APPROACHES IN STUDYING THE DEGRADATION OF STEROIDS BY HUMAN INTESTINAL BACTERIA

Administration of labeled steroids

Labeled steroids can be given orally or intravenously. The label should be situated in the steroid ring or on the side chain (e.g., C24) in a position refractory to chemical or biological removal. The dynamics of EHC of any given steroid can be studied by draining the bile through a fistula or removing bile-rich duodenal samples periodically. The bile is then fractionated to measure the specific activity of the substance in question. Parameters such as pool size, turnover rate, and synthesis rate can be measured in the intact animal or human using the isotope dilution principle (5, 6). Alternately, bile can be drained and the total product (e.g., bile acid) can be measured. If the label appears in fraction(s) other than the administered compound, the new metabolite can be identified and its rate of formation can be calculated. Labeled products or their metabolites leaving the EHC may be detected in urine or feces.

Analysis of steroids in bile, urine and feces

Cholesterol. A number of publications have described the determination of cholesterol in bile by GLC (26), HPLC (27), and enzymic methods (28). The determination of total sterols in feces is difficult because in the gut, cholesterol is partially degraded to coprostanol and coprostanone. These analyses are rendered more complex by the presence of the corresponding degradation products of plant sterols, e.g., coprositosterol (24-ethyl-5β-cholestan-3β-ol) and by high concentrations of pigmented material. Elaborate and reliable techniques for the determination of fecal neutral sterols by a combination of solvent extraction, TLC, and GLC were developed by Miettinen, Ahrens, and Grundy (29). Small amounts of cholesterol in urine are detectable by GLC (30).

Bile acids. Methods for the analysis of biliary bile acids, widely used at present, employ deproteinization with methanol, and alkaline or enzymatic hydrolysis followed by GLC. Final confirmation of bile acid structures often requires MS. A reliable method for the analysis of total fecal bile acids based on solvent extraction, TLC, and GLC has been published by Grundy, Ahrens, and Miettinen (31). Urine contains small amounts of a variety of unusual bile acids. The analytical procedures have been worked out (10, 12) and similar techniques have been used to analyze fecal bile acid composition (32). The above-mentioned techniques, although sophisticated, are not without artifacts such as the destruction of 3-keto bile acids by alkaline hydrolysis of conjugates which are found in trace amounts in feces (32) and higher amounts in urine (10–12).

Steroid hormones. Bile contains many different types of steroid hormones ranging from the C_{18} estrogens with phenolic hydroxyl groups, the C_{19} androgens, and C_{21} progestational hormones to the C_{21} corticosteroids with various oxygenated substituents. These classes of steroids exhibit considerable differences in polarity, solubility, and stability which require a variety of specialized procedures for their isolation and identification (33, 34).

Animal models

In order to determine whether or not a given metabolic process is carried out by the intestinal flora, the metabolism of labeled steroids can be studied in germ-free animals and compared to the metabolism in conventional animals. The appearance of labeled transformation products in the feces or urine of conventional animals and their absence in germ-free animals strongly suggest the involvement of the microbial flora. This principle was first employed in the field of bile acid metabolism (35–37). By monoinfecting germ-free animals with a pure bacterial culture and examining the metabolic products in the feces, the formation of distinct metabolites could be directly associated with a given organism. Similar techniques were applied to the study of steroid hormones. For example, it was shown that 21-dehydroxylated derivatives of certain corticoids required the existence of normal intestinal microflora (16, 38). Such observations in animal models have led to the isolation from human fecal flora of microorganisms capable of carrying out a variety of transformations (39, 40). Wostmann and coworkers (41, 42) compared the composition of bile acids in the feces and bile of germ-free rats and conventional animals. Fecal bile acids were analyzed by a modification of the method by Grundy et al. (31). Briefly, this consisted of solvent extraction and separation of the bile acids by two TLC systems followed by GLC analysis; a trace label of [^{14}C]labeled CA was used as an internal standard. The fecal bile acid composition of germ-free and conventional Wistar rats is summarized in **Table 1,** adopted from Madsen et al. (41). Similarly, **Table 2** summarizes the biliary bile acid composition of germ-free dogs, rats, rabbits, and man. Data for the rat and mouse are adapted from Beaver, Wostmann, and Madsen (42), for the rabbit from Hofmann et al. (43, 44), and for man from Garbutt et al. (45). From the bile acid composition listed in Table 2, it is evident that in the rat, DC, ω-muricholic acid (3α,6α,7β-trihydroxy-5β-cholan-24-oic acid), hyodeoxycholic acid (3α,6α-dihydroxy-5β-cholan-24-oic acid), and the ketonic bile acids are secondary bile acids (Table 1). Also muricholic acids (3,6,7-trihydroxy-5β-cholan-

TABLE 1. Percent composition of fecal acids in germ free conventional Wistar rats[a]

Bile Acid	GF[b]	CV[b]
CA	41	4.0
CDC	1.4	0
DC	0	16
LC	0	1.2
Hyodeoxycholic[c]	0	34
β-Muricholic[d]	56	2.2
ω-Muricholic[e]	0	19
Total keto-bile acids	tr.	24

[a] Adapted from Madsen et al. (41).
[b] GF, germ free; CV, conventional.
[c] Hyodeoxycholic, 3α,6α-dihydroxy-5β-cholan-24-oic acid.
[d] β-Muricholic, 3α,6β,7β-trihydroxy-5β-cholan-24-oic acid.
[e] ω-Muricholic, 3α,6α,7β-trihydroxy-5β-cholan-24-oic acid.

24-oic acids) are found largely in mice and rats but are absent in the other species including humans. Formation of hyodeoxycholic acid appears to be largely by 7-dehydroxylation of ω-muricholic acid (42, 43). It is apparent that β-muricholic acid (3α,6β,7β-trihydroxy-5β-cholan-24-oic acid) may be epimerized to ω-muricholic acid by the rat intestinal flora. Confirming this proposal, a strain of Clostridium group III, which transforms β-muricholic acid to ω-muricholic acid, has been isolated from rat feces by Sacquet and coworkers (46); In contrast to 7-hydroxyl epimerization (See Epimerization of 3α- and 7α-hydroxyl groups.), this reaction results in formation of a 6α-hydroxyl group and is peculiar to the fecal flora of rats and mice. Rabbits, on the other hand, make some allo-CA(5α-) as a primary bile acid, (as observed in germ-free animals) and in conventional animals this is quantitatively 7-dehydroxylated to allo-DC by the flora (Table 2) (See also below, Formation of 5α-(allo) bile acids.)

Metabolism of steroids by mixed fecal flora and pure cultures in vitro

The bacterial metabolism of steroids may also be studied in vitro. By incubating a steroid precursor with a culture of mixed fecal flora or with a pure bacterial culture it is possible to follow the sequential alterations of the substrate (16). The cultures are sampled at specific intervals and the steroids are extracted, identified, and quantified.

In vitro techniques enable the investigator to identify specific microorganisms and isolate enzymes associated with a given steroid transformation (39, 47). This approach has provided information on metabolic pathways of steroid compounds (39), and microbiologists have used the pertinent enzymes as a basis for bacterial classification (48).

Studies with crude and purified enzyme systems in vitro

Studying enzyme systems in vitro throws some light on the mechanism by which certain bacterial transformations take place in vivo. Example 1: Ursodeoxycholic acid (UDC) is a fecal bile acid and is also found as a minor biliary bile acid. Yet the 12α-hydroxy analogue, UC, is scarce in both bile and feces. This finding can be explained in terms of a bacterial 7α-HSDH which has a greater affinity (lower K_m value) for CDC than for CA (49). It is also possible that CDC is a better inducer of the epimerizing enzymes, 7α- and 7β-HSDH, than CA (49), or that UC is more rapidly 7-dehydroxylated to give DC than CA. (for details see below, Epimerization of 3α- and 7α-hydroxyl groups.)

Example 2: Deoxycholic acid (DC) is the most common secondary bile acid. Yet Stellwag and Hylemon (50) found that the number of organisms capable of synthesizing the specific 7-dehydroxylase is only about 10^4–10^6 organisms per g wet feces. Studies on *C. leptum*

TABLE 2. Percent composition of biliary bile acids in germ-free dogs, rats, mice, rabbits, and humans[a]

	Mouse		Rat		Rabbit		Dog		Human	
Bile Acid	GF[b]	CV[b]	GF	CV	GF	CV	GF	CV	GF	CV
CA	25	53	50	75	94	0	95	84	Present	45
CDC	1.5	0	1	4	1	0	4.6	3.7	Present	35
DC	0	3.5	0	1.0	0	89	0	12	0	20
allo-CA	NR[c]	NR	NR	NR	5	0	NR	NR	NR	NR
allo-DC	NR	NR	NR	NR	0	6.4	NR	NR	NR	NR
β-Muricholic[d]	68	38	49	15	0	0	0	0	0	0
Hyodeoxycholic[e]	0	trace	0	3	0	0	0	0	0	0

[a] Adapted from Beaver et al. (42) and Hofmann et al. (43).
[b] GF, germ-free; CV, conventional.
[c] NR, not reported.
[d] β-Muricholic acid, 3α,6α,7β-trihydroxy-5β-cholan-24-oic acid.
[e] Hyodeoxycholic acid, 3α,6α-dihydroxy-5β-cholan-24-oic acid.

show that the affinity of this enzyme toward the substrate is extremely high (K_m value of the order of 10^{-7} M) (50, 51) and the reaction occurs on the cell surface so that the substrate need not even enter the cell for transformation.

Example 3: Pregnanolone and 16α-hydroxypregnanolone are known to undergo EHC. In these metabolites, the side chain normally has the 17β-configuration. Human urine contains both 17α- and 17β-pregnanolone. Studies with pure cultures and isolated enzymes explain the origin of the 17α-isomer (52–54). It results from the 16α-dehydroxylation reaction of 16α-hydroxypregnanolone. In the gut, *E. lentum* synthesizes a 16α-dehydrase which converts the 16α-hydroxypregnanolone to the intermediary, Δ^{16}-pregnanolone. This in turn, is metabolized by a specific reductase to the 17α-isomer (54). 17β-Pregnanolone arises from an entirely different source. It is derived either from the ring A reduction of progesterone or from the 21-dehydroxylation of THDOC. In this case, the stereochemistry of the urinary steroids discloses the origin of a given metabolite.

METABOLISM OF CHOLESTEROL

Outlined in **Fig. 3** are the major known degradative products of cholesterol. The reactions are presented in **Table 3,** describing the microorganisms and enzyme systems responsible.

Reduction of cholesterol to coprostanol

Depending upon the diet, 50% or more of the total fecal sterol can be present in the form of coprostanol. Coprostanone and lesser amounts of cholestanol can be detected in normal feces. The latter saturated sterol arises predominantly from the bacterial reduction of cholestenone (55, 56).

The early observations (57, 58) that mixed fecal cultures of human or rat readily transform cholesterol to coprostanol led to later attempts to isolate the microorganism responsible for this transformation. Crowther et al. (59) claimed that this reaction was carried out by strains of common intestinal bacteria; *Bifidobacterium sp.*, *Clostridium sp.*, and *Bacteroides spp.* of which *Bacteroides thetaiotaomicron* produced the largest amount of coprostanol (59). In contrast, Sadzikowski, Sperry, and Wilkins (60) and Eyssen et al. (38, 61) isolated microorganisms from human and rat feces that reduced Δ^5-3β-hydroxysteroids to 5β-saturated derivatives. These organisms were identified as *Eubacterium* species; according to the authors (38, 60, 61) they should be solely responsible for the conversion of cholesterol to coprostanol. The discrepancy remains to be resolved.

Two major pathways have been postulated for the conversion of cholesterol to coprostanol. The first involves direct reduction of the double bond at C-5. The second pathway involves the initial oxidation of the 3β-OH group and isomerization of the double bond to form the intermediate 4-cholesten-3-one which then undergoes nuclear reduction to 5β-cholestan-3-one and further reduction of the ketone to yield coprostanol. Evidence has been presented for both alternatives. Rosenfeld and Gallagher (62) incubated [3α-^{3}H]cholesterol with human feces and observed that the resulting co-

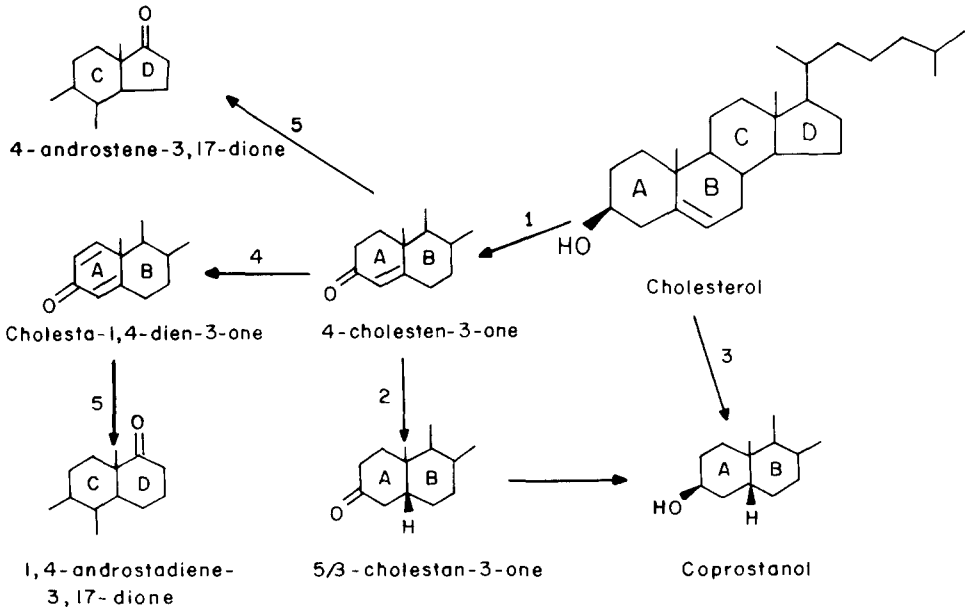

Fig. 3. Metabolism of cholesterol.

TABLE 3. Metabolism of cholesterol

#	Steroid Transformation	Enzyme(s)	Organism(s)	Comments	References
1	Cholesterol → 4-cholesten-3-one	Cholesterol-dehydrogenase	*E. coli* *Eubacterium 21408*	This product is the proposed intermediate for conversion of cholesterol to coprostanol.	76 77
2	4-Cholesten-3-one → 5β-cholestan-3-one	Δ^4-NDH	*Eubacterium* *Bacteroides* *Clostridium* *Bifidobacterium*		59 61 76
3	Cholesterol → coprostanol	Δ^5-NDH	*Eubacterium*	Direct conversion of cholesterol to coprostanol may not be the common pathway. Most organisms require cholesterol and plasmalogens for specific growth factors.	61 65–69
4	4-Cholestan-3-one → cholesta-1,4-dien-3-one	Δ^1-Dehydrogenase	*E. coli*	Four products can be obtained from this reaction (see Fig. 3).	76
5	Cholesta-1,4-dien-3-one → androsta-1,4-dien-3,17-dione	Desmolase	human fecal flora *E. coli*		75 76

prostanol retained most of the label of C-3, indicating a direct reduction of the double bond. On the other hand, experiments with the cholesterol-reducing *Eubacterium* species from rat and human (38, 60, 61) indicate that a major pathway leading from cholesterol to coprostanol involves the intermediate formation of 4-cholesten-3-one (Fig. 3), followed by the reduction of the latter to coprostanol. Determination of the validity of either or both mechanisms was undertaken by Björkhem and Gustafsson (63) with rat fecal flora, and similarly by Parmentier and Eyssen (64) with *Eubacterium* ATCC 21408. Using [3α-^{3}H,4-^{14}C]cholesterol as a substrate, Parmentier and Eyssen (64) found that conversion to coprostanol occurred with a loss of about 50% of the tritium, the remainder being located in the 3α-position. However, Björkhem and Gustafsson (63) proposed that during the microbial conversion of [3α-^{3}H]cholesterol to coprostanol, 3α-tritium can be removed and then re-inserted into the same position. Thus, on the basis of experiments with [3α-^{3}H]cholesterol it is not possible to decide which of the two proposed mechanisms predominates. Using [4β-^{3}H,4-^{14}C]cholesterol as a substrate, the resulting coprostanol retained 60% of the tritium, most of which had been transferred to the C-6 position. This implies that the conversion of cholesterol to coprostanol involves isomerization of the double bond from C5 to C4 and implicates 4-cholesten-3-one as intermediate. Björkhem and Gustafsson (63) concluded that both pathways were of equal importance, whereas Parmentier and Eyssen (64) determined that the second pathway involving 4-cholesten-3-one predominated.

Cholesterol-reducing *Eubacteria* have been isolated from rat cecum (61), and feces of human (60) and baboon (65, 66). Most isolates (60, 65–69) have been reported to require the presence of alkenyl ether lipids (plasmalogens) as well as cholesterol or other steroids in the medium as specific growth factors. Nine *Eubacteria* isolates from baboons (66) did not require plasmalogen and cholesterol for growth; only two of the nine strains reduced cholesterol in the absence of plasmalogen.

Nonspecific inhibition of the transformation of cholesterol to coprostanol has been demonstrated. Cohen, Raicht, and Mosbach (70) showed that in rats, addition of tauro-CDC to the diet resulted in a 5-fold reduction of coprostanol formation. Subbiah et al. (71) found that certain sugars and milk inhibited coprostanol formation by human fecal homogenates. It is doubtful that the inhibition was specific; more likely, the sugars exerted their effect by lowering the pH. Eyssen, De Pauw, and Parmentier (72) noted that cholesterol could be metabolized to coprostanol by pure cultures of *Eubacterium* ATCC 21408 even in the presence of lactose. Addition of *E. coli*, a *Clostridium* sp. or *Streptococcus fecalis* (all forming acids from sugars) inhibited the reaction which could not be restored by buffering the medium above pH 6.5. Thus, the mechanism(s) of sugar inhibition is not known. Eyssen (72) further found that *Eubacterium* 21408 which transformed 4-cholesten-3-one to coprostanol did not reduce 4-cholestene, epicholesterol, or cholesterol esters, indicating that the 3β-hydroxyl group is required for the biohydrogenation process (61). At present, therefore, there is a paucity of information concerning the major mechanism(s) involved in the microbial transformation of cholesterol to coprostanol. The relative importance of different bacterial species in catalyzing this reaction remains to be established, although most known isolates performing this conversion are *Eubacteria* (60, 61, 65–69).

Coprostanone from cholesterol

Coprostanone is a relatively minor constituent of the fecal sterol fraction. It is readily reduced by rat cecal contents to coprostanol (73). In vivo conditions may favor this reaction and minimize the accumulation of coprostanone.

Side chain cleavage of cholesterol

Though well documented for fungi and various soil microorganisms, there is little evidence of a side chain cleavage of the cholesterol molecule by human intestinal bacteria (74). The studies summarized below are preliminary and unconfirmed data from animal experiments.

Following intracecal administration of [^{14}C]cholesterol to guinea pigs, Goddard and Hill (75) found that 1.7% appeared in the urine as estrogens. In vitro conversion of cholesterol by gut flora to estrone and estradiol has also been observed (75). In contrast, the rat excreted little of the intracecally administered cholesterol in the urine. The bulk appeared in the feces, with 6% as LC and iso-LC. The authors concluded that the gut flora of guinea pig is capable of removing the side chain of cholesterol, whereas in the rat only a 3-carbon unit is removed. Both activities were suppressed by administering antibiotics to the animals, further emphasizing the role of bacteria in the conversion.

A strain of *E. coli* isolated from a patient with colorectal cancer was reported by Owen et al. (76) and Tenneson, Owen, and Mason (77) to cleave the side chain of cholesterol and CA, nuclear dehydrogenate ring A, and dehydroxylate CA at C-7 (76). The metabolic products of cholesterol were 4-cholesten-3-one, androsta-4-en-3,17-dione, cholesta-1,4-dien-3-one, and androsta-1,4-dien-3,17-dione. All four metabolites were produced in aerobic incubation over a 14-day period while only the two latter compounds were obtained under anaerobic conditions.

The clinical significance of cholesterol and its metabolites coprostanol and coprostanone in the human intestine is still far from clear. Reddy et al. (78–80) have shown that the fecal levels of both cholesterol and its two major microbial products are higher in patients with bowel cancer, adenomatous polyps, and ulcerative colitis compared to age- and sex-matched controls. The same researchers (81) surprisingly, have shown that the degree of degradation of cholesterol was lower in a population hereditarily predisposed to bowel cancer. Cruse et al. (82, 83) have proposed that cholesterol, and perhaps its metabolites, may play a role as co-carcinogen(s) in colon cancer. This hypothesis hinges mainly on an epidemiological link and remains to be substantiated (84).

METABOLISM OF BILE ACIDS

The major known degradative reactions of CA are outlined in **Fig. 4.** Analogous reactions with the exception of 12α-OH dehydrogenation (reaction 4) can be listed for CDC. **Table 4** describes the organisms and

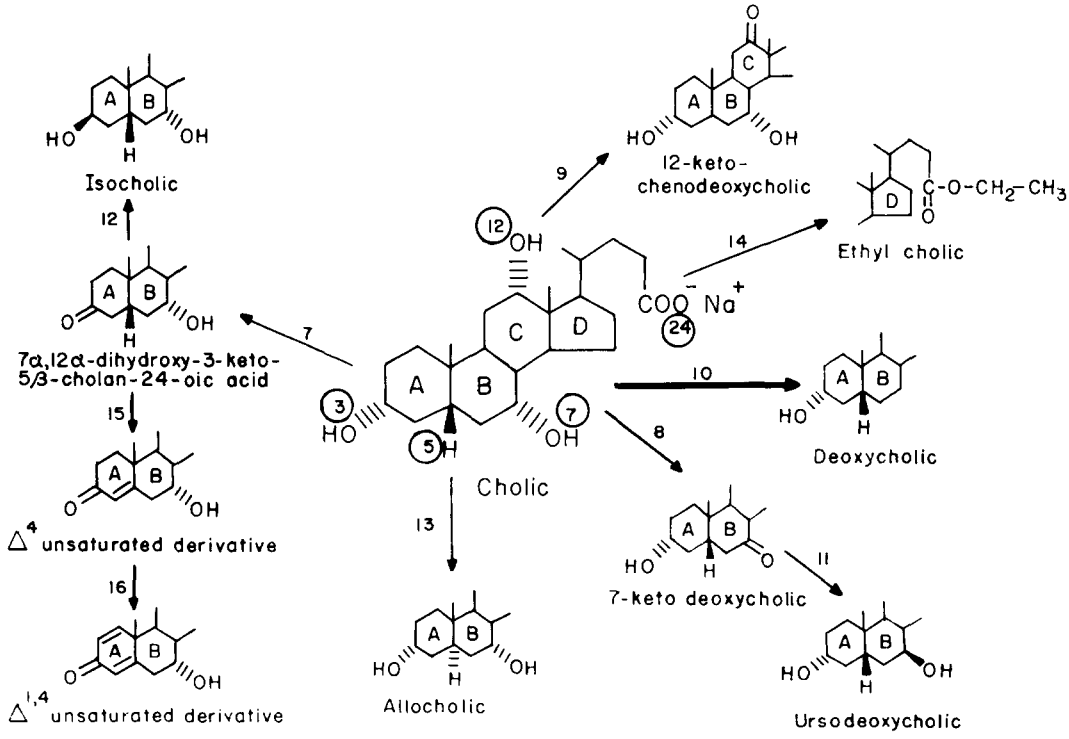

Fig. 4. Metabolism of cholic acid.

TABLE 4. Metabolism of bile acids

#	Steroid Transformation	Enzyme(s)	Organism(s)	Cofactor	pH	Comments	References
6	Hydrolysis of glycine or taurine conjugates	Bile acid hydrolase	*C. perfringens* *B. fragilis* many other orgs.		5.5 4.2	This is a very rapid and complete reaction in the colon.	86–93
7	3α-OH group → 3-ketone	3α-HSDH	*C. perfringens* *E. lentum*	NADP NAD	11.4 11.4		100, 101, 144 102, 103, 110, 111
8	7α-OH group → 7-ketone	7α-HSDH	*E. coli* *B. fragilis* *E. lentum*	NAD NAD(P) NAD	9.2 8.9	This is the most common of the human fecal flora.	105–107 108–111
9	12α-OH group → 12-ketone	12α-HSDH	*C. leptum* *Clostridium group P.* *E. lentum*	NADP NADP NAD	8.5 9.0 10.0		112–114 102, 103, 110, 111
10	7-OH group → alkane	7-Dehydroxylase	*C. leptum* *Eubacterium sp.* *C. bifermentans*	FMN/ NADH	≃7.0	This is a very efficient reaction with a low K_m value associated with the *Eubacterium* enzyme. A very low population (e.g., 10^4–10^6 org./ml feces) perform this reaction.	30, 51 120–128 130
11	7-Ketone → 7β-OH group	7β-HSDH	*C. absonum* *E. aerofaciens* lipase-lecithinase-neg. *Clostridia*	NADPH	≃6.0	This enzyme is associated with a 7α-HSDH in some organisms, e.g., *C. absonum*, and participates in the epimerization of the 7α-OH group.	49 148–155
12	3-Ketone → 3β-OH group	3β-HSDH	*C. perfringens* *E. lentum*	NAD(P)H	≃6.0	This enzyme has not been isolated from *C. perfringens*, *E. lentum*, or other organisms but should theoretically be present and participate in 3α-OH epimerization.	145 110, 111
13	5β → 5α (allo) H	"5β-Proton-isomerase"	?	?	?	The only evidence for the occurrence of this reaction is the presence of allocholic acid in human feces.	140 171–178
14	Bile acid → Ethyl ester	Bile salt–ethyl transferase	*B. fragilis* *Citrobacter sp.* *Peptostreptococcus productus T.*	EtOH	≃7.0	Ethylated bile acids have yet to be found in human feces.	181
15	3-Ketone → Δ^4 derivative	3-Ketone-bile-salt Δ^4 NDH	*C. paraputrificum*	Vit. K_3	≃7.0	Unsaturated bile acids are rarely found in human feces.	182
16	Δ^4 Derivative → $\Delta^{1,4}$ derivative	3-Keto bile salt $\Delta^{1,4}$ NDH	*C. paraputrificum*	Vit. K_3	≃7.0	Unsaturated bile acids are rarely found in human feces.	182

enzyme systems responsible for a given transformation. These reactions have been reviewed by Hayakawa (85), Midtvedt (86), Lewis and Gorbach (87) and Hill (88); important advances are summarized below.

Hydrolysis of conjugated bile acids

Bacterial deconjugation by "*B. coli*" was first described in 1934 by Basu and Chakravarty (89). Later Norman and Grub (90) reported deconjugation by a number of *Clostridia* and *Enterococci.* It is now known that bile acid hydrolysis may be caused by many intestinal organisms including *Bacteroides, Clostridium, Eubacterium, Lactobacillus,* and *Streptococcus,* but it has not been demonstrated in mammalian cells (85–88). Bacterial deconjugation in the gut is so thorough that hydrolysis of both taurine and glycine conjugates goes to completion in the colon (5, 6). The enzyme "bile acid hydrolase" (cholylglycine hydrolase) from *C. perfringens* (91) and *B. fragilis* (92) has been purified and characterized. The *C. perfringens* and the *B. fragilis* enzymes are both intracellular and have pH optima around 5.5 and 4.2, respectively (91, 92).

Among deconjugating microorganisms there is considerable variation in preference for taurine- versus glycine-conjugated bile acids. For example, a bile acid hydrolase from *Peptostreptococcus intermedius* displays a specificity for taurine conjugates while the enzyme from *S. fecalis* and *L. brevis* will hydrolyze chiefly glycine conjugates (93). In contrast, the *C. perfringens* enzyme will hydrolyze both forms of conjugates with some preference for the glycine derivatives (91).

It must be emphasized that the physical and chemical properties of bile acids change drastically when they are hydrolyzed (92). The solubility decreases, particularly at low pH. Free bile acids are poorer detergents than their conjugates and are far less efficient in forming mixed micelles (94, 95). Bacterial overgrowth in the small intestine can lead to premature deconjugation (95). Thus conjugated ionized forms are not available for fat absorption and digestion leading to fat malabsorption and steatorrhea (95). Premature deconjugation can also lead to some passive reabsorption of the unionized forms of free bile acids, which will not occur under physiological pH with conjugated bile acids.

Oxidation of 3α-, 7α-, and 12α-hydroxyl groups to ketones

Ketonic bile acids are not found in normal human bile in appreciable quantities but they may represent a significant fraction of human fecal bile acids. The bile of cholecystectomized patients contains small amounts of keto bile acids (96), presumably because the bile acids in the absence of the gallbladder are exposed more intensively to bacterial action. The presence of ketonic functions reduces the solubility, polarity, and detergency of the 5β-cholanoic acids. Ketonic bile acids are produced exclusively by bacterial NAD- or NADP-dependent hydroxysteroid dehydrogenases (HSDH). If they are reabsorbed and returned to the liver, the keto-groups are reduced primarily to the α-hydroxyl conformation by hepatic enzymes, which accounts for the low levels of keto-bile acids normally in bile (96).

Cell-free preparation of 3α-, 7α-, and 12α-HSDH were first reported by Aries and Hill (97) who studied these enzymes in isolates of *Clostridia, Bacteroides, Bifidobacterium,* and *Enterobacter.* In the human intestinal flora, the 7α-HSDH was much more abundant than the 3α-HSDH or the 12α-HSDH. More recently, the positional- and stereo-specificity of HSDH associated with specific microorganisms have been characterized. A given organism may elaborate a single enzyme acting solely upon a single portion of the bile acid molecule, while others may produce several HSDHs that can oxidize hydroxyl groups at positions 3, 7, and 12. Organisms known to elaborate these enzymes (98–115) are summarized on Table 4 and Fig. 4. The HSDH enzymes usually have different pH optima for oxidation and reduction; alkaline conditions favor oxidation while pH values below 7 promote reduction (99, 115). These enzymes are intracellular and possess a high degree of positional and stereochemical specificity. However, it is reasonable to assume that the 3α-HSDH acting upon bile acids and neutral steroids is one and the same enzyme (48, 103). Organisms synthesizing HSDH are thought to obtain metabolic energy by the formation of intracellular reduced nucleotides (NADH, NADPH) by hydride ion transfer (105).

7-Dehydroxylation

7-Dehydroxylation of the primary bile acids CA and CDC is quantitatively the most important transformation giving rise to DC and LC. In man, DC accounts for about 20% of the total biliary bile acids, with CDC and CA making up most of the remainder. It is known that DC undergoes EHC. Man is incapable of rehydroxylating the 7-position of DC to CA as is known to occur in rats (116). In rabbits, 7α-dehydroxylation of CA by anaerobes primarily concentrated in the cecum is so thorough that as much as 95% of the rabbits' biliary bile acids is, in fact, DC (117). In contrast to DC, LC, the product of 7α-dehydroxylation of CDC, is not efficiently conserved in the EHC of most animal species including man (118) in whom it accounts for about 2% of biliary bile acids. LC is poorly absorbed from the ileum because it is present mainly as a 3-sulfate ester. The compound is hydrolyzed in the colon by bacterial enzymes and the LC formed is mainly adsorbed to bacterial debris and excreted in the feces.

7α-Dehydroxylation can readily be demonstrated in mixed fecal cultures of man (99) and laboratory animals (119). Pure cultures of anaerobic bacteria synthesizing 7α-dehydroxylase were first isolated by Gustafsson, Midtvedt, and Norman (120) from feces and classified as "*Lactobacilli*" (120). Transformation studies were further performed on these isolates by Midtvedt (121).

Independently, both Bokkenheuser, Hoshita, and Mosbach (122) and Aries and Hill (97) isolated a 7α-dehydroxylating strain of *Bacteroides*. 7α-Dehydroxylase activity has more recently been demonstrated in cultures of *C. bifermentans* (123), *C. leptum* (50, 51), a *Eubacterium* species (124), and a variety of other anaerobes including *Clostridial* and non-*Clostridial* isolates from human feces and from sewers (125). Attempts to purify the *C. leptum* enzyme have been unsuccessful (50, 51). However, the *Eubacterium* enzyme is strongly inducible by CA and weakly inducible by CDC (124). It has been purified 7-fold (124) and studied in some detail by White and co-workers (126, 127). It has a molecular weight of 114,000 by gel filtration (127) and is activated 4- to 6-fold by NAD. Low concentrations of NADH further enhance activity (128) while higher concentrations are strongly inhibitory, suggesting that the intracellular ratio of NAD/NADH may be a controlling factor for the cellular level of activity. Moreover, kinetics for the NAD saturation curve do not obey the Michaelis-Menton equation in the presence of NADH, (but do so in its absence) suggesting allosteric binding of NADH to the enzyme (128). The same workers (126, 128) showed by two-dimensional electrophoresis that five polypeptides of molecular weights 77,000, 55,000, 55,000, 27,000, and 23,500, respectively, are associated with enzyme induction. Purification of the enzyme by HPLC and subsequent electrophoresis showed that all of the above peptides except the one with the lowest molecular weight are associated with enzyme activity. In addition, a substance of even smaller molecular weight is apparently associated with the enzyme in the intact bacterial cell and may act as an activator.[5]

The purified enzyme 7-dehydroxylated CA, CDC, and UDC but the reaction with the 7β-epimer (UDC) was somewhat slower (127). This observation is in agreement with findings by Fedorowski et al. (129) that dehydroxylation of both CDC and UDC occurred in mixed fecal cultures but the reaction with CDC was about 5-fold faster. Presumably, the rates of 7-dehydroxylation of UDC and CDC in mixed fecal cultures will also depend upon the relative concentrations of the organisms involved, some of which do not react with UDC (129). Interestingly, 7-dehydroxylation of CA by cultures of *Eubacterium sp.* can be enhanced by coculturing this organism with a variety of species of *Bacteroides* (130).

There is some controversy regarding the existence of intestinal microorganisms capable of 7-dehydroxylating conjugated bile acids. The organisms studied by White and co-workers (128) did not produce enzymes that reacted with conjugated, methylated, or unsaturated bile acids. Other workers have found, largely on the basis of experimental evidence in humans, that conjugated bile acids can be dehydroxylated prior to hydrolysis (131, 132). The reason for this discrepancy is not clear, unless other organism(s) yet to be studied in vitro can 7-dehydroxylate conjugated bile acid.

Samuelsson (133) proposed that the first step of the 7α-dehydroxylation reaction is the diaxial *trans*-elimination of water from the 6,7-position of CA, yielding a 6,7-unsaturated intermediate. The second step is the reduction of the intermediate to form DC. So far, it has not been possible to isolate the intermediate but White and co-workers (126, 128) have shown that the synthetic unsaturated intermediate, 3α-hydroxy-5β-6-cholen-24-oic acid, was rapidly converted to LC by *Eubacterium* 7-dehydroxylase (128) lending support to Samuelsson's proposal (133). No evidence of hydration of the Δ^6-intermediate to give CDC or UDC was found (128), suggesting the first step in the process is irreversible. 7-Dehydroxylation may be a source of metabolic energy to the bacterium but the significance of 7α-dehydroxylation for the host is not clear. Drasar and Hill (115) and Hill (134) noted a positive correlation between the proportion of DC in bile and the risk of developing colon cancer. Moreover, LC, the 7-dehydroxylation product of CDC, is a liver toxin for animals (135, 136) and possesses comutagenic properties (137, 138). On the other hand, Hofmann (139) has suggested that 7-dehydroxylation of bile acids may have some benefit for the host, for example the formation of insoluble LC from CDC can result in removal of bile acids from solution thus preventing their cathartic effect. It is interesting to note that germ-free animals have watery stools that can be restored to normal consistency by feeding a bile acid sequestrant such as cholestyramine (139).

Epimerization of 3α- and 7α-hydroxyl groups

The transformation of the 3α- and 7α-hydroxyl groups of primary bile acids to 3β- and 7β-epimers by intestinal microorganisms has been suggested by the presence of 3β- and 7β-epimers occurring in the feces of laboratory animals (140) and UDC in the bile of man (141) and some species of bears (142). Several studies also demonstrate 3 and 7 epimerization by human intestinal microorganisms in vitro (129, 143, 144). In 1970, Hayakawa (85) proposed that 3α-hydroxyl epi-

[5] Hylemon, P. B. Private communication.

merization occurred by oxidation of the 3α-hydroxyl group to the 3-ketone, then subsequent reduction of the ketone to the 3β-hydroxyl group. An analogous mechanism can be assumed for 7α-hydroxyl epimerization. Recently 3α-hydroxyl epimerization of CDC has been shown with *C. perfringens* (145, 146) and some strains of *E. lentum* (110, 111), implying the presence of a 3α- and 3β-HSDH and a 3-keto-intermediate. 3β-HSDH has not been clearly demonstrated in either *C. perfringens* (100) or *E. lentum* (103–105) although theoretically this enzyme should be present. The reverse reaction, the transformation of 3β-hydroxyl (iso) bile acid to 3α-hydroxyl bile acid can also be observed in *C. perfringens* (146) and in rat liver (147). The hepatic enzyme appears to be physiologically more significant than the microbial enzyme for the reverse reaction.

In the case of the epimerization of the 7α-hydroxyl group, the number of participating anaerobes in pure culture include *C. absonum* (49, 148–150), lecithinase-lipase-negative *Clostridia* (151, 152), *Eubacterium aerofaciens* (153, 154), and a gram-positive unidentified anaerobe (155). *Eubacterium aerofaciens* and the gram-positive anaerobe possess only the 7β-HSDH and, therefore, must be co-cultured with an organism synthesizing 7α-HSDH to epimerize the 7α-OH group (153–155).

Both *C. absonum* (49, 148) and *E. aerofaciens* (154) oxidize UDC and UC to the 7-keto bile acid intermediate when the Eh (redox potential) of the culture rises to above −100 mV (i.e., the medium is less reduced as a result of oxygen diffusion). This observation suggests that high redox potential lowers the intracellular NADPH/NADP ratio, a controlling factor in the ratio of hydroxy-bile acid/keto bile acid (149, 150).

The enzymes 7α- and 7β-HSDH in *C. absonum* are inducible by CDC and DC. Thus a two-enzyme pathway consisting of (a) 7α-HSDH (oxidative direction) and (b) 7β-HSDH (reductive direction) is, in effect, induced (i.e., CDC $\overset{(a)}{\rightleftarrows}$ 7-KLC $\overset{(b)}{\rightleftarrows}$ UDC). These enzymes are also inducible by 12-KLC and 7-KLC but repressed by the endproduct UDC. (149, 150). It is unexpected that DC and 12-KLC, which are *not* reactants, are both good inducers. Epimerization of bile acids at the 7-position may represent a detoxification process for the bacterium as UDC is less hydrophobic (less toxic) than CDC (156). In the human host, however, UDC, although less toxic than CDC, represents only a very small portion of biliary bile acids (141).

Two groups of investigators have proposed that at least part of the 7-epimerization occurring in mixed fecal cultures may proceed directly without a 7-keto intermediate (129, 143). Evidence for this lies in the failure to detect the 7-keto intermediate (143) and the conservation of tritium in the transformation of [7β-^{3}H]CDC to [7α-^{3}H]UDC by human fecal bacteria (129). 7-Keto-intermediates may escape detection because their conversion to 7β-OH bile acids is very rapid. Conservation of tritium at the 7-position during epimerization does not exclude the existence of a 7-keto-intermediate since the label could be recycled by bacterial intracellular nucleotides (63). Present evidence therefore favors the theory that 7-OH epimerization takes place via the 7-keto-intermediate. In mammals, epimerization of the 7α-hydroxyl group may result from oxidation of primary bile acid by bacterial 7α-HSDH followed by either hepatic or bacterial reduction of the 7-keto-intermediate to 7β-OH bile acid (8, 35, 157). The hepatic participation can be illustrated in germ-free animals monoinfected with *E. coli* which has no 7β-HSDH (35, 105).

Evidence for 12α-OH epimerization lies only in one report of the presence of 12β-OH bile acids in human feces (158). An organism elaborating 12β-HSDH remains to be isolated. Two established 12α-HSDH containing organisms have been shown to be devoid of activity with 12β-OH-containing substrates (104).

The clinical significance of in vivo formation of ursobile acids is somewhat controversial. Fedorowski et al. (129) showed that UDC was about five times as resistant as CDC to 7-dehydroxylation in mixed fecal cultures. On the other hand, Bazzoli et al. (159) were unable to detect significant differences in the rates of 7-dehydroxylation of UDC and CDC whether in fecal cultures or following colonic instillation experiments in human volunteers. Both CDC and UDC are potent gallstone-dissolving agents (160–162) but UDC is thought to be somewhat efficacious (160–162). Since UDC is less hydrophobic (less toxic) (156), it should be the safer agent for gallstone dissolution. However, Barns and Powrie (163) showed that UDC and to a small extent CA (but not CDC, DC, or LC) were clastogenic (chromosome breaking) to Chinese hamster ovary cells. In animal models, UDC does not appear to be co-carcinogenic for colon cancer (164) as does CDC (164, 165). VanTassell, MacDonald, and Wilkins (166) recently showed that a number of bile acids including CA, DC, and CDC (but not LC) stimulate formation of a mutagen when incubated with human feces; UDC and UC remain to be tested in their system.

Two model systems attempting to enhance 7-epimerization of bile acids at the expense of 7-dehydroxylation are described (167, 168). In vitro, Macdonald and Hutchison (167) failed to reduce 7-dehydroxylation of CDC or CA in mixed fecal cultures by inoculation of *C. absonum* into such cultures. Although UDC and UC formation were enhanced, this occurred primarily at the expense of 7-KLC and 7-KDC formation. In vivo, Yahiro, Setoguchi, and Katsuki (168), gave CDC orally to rabbits that had been cecectomized and appendectomized. They found a marked enhancement of 7-epimerization and a depression of 7-dehydroxylation as

reflected by the increased levels of UDC and decreased levels of DC and LC in the bile. This altered microbial metabolism presumably reflects changes both in the flora and the transit time resulting from surgery. Oral administration of CDC to human subjects enhances epimerization of the 7α-OH group in some patients, but in most cases as much as 90% of the bile acid pool becomes CDC (169, 170).

Formation of 5α-(allo) bile acids

In 1936, allo-DC (termed "lagodeoxycholic acid") was isolated by Kishi (171) from rabbit bile. Allo-DC has since been found in small amounts in rat (172) and rabbit feces (173); allo-CA occurs in reptile bile as a major constituent (174) and in human feces in minor amounts (140, 175). Allo-CDC has been identified as a minor constituent in the bile of the giant salamander (176). The origin of such bile acids is not clear. Some of the 5α-cholanoic acid in reptilian bile can probably be formed without bacterial intervention via the stereospecific reduction of 7α,12α-dihydroxy-4-cholesten-3-one to a 5α-intermediate (177). Kallner (172) showed that rats could transform intracecally administered ^{14}C-labeled DC to allo-DC. He later demonstrated that labeled 12α-hydroxy-3-keto-5β-cholanoic, 12α-hydroxy-3-keto-5α-cholanoic, and 12α-hydroxy-3-keto-chol-4-en-oic acids similarly administered to rats could all be transformed to both DC and allo-DC (178). Although metabolism by the rat intestinal flora is implicated here, similar experiments with mixed fecal cultures have not been published and organism(s) capable of carrying out this transformation have not been isolated.

The significance of allo-bile acids is not known but they are undoubtedly less soluble and probably more toxic than 5β-bile acids. Cholestanol-fed rabbits form gallstones containing a high proportion of the calcium salts of deoxycholyl- and allodeoxycholyl-glycine (179). The insoluble allo-salt arises from a combination of hepatic and microbial transformations (44). With cholestanol feeding, allo-CA acid is formed in the liver of rabbits as a primary bile acid and then transformed by microbial 7-dehydroxylation to allo-DC. Similarly, the small amounts of allo-CA found in human bile (180) may in part, be derived directly from hepatic transformation of cholestanol.

Other bile acid transformations

A number of bile acid transformations can be demonstrated either in mixed fecal culture or in pure culture. These include: *a*) formation of C24-ethyl esterified bile acids, *b*) formation of unsaturated bile acids, and *c*) hydrolysis of a 3-sulfated bile acid. The first two reactions have been illustrated in Table 2 and Fig. 4. The occurrence of these transformations in the human intestine have to be established and the medical relevance remains at best conjectural.

Formation of C-24 ethylesters. Bile acid ethyl esters have been shown to form in rat fecal cultures (181) and in cultures of three rat fecal isolates: *B. fragilis, Citrobacter.* sp., and *Peptostreptococcus productus T* (181). To the authors' knowledge these derivatives have not been observed in human feces.

Formation of unsaturated bile acids. Hill and coworkers (182–185) have shown that lecithinase-negative organisms, primarily *C. paraputrificum, C. indolis,* and *C. tertium,* contain nuclear dehydrogenases (NDH) which, in the presence of a suitable hydrogen acceptor such a menadione, can introduce double bonds at the 1, 2, and 4, 5 positions of 3-keto bile acids (Fig. 4). This reaction, however, requires nonphysiological amounts of menadione and a considerably higher Eh value than that found in the colon. Moreover, the bile acid substrate must be first oxidized in the 3-position since nuclear dehydrogenating clostridia (NDC) are lacking 3α-HSDH (186). A second possible mechanism of obtaining an unsaturated bile acid could be via hydrolysis of bile acid 3-sulfates (see below). Until recently, unsaturated bile acids have not been found in the feces or bile of man. Tanida et al. (32) were able to show the presence of a cholenoic acid in the feces of one out of four healthy subjects analyzed in detail. Indirect evidence for the formation of unsaturated bile acids was provided by Larusso, Hoffman, and Hofmann (187) who showed that about 10% of tritium from [2,2′,4,4′-^{3}H]CDC was removed in human subjects and could be recovered in the urine as ^3H_2O. A much higher percentage of the tritium (20–35%) was removed when this substrate was incubated with feces from the same subjects or with 7 of 24 anaerobic fecal isolates (187). Thus it appears that desaturation at the Δ 1,2 and Δ 4,5 position of CDC may be catalyzed by the human fecal flora, (presumably by NDC) but this reaction is reversible and saturated bile acid predominates (32). Additionally, [11,12-^{3}H]-CDC appears to be stable to tritium loss in man (188); but this does not appear to be the case with [11,12-^{3}H]-LC which looses tritium very readily (188). The stability of 11,12-tritiated bile acids in mixed fecal cultures remains to be studied.

Bowel cancer patients in the United Kingdom have a significantly higher number of NDC than do age- and sex-matched controls (183, 189). Blackwood et al. (190) confirmed this observation and noted a similar relationship with breast cancer patients (as well as obtaining confirmatory data with colon cancer patients). However, this finding may not have general significance as very few NDC could be isolated from populations of Hong Kong (191).

Hydrolysis of bile acid 3-sulfate. Kelsey, Muschik, and Sexton (192) and Borriello and Owen (193) showed that

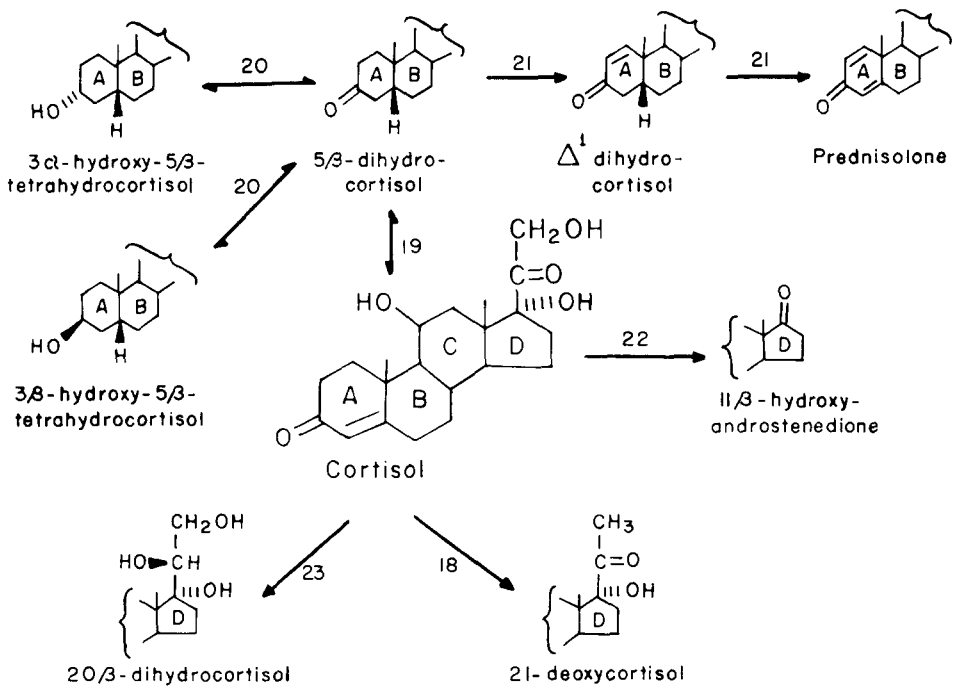

Fig. 5. Metabolism of cortisol.

LC-3-sulfate was metabolized by human mixed fecal cultures to LC, 3-keto-LC, iso-LC (3β-OH), and a 5β-cholenoic acid (193). Two organisms from rat feces, *Ps. aeruginosa* (194) and a *Clostrididum strain "S"* (195) have been found to synthesize a 3-sulfatase. The latter sulfatase was shown to be specific for equatorial-3 sulfates of C_{24}- or C_{26}-bile acids and yields nonsulfated cholanoate and sulfate ions on hydrolysis (195–197). Such organisms have not yet been recovered from human feces. It is not known whether the elaboration of 3-sulfatase has physiological significance.

Effect of bile acids on intestinal bacteria

Bile acids, particularly the dihydroxycholanoic acids are bacteriostatic against certain intestinal bacteria (198, 199). It must be expected therefore, that both composition and concentration of bile acids in the gut influence the flora selectively.

METABOLISM OF NEUTRAL STEROIDS

The major known catabolic reactions of neutral steroids are shown in **Fig. 5** and **Fig. 6. Table 5** lists the same reactions with the organisms responsible and the enzyme systems involved.

Hydrolysis of glucuronides and sulfates

Steroid hormones undergoing EHC are reduced in the liver in ring A, conjugated with sulfate or glucuronide, and excreted in the bile. In the normal human colon these compounds are deconjugated by the action of sulfatases and glucuronidases. Sulfatases, exclusively of bacterial origin (18, 200–210), can hydrolyze steroid sulfates conjugated at the 3α-, 3β-, 17β-, and 21-hydroxyl positions, and it is not known if they are active at other positions e.g., 16. Glucuronidases are synthesized both in the intestinal wall and by bacteria (201).

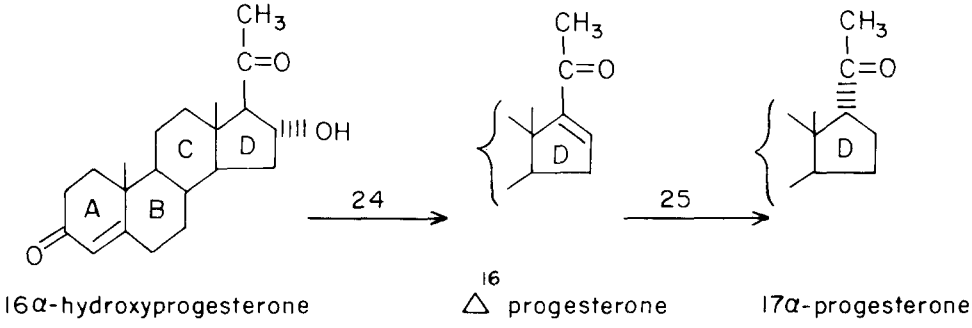

Fig. 6. Metabolism of 16α-hydroxyprogesterone.

TABLE 5. Metabolism of steroids

#	Steroid Transformation	Enzyme(s)	Organism(s)	Cofactor	pH	Comments	References
17	Hydrolysis of sulfates and glucuronides	Sulfatase and glucuronidase	Human fecal flora *Bacteroides sp.* *E. coli*				18, 201 202
18	21-OH group → 21-CH_3	21-Dehydroxylase	*E. lentum* and phenotypically similar orgs.	NAD(P)H FMN OR $FMNH_2$	6.4–6.8	The enzyme is of bacterial origin only. It requires an α-ketol group and deep anaerobic conditions. Reaction goes independently of ring A configuration.	38, 47 203, 206
19	4-En → 5β-H	Δ^4-5β-NDH	*C. paraputrificum* *L. leichmanii* *Bifidobacterium* *Peptostreptococcus*	NADPH	≃7.0	When *C. paraputrificum* is employed, reduction takes place in the β position.	52, 204
20	3-One → 3α(3β)OH group	3α-(β-)HSDH	*E. lentum* *C. paraputrificum* *Pseudomonas sp.*	NAD(P)H	≃7.0	These organisms are responsible for the isomerization of 3α ↔ 3β-OH groups via 3-one intermediate.	52, 103 203
21	5β-3-One → 1-en-3-one 1-en-3-one → 1,4-dien-3-one	Δ^1NDH Δ^4NDH	*C. perfringens* *C. perfringens* *C. paraputrificum*	NAD(P) NAD(P)	>7.0 >7.0	This reaction specifically requires 5β steroids.	67, 182
22	C_{17}-side chain → 17-one or 17-OH	17α, 20-Desmolase	Human fecal flora	NADPH and O_2	?	An OH group in the 17 position is required.	218, 219
23	20-one → 20-OH group	20-HSDH	*B. fragilis* *Pseudomonas sp.* *Bifidobacteria sp.*		6.0–7.4	A 20,21 diol protects the molecule from further degradation.	203, 205
24	16α-OH group → Δ^{16}	16α-Dehydratase	*E. lentum* and phenotypically similar organisms.		6.5–7.5		222, 223
25	Δ^{16} → 17α-Pregnanes	Δ^{16}-NDH	*E. lentum* and phenotypically similar organisms.		≃7.0	This reaction takes place with the isomerization of the side chain from β → α.	53, 221

The enzymes are absent from human feces at birth but present in increasing concentrations with age. In the rat, glucuronidase activity is related to the presence of *Bacteroides;* similar types of *Bacteroides* are also present in human fecal flora (18). The hydrolysis of glucuronides or sulfates is very important because bacteria cannot modify the conjugated molecule. For example, *E. lentum* is incapable of 21-dehydroxylating DOC-21-sulfate, but does so in the presence of sulfatase.[6]

Dehydroxylation of the 21-hydroxyl group

21-Hydroxycorticoids undergoing EHC lose their 21-hydroxyl group. In contrast, those not undergoing EHC are excreted in the urine with the 21-hydroxyl group intact albeit conjugated. The enzyme responsible for this conversion is exclusively of bacterial origin (39). 21-Dehydroxylating bacteria are present in stools of normal subjects regardless of the diet.[6] The enzyme is synthesized by *Eubacterium lentum* (203) and phenotypically similar organisms (48) of which there are about 10^7/g of human feces (203, 204). Colonization takes place early in life, usually before 1 year of age, and may well be achieved at birth since the organisms have been isolated from the vaginal secretion of 27% of pregnant women.[6] 21-Dehydroxylase is a constitutive enzyme with a pH optimum between 6.4 and 6.8. It requires an α-ketol group at C-20–21; a hydroxyl group at C-20 protects the molecule against the action of the enzyme (205). The enzyme has no effect on hydroxyl groups at C-11 and C-17, and it acts independently of the configuration of ring A (40). The enzyme has been extracted and partially purified (206). It is inhibited by both water-soluble and lipophilic metal ion-chelators and is inactivated by oxygen in a matter of seconds (47). Interestingly, the enzyme functions equally well whether the medium is reduced by mechanical and chemical means or is reduced by co-culturing with a rapidly growing, fermenting organism (203, 206). $FMNH_2$ or NAD(P)H and FMN, required as coenzymes, are usually synthesized by the bacteria manufacturing the 21-dehydroxylase. The molecular weight of the enzyme determined by chromatography on Sepharose 6B is 582,000 (203, 206).

Reduction of ring A

Saturation of ring A in Δ^4-3-keto steroids proceeds in two steps. Frequently, both reactions can be carried out by enzymes secreted by a single microbial strain but others synthesize enzymes catalyzing only one of these reductions (207, 208). Schubert et al. (209) demonstrated that Δ^4-3-keto steroids incubated with intestinal flora were stereospecifically reduced to the 3α-hydroxy-5β-configuration. Quantitative reduction is performed by *C. paraputrificum* while partial reduction may be accomplished by *L. leichmanii, Bifidobacterium adolescentis,* and *Peptostreptococcus* (204). With *C. paraputrificum,* an in vitro substrate concentration of 200 μg/ml or more inhibits the reduction of the 3-keto group. This principle has been utilized in the biosynthesis of rare and expensive reference compounds, e.g., 3α-hydroxy, 5β-derivatives of 18-hydroxylated corticoids (210, 211) and 3-keto, 5β-derivatives of medroxyprogesterone acetate.[5]

Kinetic studies with *C. paraputrificum* (204) showed that the Δ^4-3-keto reduction begins with hydrogenation of the double bond which is then followed by the formation of a hydroxyl group at C-3. The structure of rings A and B influences the rate of the reaction. For example, a methyl function at C-6 together with a 6, 7 double bond increases the resistance of the molecule to bacterial reduction (212). Moreover, 1,4-dienes and 4,6-dienes are more resistant to *C. paraputrificum* than 4-mono-ene steroids (213, 214) and megestrol acetate, the most active oral progestin, owes its resistance to the 6-methyl, 4-6-diene configuration rendering it refractory to both hepatic and bacterial reduction (18).

The preservation of the 3-keto-4-ene structures is essential for the hormonal function of the molecule. It follows, therefore, that hepatic or bacteriologic reduction of ring A plays a profound role in the biological activity of these steroid structures.

Epimerization of 3α-hydroxyl group

E. lentum and phenotypically similar organisms possess a 3α-HSDH responsible for the epimerization of the 3-hydroxyl group from α to β. Incubation of pregnanolone (3α-) with *E. lentum* strains yields pregnanedione, 3β- and 3α-pregnanolone under mild anaerobic conditions (Eh, -150 mV $\mp$ 20) (48, 202). The epimerization may proceed via oxidation to a 3-keto group, followed by reduction to the 3α- or 3β-compound. Alternatively, the reaction may involve the formation of an unsaturated intermediate which is rapidly converted to 3α- or 3β-pregnanolone without accumulation of the intermediate. It should be noted that the 3α-HSDH requires a substrate with a 3α-OH group and has no effect on substrates with 3β-OH groups.

Introduction of a double bond conjugated to a keto group

Nuclear dehydrogenation may be carried out in vitro by some strains of *C. welchii* and *C. paraputrificum.* The inducible enzyme synthesized by the latter organism requires a hydrogen acceptor such as menadione or

[6] Bokkenheuser, V. D., and J. Winter. Unpublished observations.

phenazine methosulfate (215). The unsaturated compound may be observed only within the first 24 hr; thereafter it becomes reduced to the original substrate. It seems unlikely that nuclear dehydrogenation takes place in the gut because of the highly reduced environment (Eh less than −300 mV). (See also Formation of unsaturated bile acids.)

Side chain cleavage

Formation of 17-keto steroids (210) from cortisol by slurries of human feces was observed by Wade et al. (216), Gustafsson (217), and Eriksson and Gustafsson (218). Recently, C_{19} metabolites, 5ξ-androstane-3,-11,17-triol and 5ξ-androstane-3α,11β-diol-17-one, were isolated from incubation of cortisol with human fecal flora (219). The prevalence of converting organisms was approximately 10^6/g of feces. The conversion required an Eh below −130 mV, and an initial pH of 7.0. Preliminary investigation indicated that the ability of converting organisms to form colonies on solid media was related to the composition of the media and the gaseous environment. Only corticoids with a hydroxyl group at the C_{17} position were transformed to C_{19} steroids by fecal flora. Recently, two of us[6] recovered from human feces a gram-positive obligate anaerobic rod that synthesizes desmolase. This organism is yet to be identified. Free-living soil organisms can also remove the side chain of C_{21} steroids in the absence of the 17-hydroxyl group (52, 207).

Reduction of a 20-ketone to an alcohol

Bacteroides fragilis, present in normal human fecal flora, converts DOC to a metabolite tentatively identified as 20,21-dihydroxy-5β-pregnan-3-one (205). This metabolite, resistant to further alteration by fecal bacteria (204) is formed in yields of 10% when DOC is the substrate; it cannot be produced from such closely related structures as THDOC and pregnanolone (204). These experiments suggest that the 20-HSDH of *B. fragilis* requires an intact 3-keto-4-ene structure for its action.

A different 20-HSDH is synthesized by *Bifidobacterium adolescentis,* a common anaerobe in the intestinal flora of human and rat (220). It is more efficient than the enzyme elaborated by *B. fragilis* and acts regardless of an unsaturation in the A ring. The enzyme shows a wide substrate specificity and reduces the 20-keto group to a 20β-hydroxyl group. It neither metabolizes, nor is affected by, hydroxyl groups at C3, C11, C17, or C21. Once formed, 20-hydroxyl compounds are resistant to further bacterial metabolism of the side chain. However, existing Δ^4-3-keto groups may be reduced by *C. paraputrificum* to entirely refractory compounds, e.g., cortol (220).

16α-Dehydroxylation

As with most other bacteria capable of altering ring D, the organisms responsible for the conversion of 16α-hydroxy progesterone to 17α-progesterone also belong to the *Eubacteria* (53, 221). They are present in human feces in a concentration of 10^5/g. Two strains, #144 and #146, have been isolated from fecal flora of the rat. Although phenotypically identical, strain #144 synthesizes a 16α-dehydratase and a Δ^{16} nuclear dehydrogenase while a strain #146 also manufactures a 21-dehydroxylase (53, 221).

The transformation of 16α-OH progesterone to 17α-progesterone is a two-step reaction. The first, the removal of the hydroxyl group with formation of Δ^{16}-progesterone, is completed within 9–12 hr of incubation. The second step, reduction of the Δ^{16} bond, takes place only after 24 hr of incubation and results in the isomerization of the side chain in the 17 position from β to α (54, 222). This mechanism, proposed by Calvin and Lieberman (223) was confirmed by isolation of Δ^{16} derivatives from incubations of strain #144 with 16α-OH progesterone; moreover, 17α-progesterone was formed in cultures supplemented with Δ^{16}-progesterone (54). Thus, the position of the side chain of urinary metabolites reveals which steroids have been 16α-dehydroxylated. The 16α-dehydratase is unusually resistant to oxygen as evidenced by the specific enzymatic activity of cell fractions whether incubated aerobically or anaerobically (54).

METABOLISM OF PHENOLIC STEROIDS

The metabolism of these compounds was reviewed in detail by Adlercreutz et al. (17), Diczfalusy and Levitz (224), and Taylor (16). Some important transformations are outlined below.

Like other steroids, conjugated estrogens are hydrolyzed by the intestinal microflora; as expected, administration of antibiotics causes a huge increase in the excretion of conjugates in the feces. Estrone and 15α-hydroxy-estrone can be reduced in the 17-position. The reduction can take place also in the 16-position converting 16-oxo-estradiol to 16-epiestriol. Adlercreutz et al. (225) suggested that the transformation of 16α-hydroxy-estrone to the 15α-compound takes place in two steps: first, 16α-dehydration due to the intestinal microflora and second, 15α-hydroxylation which can take place in the intestinal wall and the liver.

Experiments with fecal flora or pure bacterial cultures are needed to clarify the precise mechanism and site of the transformation.

CONCLUSION

We have described a number of microbial transformations of the three classes of steroids occurring in the

human intestine. These include: hydrolysis, dehydrogenation (hydroxyl and nuclear), dehydroxylation, epimerization, and side chain cleavage reactions all leaving the steroid nucleus intact. In many cases the responsible organism has been isolated and the enzyme(s) has been purified and studied. Areas of paucity in information are nonetheless evident. Isolation of unknown organisms, details of the mechanism of action of most of the described enzymes, and a thorough understanding of the biological significance of the reaction products to the host must await further research. New applications of these enzymes remain in the future.

The authors wish to acknowledge financial support from the following granting agencies: the National Institute of Health (grants #AM-25324 and HL-24061), the National Cancer Institute (grant CA-25763), the Medical Research Council of Canada (grant MA 5075), and the National Cancer Institute of Canada.

Manuscript received 18 May 1982 and in revised form 18 January 1983.

REFERENCES

1. Holdeman, L. V., I. J. Good, and W. E. C. Moore. 1976. Human fecal flora: a variation in bacterial composition within individuals and a possible effect of emotional stress. *Appl. Environ. Microbiol.* **31:** 359–375.
2. Hentges, D. J., B. R. Maier, G. C. Burton, M. A. Flynn, and R. T. Tsutakawa. 1977. Effect of a high-beef diet on the fecal bacterial flora of humans. *Cancer Res.* **37:** 568–571.
3. Moore, W. E. C., E. P. Cato, and L. V. Holdeman. 1978. Some current concepts in intestinal bacteriology. *Am. J. Clin. Nutr.* **31:** S33–S42.
4. Hislop, I. G. 1970. The absorption and enterohepatic circulation of bile salts: an historical review. *Med. J. Aust.* **1:** 1223–1226.
5. Hofmann, A. F. 1979. The enterohepatic circulation of bile acids in man. *In* Bile Acids Clinics in Gastroenterology. Vol. 6. G. Paumgartner, editor. Saunders, Philadelphia. 3–24.
6. Carey, M. C. 1982. The enterohepatic circulation. *In* The Liver, Biology and Pathobiology. I. Arias, H. Popper, D. Schachter, and D. A. Shafritz, editors. Raven Press, New York. 429–465.
7. Hofmann, A. F. 1976. The enterohepatic circulation of bile acids in man. *Adv. Intern. Med.* **21:** 501–534.
8. Hofmann, A. F. 1977. The enterohepatic circulation of conjugated bile acids in healthy man: quantitative description and function. *In* Exposés Annuels de Biochimie Medicale (33^{e} serie). Cholesterol Metabolism and Lipolytic Enzymes. J. Polonovsky, editor. Masson Publishing USA Inc., New York. 69–86.
9. Ahlberg, J., B. Angelin, I. Björkhem, and K. Einarsson. 1977. Individual bile acids in portal venous and systemic blood serum in fasting man. *Gastroenterology.* **73:** 1377–1382.
10. Almé, B., A. Bremmelgaard, J. Sjövall, and P. Thomassen. 1977. Analysis of metabolic profiles of bile acids in urine using lipophilic anion exchanger and computerized gas–liquid chromatography–mass spectrometry. *J. Lipid. Res.* **18:** 339–362.
11. Amuro, Y., T. Endo, K. Higashino, K. Uchida, and Y. Yamamura. 1981. Urinary and fecal keto bile acids in liver cirrhosis. *Clin. Chim. Acta.* **114:** 137–147.
12. Bremmelgaard, A., and J. Sjövall. 1974. Bile acid profiles in urine of patients with liver diseases. *Eur. J. Clin. Invest.* **9:** 341–348.
13. Palmer, R. H., and C. K. McSherry. 1982. Lithocholate metabolism in baboons fed chenodeoxycholate. *J. Lab. Clin. Med.* **99:** 533–538.
14. Allan, R. N., J. L. Thistle, A. F. Hofmann, J. A. Carter, and P. Y. S. Yu. 1976. Lithocholate metabolism during chenotherapy for gallstone dissolution. 2. Absorption and sulphation. *Gut.* **17:** 413–419.
15. Laatikainen, T., and R. Vikko. 1970. Identification of $C_{19}O_2$ and $C_{21}O_2$ steroids in the mono- and disulphate fractions of human faeces. *Eur. J. Biochem.* **13:** 534–538.
16. Taylor, W. 1971. The excretion of steroid hormone metabolites in bile and feces. *Vitam. Horm.* **29:** 201–285.
17. Aldercreutz, H., F. Martin, R. Jarvenpaa, and T. Fotsis. 1979. Steroid absorption and enterohepatic recycling. *Contraception.* **20:** 201–223.
18. Adlercreutz, H., and F. Martin. 1980. Biliary excretion and intestinal metabolism of progesterone and estrogens in man. *J. Steroid Biochem.* **13:** 231–244.
19. Sandberg, A. A., W. R. Slaunwhite, Jr. and R. Y. Kirdani. 1970. Metabolic Conjugation and Metabolic Hydrolysis. W. M. Fishinan, editor. Academic Press, New York. 123–152.
20. Levitz, M., and B. K. Young. 1977. Estrogens in pregnancy. *Vitam. Horm.* **35:** 109–147.
21. Adlercreutz, H., and T. Luukkainen. 1967. Biochemical and clinical aspects of enterohepatic circulation of estrogens. *Acta. Endocrinol. (Copenhagen) Suppl.* **124:** 101–140.
22. Sandberg, A. A., and W. R. Slaunwhite, Jr. 1951. Studies on phenolic steroids in human subjects. II. The metabolic fate and hepatobiliary-enteric circulation of C^{14}-estrone and C^{14}-estradiol in women. *J. Clin. Invest.* **36:** 1266–1278.
23. Reed, M. J., K. Fotherby, and S. J. Steele. 1972. Metabolism of ethynyloestradiol in man. *J. Endocrinol.* **55:** 351–361.
24. Huempel, M., M. Wendt, G. Jogs, C. Weiss, S. Reitz, and U. Speck. 1977. Intraindividual comparison of pharmacokinetic parameters of d-norgestrel, lynestienol and cyproterone acetate in six women. *Contraception.* **16:** 199–215.
25. Smith, R. L. 1974. Biliary excretion and hepatotoxicity of contraceptive steroids. Pharmacological models in contraceptive development. *Acta Endocrinol. Suppl.* **185:** 149–161.
26. Grundy, S. M. 1975. Effects of polyunsaturated fat on lipid metabolism in patients with hypertriglyceridemia. *J. Clin. Invest.* **55:** 269–282.
27. Duncan, I. W., P. H. Culbreth, and C. A. Burtis. 1979. Determination of free, total and esterified cholesterol by high pressure liquid chromatography. *J. Chromatogr.* **162:** 281–292.
28. Deacon, A. C., and P. J. G. Dorsonn. 1979. Enzymic assay of total cholesterol involving chemical or enzymic hydrolysis: a comparison of methods. *Clin. Chem.* **25:** 976–984.

29. Miettinen, T. A., E. H. Ahrens, Jr., and S. M. Grundy. 1965. Quantitative isolation and gas–liquid chromatographic analysis of total dietary and fecal neutral steroids. *J. Lipid Res.* **6:** 411–424.
30. Juengst, D., A. Pickel, E. Elsaesser, F. J. Marx, and H. J. Karl. 1979. Urinary cholesterol excretion in man with benign prostatic hyperplasia and carcinoma of the prostate. *Cancer.* **43:** 353–359.
31. Grundy, S. M., E. H. Ahrens, Jr., and T. A. Miettinen. 1965. Quantitative isolation and gas–liquid chromatographic analysis of total fecal bile acids. *J. Lipid Res.* **6:** 397–410.
32. N. Tanida, Y. Hikasa, M. Hosomi, M. Satoni, I. Oohama, and T. Shimoyama. 1981. Fecal bile acid analysis in healthy Japanese subjects using a lipophilic anion exchanger, capillary column gas chromatography and mass spectrometry. *Gastroenterol Jpn.* **16:** 363–371.
33. Heftmann, E., editor. 1973. Modern Methods of Steroid Analyses. Academic Press, New York.
34. Karger, B. L., L. R. Snyder, and C. Horvath, editors. 1973. Introduction to Separation Science. John Wiley & Sons.
35. Gustafsson, B. E., A. Norman, and J. Sjövall. 1960. Influence of *E. coli* infection on turnover and metabolism of cholic acid in germ-free rats. *Arch. Biochem. Biophys.* **91:** 83–100.
36. Norman, A., and S. Bergman. 1960. The action of intestinal microorganisms on bile acids: bile acids and steroids 101. *Acta Chem. Scand.* **14:** 1781–1789.
37. Gustafsson, B. E., T. Midtvedt, and A. Norman. 1968. Metabolism of cholic acid in germ-free animals after the establishment in the intestinal tract of deconjugating and 7α-dehydroxylating bacteria. *Acta Pathol. Microbiol. Scand.* **72:** 433–443.
38. Eyssen, H., and G. Parmentier. 1977. Biohydrogenation of sterols and fatty acids by the intestinal microflora. *Am. J. Clin. Nutr.* **27:** 1329–1340.
39. Winter, J., and V. D. Bokkenheuser. 1978. 21-Dehydroxylation of corticoids by anaerobic bacteria isolated from human fecal flora. *J. Steroid Biochem.* **9:** 379–384.
40. Winter, J., V. D. Bokkenheuser, and L. Ponticorvo. 1979. Bacterial metabolism of corticoids with particular reference to the 21-dehydroxylation. *J. Biol. Chem.* **254:** 2626–2629.
41. Madsen, D., M. Beaver, L. Chang, E. Bruckner-Kardoss, and B. Wostmann. 1976. Analysis of bile acids in conventional and germ-free rats. *J. Lipid Res.* **17:** 107–111.
42. Beaver, M. H., B. S. Wostmann, and D. C. Madsen. 1978. Bile acids in bile of germ-free and conventional dogs. *Proc. Soc. Exp. Biol. Med.* **157:** 386–389.
43. Hofmann, A. F., E. H. Mosbach, and C. C. Sweeley. 1969. Bile acid composition of bile from germ-free rabbits. *Biochim. Biophys. Acta.* **176:** 204–207.
44. Hofmann, A. F., V. Bokkenheuser, R. L. Hirsch, and E. H. Mosbach. 1968. Experimental cholelithiasis in the rabbit induced by cholestanol feeding: effect of neomycin treatment on bile composition and gallstone formation. *J. Lipid Res.* **9:** 244–253.
45. Garbutt, J. T., K. W. Heaton, L. Lack, and M. P. Tyor. 1969. Increased ratio of glycine to taurine conjugated bile salts in patients with ileal disorders. *Gastroenterology.* **56:** 711–720.
46. Sacquet, E. C., P. M. Raibaud, C. Mejean, M. J. Riottot, C. Leprince, and P. C. Leglise. 1979. Bacteriol formation of ω-muricholic acid in rats. *Appl. Environ. Microbiol.* **37:** 1127–1131.
47. Feighner, S. D., and P. B. Hylemon. 1980. Characterization of a corticosteroid 21-dehydroxylase from the intestinal anaerobic bacterium, *Eubacterium lentum. J. Lipid Res.* **21:** 585–593.
48. Bokkenheuser, V. D., J. Winter, S. M. Finegold, V. L. Sutter, A. E. Ritchie, W. E. C. Moore, and L. V. Holdeman. 1979. New markers for *Eubacterium lentum. Appl. Environ. Microbiol.* **37:** 1001–1006.
49. 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.
50. Stellwag, E. J., and P. B. Hylemon. 1979. 7α-Dehydroxylation of cholic acid and chenodeoxycholic acid by *Clostridium leptum. J. Lipid Res.* **20:** 325–333.
51. Stellwag, E. J., and P. B. Hylemon. 1978. Characterization of 7α-dehydroxylase in *Clostridium leptum. Am. J. Clin. Nutr.* **31:** S243–S247.
52. Dorfman, R. I., and F. Ungar. 1965. Metabolism of Steroid Hormones. Academic Press, New York. 204–288.
53. Bokkenheuser, V. D., J. Winter, S. O'Rourke, and A. E. Ritchie. 1982. Isolation and characterization of fecal bacteria capable of 16α-dehydroxylating corticoids. *Appl. Environ. Microbiol.* **40:** 803–808.
54. Winter, J., S. O'Rourke, V. D. Bokkenheuser, P. B. Hylemon, and T. L. Glass. 1982. 16α-Dehydroxylation of corticoids by bacteria from rat fecal flora. *J. Steroid Biochem.* **16:** 231–238.
55. Heftman, E. 1970. Steroid Biochemistry. 1st edition. Academic Press, New York. 115–119.
56. Miettinen, T. A., A. Proia, and D. J. McNamara. 1981. Origins of fecal neutral steroids in rats. *J. Lipid Res.* **22:** 485–495.
57. Dam, H. 1934. The formation of coprostanol in the intestine. II. The action of intestinal bacteria on cholesterol. *Biochem. J.* **28:** 820–825.
58. Snog-Kjaer, A., J. Prange, and H. Dam. 1956. Conversion of cholesterol to coprostanol by bacteria. *J. Gen. Microbiol.* **14:** 256–260.
59. Crowther, J. S., B. S. Drasar, P. Goddard, M. J. Hill, and K. Johnson. 1977. The effect of a chemically defined diet on the faecal flora and a faecal steroid concentration. *Gut.* **14:** 490–493.
60. Sadzikowski, M. R., J. F. Sperry, and T. D. Wilkins. 1977. Cholesterol-reducing bacterium from human feces. *Appl. Environ. Microbiol.* **34:** 355–362.
61. Eyssen, H. J., C. G. Parmentier, F. C. Compernolle, G. De Pauw, and M. Piessens-Denef. 1973. Biohydrogenation of sterols by *Eubacterium* ATCC 21,408—*Nova* species. *Eur. J. Biochem.* **36:** 411–421.
62. Rosenfeld, R. S., and T. F. Gallagher. 1964. Further studies of the biotransformation of cholesterol to coprostanol. *Steroids.* **4:** 515–520.
63. Björkhem, I., and J. A. Gustafsson. 1971. Mechanism of microbial transformation of cholesterol into coprostanol. *Eur. J. Biochem.* **21:** 428–432.
64. Parmentier, G., and H. Eyssen. 1974. Mechanism of biohydrogenation of cholesterol to coprostanol by *Eubacterium* ATCC 21,408. *Biochim. Biophys. Acta.* **348:** 279–284.
65. Brinkley, A. W., and G. E. Mott. 1978. Anaerobic fecal

bacteria of the baboon. *Appl. Environ. Microbiol.* **36:** 530–532.
66. Brinkley, A. W., A. R. Gottesman, and G. E. Mott. 1982. Isolation and characterization of new strains of cholesterol-reducing bacteria from baboons. *Appl. Environ. Microbiol.* **43:** 86–89.
67. Brinkley, A. W., A. R. Gottesman, and G. E. Mott. 1980. Growth of cholesterol-reducing *Eubacterium* on cholesterol-brain agar. *Appl. Environ. Microbiol.* **40:** 1130–1132.
68. Mott, G. E., and A. W. Brinkley. 1979. Plasmenylethanolamine: growth factor for cholesterol-reducing *Eubacterium*. *J. Bacteriol.* **139:** 755–760.
69. Mott, G. E., A. W. Brinkley, and C. L. Mersinger. 1980. Biochemical characterization of cholesterol-reducing *Eubacterium*. *Appl. Environ. Microbiol.* **40:** 1017–1022.
70. Cohen, B. I., R. F. Raicht, and E. H. Mosbach. 1974. Effect of dietary bile acids, cholesterol, and β-sitosterol upon formation of coprostanol and 7-dehydroxylation of bile acids by rat. *Lipids.* **9:** 1024–1029.
71. Subbiah, M. T. R., M. C. Naylor, J. Schumacher, and B. A. Kottke. 1974. 5β-Reduction of (^{14}C)cholesterol by human feces in vitro: nonspecific inhibition by sugars. *Experientia.* **30:** 249–250.
72. Eyssen, H., G. De Pauw, and C. Parmentier. 1974. Effect of lactose on Δ^5-steroid-reducing activity of intestinal bacteria in gnotobiotic rats. *J. Nutr.* **104:** 605–612.
73. Björkhem, I., J. A. Gustafsson, and O. Wrange. 1973. Microbial transformation of cholesterol into coprostanol. *Eur. J. Biochem.* **37:** 143–147.
74. Martin, C. K. 1977. Cleavage of sterol side chains. *Adv. Appl. Microbiol.* **22:** 29–58.
75. Goddard, P., and M. J. Hill. 1974. The in vivo metabolism of cholesterol by gut bacteria in the rat and guinea pig. *J. Steroid Biochem.* **5:** 569–572.
76. Owen, R. W., M. E. Tenneson, R. F. Bilton, and A. N. Mason. 1978. The degradation of cholesterol by *Escherichia coli* isolated from human feces. *Biochem. Soc. Trans.* **6:** 377–379.
77. Tenneson, M. E., R. W. Owen, and A. N. Mason. 1977. The anaerobic side-chain cleavage of bile acids by *Escherichia coli* isolated from human feces. *Biochem. Soc. Trans.* **5:** 1758–1760.
78. Reddy, B. S., and E. L. Wynder. 1977. Metabolic epidemiology of colon cancer: fecal bile acids and neutral sterols in colon cancer patients and patients with adenomatous polyps. *Cancer.* **39:** 2533–2539.
79. Wynder, E. L., and B. S. Reddy. 1977. Colon cancer prevention: today's challenge to biomedical scientists and clinical investigators. *Cancer.* **40:** 2565–2571.
80. Reddy, B. S., C. W. Martin, and E. L. Wynder. 1977. Fecal bile acids and cholesterol metabolites of patients with ulcerative colitis, a high risk group for the development of colon cancer. *Cancer Res.* **37:** 1697–1701.
81. Lipkin, M., B. S. Reddy, J. Weisburger, and L. Schechter. 1981. Nondegradation of fecal cholesterol in subjects at high risk for cancer of the large intestine. *J. Clin. Invest.* **67:** 304–307.
82. Cruse, J. P., M. R. Lewin, and C. G. Clark. 1979. Dietary cholesterol is cocarcinogenic for human colon cancer. *Lancet.* **i:** 752–755.
83. Cruse, J. P., M. R. Lewin, G. P. Ferulano, and C. G. Clark. 1978. Cocarcinogenic effects of dietary cholesterol in experimental colon cancer. *Nature.* **276:** 822–825.
84. Macdonald, I. A., and B. G. Rao. 1979. Diet, bacteria, and the origin of bowel cancer. *Modern Med. Canada.* **34:** 136–140.
85. Hayakawa, S. 1973. Microbial transformations of bile acids. *Adv. Lipid Res.* **11:** 143–192.
86. Midtvedt, T. 1974. Microbial bile acid transformation. *Am. J. Clin. Nutr.* **27:** 1341–1347.
87. Lewis, R., and S. Gorbach. 1972. Modification of bile acids by intestinal bacteria. *Arch. Intern. Med.* **130:** 545–549.
88. Hill, M. J. 1975. The etiology of colon cancer. *CRC Crit. Rev. Toxicol.* **4:** 31–81.
89. Basu, K. P., and S. C. Chakravarty. 1934. Action of *B. coli* on conjugated bile acids. *Indian J. Med. Res.* **21:** 691–694.
90. Norman, A., and R. Grubb. 1955. Hydrolysis of conjugated bile acids by Clostridia and Enterococci: bile acids and steroids 25. *Acta Pathol. Microbiol. Scand.* **36:** 537–547.
91. Nair, P. P., M. Gordon, and J. Reback. 1967. The enzymatic cleavage of the carbon–nitrogen bond in 3α,7α,12α-trihydroxy-5β-cholan-24-oyl glycine. *J. Biol. Chem.* **243:** 7–11.
92. Stellwag, E. J., and P. B. Hylemon. 1976. Purification and characterization of bile salt hydrolase from *Bacteroides fragilis* sub. sp. *fragilis*. *Biochim. Biophys. Acta.* **452:** 165–176.
93. Kobashi, K., I. Nishizawa, T. Yamada, and J. Hase. 1978. A new hydrolase specific for taurine conjugates of bile acids. *J. Biochem. Tokyo.* **84:** 495–497.
94. Small, D. M. 1971. The physical chemistry of cholanic acids. *In* The Bile Acids. Vol. 1. P. P. Nair and D. Kritchevsky, editors. Plenum Press, New York, London. 249–256.
95. Holt, P. R. 1972. The roles of bile acids during the process of normal fat and cholesterol absorption. *Arch. Intern. Med.* **130:** 574–583.
96. Hepner, G. W., A. F. Hofmann, J. R. Malagelada, P. A. Szczepanik, and P. D. Klein. 1974. Increased bacterial degradation of bile acids in cholecystectomized patients. *Gastroenterology.* **66:** 556–564.
97. Aries, V., and M. J. Hill. 1970. Degradation of steroids by intestinal bacteria. II. Enzymes catalyzing the oxidoreduction of the 3α-, 7α-, and 12α-hydroxyl groups in cholic acid, and the dehydroxylation of the 7-hydroxyl group. *Biochim. Biophys. Acta.* **202:** 535–543.
98. Macdonald, I. A., G. R. Webb, and D. E. Mahony. 1978. Fecal hydroxysteroid dehydrogenase activities in vegetarian Seventh Day Adventists, control subjects, and bowel cancer patients. *Am. J. Clin. Nutr.* **31:** S233–S238.
99. Macdonald, I. A., G. Singh, D. E. Mahony, and C. E. Meier. 1978. Effect of pH on bile salt degradation by mixed fecal cultures. *Steroids.* **32:** 245–256.
100. Macdonald, I. A., C. E. Meier, D. E. Mahony, and G. A. Costain. 1976. 3α-, 7α-, and 12α-hydroxysteroid dehydrogenase activities from *Clostridium perfringens*. *Biochim. Biophys. Acta.* **450:** 142–153.
101. Macdonald, I. A., T. P. Forrest, G. A. Costain, and B. G. Rao. 1978. Identification of 7α,12α-dihydroxy-3-oxo-cholanoic acid as the major degradation product from cholic by *C. perfringens*. *J. Steroid Biochem.* **9:** 353–358.
102. Macdonald, I. A., D. E. Mahony, J. F. Jellett, and C. E. Meier. 1977. NAD-dependent 3α- and 12α-hydroxy-

steroid dehydrogenase activities from *Eubacterium lentum* ATCC No. 25559. *Biochim. Biophys. Acta.* **489:** 466–476.
103. Macdonald, I. A., J. F. Jellett, D. E. Mahony, and L. V. Holdeman. 1979. Bile salt 3α- and 12α-hydroxysteroid dehydrogenases from *Eubacterium lentum* and related organisms. *Appl. Environ. Microbiol.* **37:** 992–1000.
104. Macdonald, I. A., and F. C. Chang. 1982. The stereospecificity of 3α- and 12α-bile salt hydroxysteroid dehydrogenase systems from four microbial sources. *Enzyme.* **28:** 392–395.
105. 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.
106. Haslewood, E. S., and G. A. D. Haslewood. 1976. The specificity of a 7α-hydroxysteroid dehydrogenase from *Escherichia coli. Biochem. J.* **157:** 207–210.
107. Macdonald, I. A., C. N. Williams, and D. E. Mahony. 1976. The behaviour of 3α- and 7α-hydroxysteroid dehydrogenase on chenodeoxycholate-substituted sepharose. *Steroids.* **28:** 25–30.
108. 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.
109. 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.
110. Hirano, S., N. Masuda, H. Oda. and T. Imamura. 1981. Transformation of bile acids by mixed microbial cultures from human feces and bile acid transforming activities of isolated bacterial strains. *Microbiol. Immunol.* **25:** 271–282.
111. Hirano, S., and N. Masuda. 1981. Transformation of bile acids by *Eubacterium lentum. Appl. Environ. Microbiol.* **42:** 912–915.
112. Harris, J. N., and P. B. Hylemon. 1978. Partial purification and characterization of NADP-dependent 12α-hydroxysteroid dehydrogenase from *Clostridium leptum. Biochim. Biophys. Acta.* **528:** 148–157.
113. Mahony, D. E., C. E. Meier, I. A. Macdonald, and L. V. Holdeman. 1977. Bile salt degradation by nonfermentative *clostridia. Appl. Environ. Microbiol.* **34:** 419–423.
114. Macdonald, I. A., J. F. Jellet, and D. E. Mahony. 1979. 12α-Hydroxysteroid dehydrogenase from *Clostridium* group P strain C48-50 ATCC #29733: partial purification and characterization. *J. Lipid Res.* **20:** 234–239.
115. Drasar, B. S., and M. J. Hill. 1974. Human Intestinal Flora. Academic Press, London, New York, San Francisco. 194.
116. Norman, A., and J. Sjövall. 1958. On the transformation and enterohepatic circulation of cholic acid in the rat. Bile acids and steroids, 68. *J. Biol. Chem.* **233:** 872–885.
117. Lindstedt, S., and J. Sjövall. 1957. On the formation of deoxycholic acid from cholic acid in the rabbit. *Acta Chem. Scand.* **11:** 421–426.
118. Cowen, A. E., M. G. Korman, A. F. Hofmann, O. W. Cass, and S. B. Coffin. 1975. Metabolism of lithocholic acid in healthy man. II. Enterohepatic circulation. *Gastroenterology.* **69:** 67–76.
119. Morotomi, M., Y. Kawai, and M. Masahiko. 1979. Intestinal microflora and bile acids: in vitro cholic acid transformation by mixed fecal culture of rats. *Microbiol. Immunol.* **23:** 839–847.
120. Gustafsson, B. F., T. Midtvedt, and A. Norman. 1966. Isolated fecal microorganisms capable of 7α-dehydroxylating bile acids. *J. Exp. Med.* **123:** 413–432.
121. Midtvedt, T. 1967. Properties of anaerobic gram-positive rods capable of 7α-dehydroxylating bile acids. *Acta Pathol. Microbiol. Scand.* **71:** 147–160.
122. Bokkenheuser, V., T. Hoshita, and E. H. Mosbach. 1969. Bacterial 7-dehydroxylation of cholic acid and allocholic acid. *J. Lipid Res.* **10:** 421–426.
123. Ferrari, A., and L. Beretta. 1977. Activity on bile acids of a *Clostridium bifermentans* cell-free extract. *FEBS Lett.* **75:** 163–165.
124. White, B. A., R. L. Lipsky, R. J. Fricke, and P. B. Hylemon. 1980. Bile acid induction specificity of 7α-dehydroxylase activity in an intestinal *Eubacterium* species. *Steroids.* **35:** 103–110.
125. Hirano, S., R. Nakama, M. Tomaki, N. Masuda, and H. Oda. 1981. Isolation and characterization of thirteen intestinal microorganisms capable of 7α-dehydroxylating bile acids. *Appl. Environ. Microbiol.* **41:** 737–741.
126. White, B. A., A. F. Cacciapuoti, R. J. Fricke, T. R. Whitehead, E. H. Mosbach and P. B. Hylemon. 1981. Cofactor requirements for 7α-dehydroxylation of cholic and chenodeoxycholic acid in cell extracts of the intestinal anaerobic bacterium *Eubacterium* species V.P.I. 12708. *J. Lipid Res.* **22:** 891–898.
127. White, B. A., R. J. Fricke, and P. B. Hylemon. 1982. 7β-Dehydroxylation of ursodeoxycholic acid by whole cells and cell extracts of the intestinal anaerobic bacterium *Eubacterium* species V.P.I. 12708. *J. Lipid Res.* **23:** 145–153.
128. White, B. A., D. A. M. Paone, A. F. Cacciapuoti, R. J. Fricke, E. H. Mosbach, and P. B. Hylemon. 1983. Regulation of bile acid 7-dehydroxylase activity by NAD^+ and NADH in cell extracts of *Eubacterium* species V.P.I. 12708. *J. Lipid Res.* **24:** 20–27.
129. 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.
130. Hirano, S., and N. Masuda. 1982. Enhancement of the 7α-dehydroxylase activity of a gram-positive intestinal anaerobe by *Bacteroides* and its significance in the 7-dehydroxylation of ursodeoxycholic acid. *J. Lipid Res.* **23:** 1152–1158.
131. Hepner, G. W., A. F. Hofmann, and P. J. Thomas. 1972. Metabolism of steroid and amino acid moieties of conjugated bile acids in man. *J. Clin. Invest.* **51:** 1889–1897.
132. Stahl, E., and B. Arnesjö, 1972. Taurocholate metabolism in man. *Scand. J. Gastroenterol.* **7:** 559–566.
133. Samuelsson, B. 1960. Bile acids and steroids. 96: On the mechanism of the biological formation of deoxycholic acid from cholic acid. *J. Biol. Chem.* **235:** 361–366.
134. Hill, M. J. 1978. The role of unsaturated bile acids in the etiology of large bowel cancer. Origins of Human Cancer. Cold Spring Harbour Symposium, Vol. XLIV. 1627–1639.
135. Palmer, R. H., and Z. Hruban. 1966. Production of bile duct hyperplasia and gallstones by lithocholic acid. *J. Clin. Invest.* **45:** 1255–1267.
136. Fisher, C. D., N. S. Cooper, M. A. Rothschild, and E. H. Mosbach. 1977. Effect of dietary chenodeoxycholic acid and lithocholic acid in the rabbit. *Am. J. Dig. Dis.* **19:** 877–886.
137. Silverman, S. J., and A. N. Andrews. 1977. Bile acids:

comutagenic activity in the *Salmonella* microsome mutagenic test: brief communication. *J. Natl. Cancer Inst.* **59:** 1557–1559.
138. Kawalek, J. C., and A. W. Andrews. 1977. The effect of bile acids on the metabolism of benzo(a)pyrene and 2-aminoanthracene to mutagenic products. *Federation Proc.* **36:** 844 (Abstract).
139. Hofmann, A. F. 1977. Bile acids, diarrhea and antibiotics: data, speculation and a unifying hypothesis. *J. Infect. Dis.* **135:** S126–S132.
140. Elliot, W. H. 1971. Allo bile acids. *In* The Bile Acids. Vol. 1. P. P. Nair, and D. Kritchevsky, editors. Plenum Press, New York, London. 47–93.
141. Igimi, H. 1976. Ursodeoxycholic acid—a common bile acid in gallbladder bile of Japanese subjects. *Life Sci.* **18:** 993–1000.
142. Kurozumi, K., T. Harano, K. Yamasaki, and Y. Ayak. 1973. Studies on bile acids in bear bile. *J. Biochem.* **74:** 485–495.
143. 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-keto lithocholic acid as an intermediate. *Gastroenterology.* **78:** 1252 (Abstract).
144. Hirano, S., N. Masuda, and H. Oda. 1981. In vitro transformation of chenodeoxycholic acid and ursodeoxycholic acid by human intestinal flora, with particular reference to mutual conversion between the two bile acids. *J. Lipid Res.* **22:** 735–743.
145. Hirano, S., N. Masuda, H. Oda, and H. Muka. 1981. Transformation of bile acids by *Clostridium perfringens. Appl. Environ. Microbiol.* **42:** 394–399.
146. Macdonald, I. A., D. M. Hutchison, T. P. Forrest, V. D. Bokkenheuser, J. Winter, and L. V. Holdeman. 1983. Metabolism of primary bile acids by *Clostridium perfringens. J. Steroid Biochem.* **18:** 94–104.
147. Shefer, S., and G. Salen. 1980. Hepatic transformation of isochenodeoxycholic acid to chenodeoxycholic acid and isoursodeoxycholic acid in the rat. *Gastroenterology.* **79:** 1053 (Abstract).
148. 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.
149. 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.
150. 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.
151. Edenharder, R., and T. Knaflic. 1981. Epimerization of chenodeoxycholic acid to ursodeoxycholic acid by human intestinal lecithinase-lipase-negative *Clostridia. J. Lipid Res.* **22:** 652–658.
152. Edenharder, P., 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. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig.* **174:** 91–104.
153. 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.
154. 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.
155. 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.
156. Armstrong, M. J., and M. C. Carey. 1982. The hydrophobic-hydrophilic balance of bile salts. Inverse correlation between reverse-phase high performance liquid chromatographic mobilities and micellar cholesterol-solubilizing capacities. *J. Lipid Res.* **23:** 70–80.
157. Fromm, H., E. L. Carlson, A. F. Hofmann, S. Farivar, and P. Amin. 1980. Metabolism in man of 7-keto lithocholic acid, precursor of cheno- and urso-deoxycholic acids. *Am. J. Physiol.* **239:** G161–G166.
158. Ali, S. S., A. Kuksis, and J. M. R. Beveridge. 1966. Excretion of bile acid by three men on a fat-free diet. *Can. J. Biochem.* **44:** 957–969.
159. Bazzoli, F., H. Fromm, R. P. Sarra, R. F. Sembrat, and S. Ceryak. 1982. Comparative formation of lithocholic acid from chenodeoxycholic and ursodeoxycholic acids in the colon. *Gastroenterology.* **83:** 753–760.
160. Stiehl, A., P. Czygan, B. Kommerell, H. F. Weir, and K. H. Holtermüller. 1978. Ursodeoxycholic acid vs. chenodeoxycholic acid, comparison of their effects on bile acid and bile lipid composition in patients with cholesterol gallstones. *Gastroenterology.* **75:** 1016–1021.
161. Salen, G., A. Colalillo, D. Verga, E. Bakan, G. S. Tint, and S. Shefer. 1980. Effect of high and low doses of ursodeoxycholic acid on gallstone dissolution in humans. *Gastroenterology.* **78:** 1412–1418.
162. Tokyo Co-operative Gallstone Study Group. 1980. Efficacy and indication of ursodeoxycholic acid treatment for dissolving gallstones. *Gastroenterology.* **78:** 542–548.
163. Barnes, W. S., and W. D. Powrie. 1982. Clastogenic activity of bile acids and organic acid fractions of human feces. *Cancer Lett.* **15:** 317–327.
164. Leuschner, U. 1982. Chenodeoxycholic acid versus ursodeoxycholic acid as cocarcinogens in Wistar rats. Falk Symposium #33. VII International Bile Acid Meeting, October 14, 1982.
165. Sarwal, A. N., B. I. Cohen, R. F. Raicht, M. Tokahashi, and E. Fazzini. 1979. Effects of dietary administration of chenodeoxycholic acid on N-methyl-N-nitrosourea-induced colon cancer in rats. *Biochim. Biophys. Acta.* **574:** 423–432.
166. vanTassell, R. L., D. K. MacDonald, and D. T. Wilkins. 1982. Stimulation of mutagen production in human feces by bile and bile acids. *Mutat. Res.* **103:** 233–239.
167. Macdonald, I. A., and D. M. Hutchison. 1982. Epimerization versus dehydroxylation of the 7α-hydroxyl group of primary bile acids: competitive studies with *Clostridium absonum* and 7α-dehydroxylating bacteria (*Eubacterium sp.*). *J. Steroid Biochem.* **17:** 295–303.
168. Yahiro, K., T. Setoguchi, and T. Katsuki. 1980. Effect of cecum and appendix on 7α-dehydroxylation and 7β-

epimerization of chenodeoxycholic acid in the rabbit. *J. Lipid Res.* **21:** 215–222.
169. Salen, G., G. S. Tint, R. Elear, N. Derring, and E. H. Mosbach. 1974. Increased formation of ursodeoxycholic acid in patients treated with chenodeoxycholic acid. *J. Clin. Invest.* **53:** 612–621.
170. Hofmann, A. F., J. L. Thistle, P. D. Klein, P. A. Szczepanik, and P. Y. S. Yu. 1978. Cheno therapy for gallstone dissolution. II. Changes in bile acid composition and gallstone response. *J. Am. Med. Assoc.* **239:** 1138–1144.
171. Kishi, S. 1936. Untersuchung der Kaninchengalle I Mitteilung: Über zwei neue Gallensäuren α- und β-Lagodesoxycholsäure und über Lithocholsäure. *Hoppe-Seyler's Z. Physiol. Chem.* **238:** 210–220.
172. Kallner, A. 1967. The transformation of deoxycholic acid into allodeoxycholic acid in the rat. *Acta Chem. Scand.* **21:** 87–92.
173. Danielsson H., A. Kallner, and J. Sjövall. 1963. On the composition of the bile acid fraction of rabbit feces and the isolation of a new bile acid 3α, 12α-dihydroxy-5α-cholanoic acid. *J. Biol. Chem.* **238:** 3846–3852.
174. Haslewood, G. A. D. 1967. The Bile Salts. Methuen and Co. Ltd., 11 New Fetter Lane. London EC-4. 78.
175. Eneroth, P., B. Gordon, and J. Sjövall. 1966. Characterization of trisubstituted cholanoic acids in human feces. *J. Lipid Res.* **7:** 524–530.
176. Hoshita, T., K. Aminoto, T. Nakagawa, and T. Kazumo. 1967. Steroids: bile acids and bile alcohols. XCIV. Synthesis of allochenodeoxycholic acid. *J. Biochem.* **61:** 750–755.
177. Hoshita, T., S. Shefer, and E. H. Mosbach. 1968. Conversion of 7α, 12α-dihydroxycholest-4-en-3-one to 5α-cholestane-3α,7α,12α-triol by iguana liver microsomes. *J. Lipid Res.* **9:** 237–243.
178. Kallner, A. 1967. On the biosynthesis and metabolism of allodeoxycholic acid in the rat. Bile acids and steroids, 175. *Acta Chem. Scand.* **21:** 315–321.
179. Hofmann, A. F., and E. H. Mosbach. 1964. Identification of allodeoxycholic acid as the major component of gallstones induced in the rabbit by 5α-cholestan-3β-ol. *J. Biol. Chem.* **239:** 2813–2821.
180. Anderson, I. G., and G. A. D. Haslewood. 1962. Comparative studies of bile salts. 15. The natural occurrence and preparation of allocholic acid. *Biochem. J.* **85:** 236–242.
181. Kelsey, M. I., and S. A. Sexton. 1976. The biosynthesis of ethyl esters of lithocholic acid and isolithocholic acid by rat intestinal microflora. *J. Steroid Biochem.* **7:** 641–647.
182. Aries, V. C., P. Goddard, and M. J. Hill. 1971. Degradation of steroids by intestinal bacteria. III. 3-Oxo-5β-steroid Δ^1-dehydrogenase and 3-oxo-5β-steroid Δ^4-dehydrogenase. *Biochim. Biophys. Acta.* **248:** 482–488.
183. Hill, M. J. 1974. Steroid nuclear dehydrogenation and colon cancer. *Am. J. Clin. Nutr.* **27:** 1475–1480.
184. Goddard, P., and M. J. Hill, 1972. Degradation of steroids by intestinal bacteria. IV. The aromatization of ring A. *Biochim. Biophys. Acta.* **280:** 336–342.
185. Goddard, P., and M. J. Hill. 1973. The dehydrogenation of the steroid nucleus by human gut bacteria. *Biochem. Soc. Trans.* **1:** 1113–1116.
186. Macdonald, I. A., and M. J. Hill. 1979. The inability of nuclear dehydrogenating clostridia to oxidize bile salt hydroxyl groups. *Experientia.* **35:** 722–723.
187. Larusso, N. F., N. E. Hoffman, and A. F. Hofmann. 1979. Validity of using 2,4-^{3}H-labeled bile acids to study bile acid kinetics in man. *J. Lab. Clin. Med.* **84:** 759–765.
188. Ng, P. Y., R. N. Allan, and A. F. Hofmann. 1977. Suitability of [11,12-$^3H_2$]chenodeoxycholic acid and [11,12-$^3H_2$]lithocholic acid for isotope dilution studies of bile acid metabolism in man. *J. Lipid Res.* **18:** 753–758.
189. Hill, M. J., B. S. Drasar, R. E. O. Williams, T. W. Meade, A. G. Cox, J. E. P. Simpson, and B. C. Morson. 1975. Faecal bile acids and *clostridia* in patients with cancer of the large bowel. *Lancet.* **i:** 535–539.
190. Blackwood, A., W. R. Murray, C. MacKay, and K. C. Calman. 1978. Faecal bile acids and *clostridia* in the aetiology of colorectal and breast cancer. *Br. J. Cancer.* **38:** 175 (Abstract).
191. Crowther, J. S., B. S. Drasar, M. J. Hill, R. MacLennan, D. Magnin, S. Peach, and C. H. Teak-Chan. 1976. Fecal steroids and bacteria and large bowel cancer in Hong Kong by socio-economic groups. *Br. J. Cancer.* **34:** 191–198.
192. Kelsey, M. I., G. M. Muschik, and S. A. Sexton. 1978. The metabolism of lithocholic acid 3α-sulfate by human intestinal microflora. *Lipids.* **13:** 152–157.
193. Borriello, S. P., and R. W. Owen. 1982. The metabolism of lithocholic acid and lithocholic acid 3α-sulfate by human fecal bacteria. *Lipids.* **17:** 477–482.
194. Imperato, T. J., G. C. Wong, L. J. Chen, and R. J. Bolt. 1977. Hydrolysis of lithocholate sulfate by *Pseudomonas aeruginosa*. *J. Bacteriol.* **130:** 545–547.
195. Eyssen, H., and S. Huijghebaert. 1981. Metabolism of bile salt sulphates by strictly anaerobic intestinal microorganisms *In* Recent Advances in Germ-Free Research. S. Sasaki et al. editors. 231–236.
196. Huijghebaert, S. M., J. A. Mertens, and H. J. Eyssen. 1982. Isolation of a bile salt sulfatase producing *Clostridium* strain from rat intestinal microflora. *Appl. Environ. Microbiol.* **43:** 185–192.
197. Huijghebaert, S., and H. J. Eyssen. 1982. Specificity of the bile salt sulphatase activity from *Clostridium Sp. strain S-1. Appl. Environ. Microbiol.* **44:** 1030–1034.
198. Floch, M. H., W. Gershengoren, S. Diamond, and T. Hersh. 1970. Cholic acid inhibition of intestinal bacteria. *Am. J. Clin. Nutr.* **23:** 8–10.
199. Binder, H. J., B. Filburn, and M. Floch. 1975. Bile acid inhibition of intestinal anaerobic organisms. *Am. J. Clin. Nutr.* **28:** 119–125.
200. Eriksson, H., J. A. Gustafsson, and J. Sjövall. 1970. Excretion of steroid hormones in adults, C_{19} and C_{21} steroids in feces from pregnant women. *Eur. J. Biochem.* **12:** 520–526.
201. Eriksson, H., and J. A. Gustafsson. 1970. Steroids in germ-free and conventional rats. Distribution and excretion of labeled pregnenolone and corticosterone in male and female rats. *Eur. J. Biochem.* **15:** 132–139.
202. Graes, V., E. Suraya, and O. Nishikiza. 1977. Hydrolysis of steroid glucuronides with β-glucuronidase preparation from bovine liver, *Helix pomatia*, and *Escherichia coli*. *Clin. Chem.* **23:** 532–535.
203. Bokkenheuser, V. D., J. Winter, P. Dehazya, and W. G. Kelly. 1977. Isolation and characterization of human fecal bacteria capable of 21-dehydroxylating corticoids. *Appl. Environ. Microbiol.* **34:** 511–515.

204. Bokkenheuser, V. D., J. Winter, P. Dehazya, O. Deleon, and W. G. Kelly. 1976. Formation and metabolism of tetrahydrodeoxycorticosterone by human fecal flora. *J. Steroid Biochem.* **7:** 837–743.
205. Bokkenheuser, V. D., J. B. Susuki, S. B. Polovsky, J. Winter, and W. G. Kelly. 1975. Metabolism of deoxycorticosterone by human fecal flora. *Appl. Microbiol.* **30:** 82–90.
206. Feighner, S. D., V. D. Bokkenheuser, J. Winter, and P. B. Hylemon. 1979. Characterization of a C_{21} neutral steroid hormone transforming enzyme, 21-dehydroxylase, in crude cell extracts of *Eubacterium lentum*. *Biochim. Biophys. Acta.* **574:** 154–163.
207. Laskee, A. I., and H. A. Lechevalier. 1974. Handbook of Microbiology. CRC Press, Ohio. *IV:* 117–442.
208. Lombardi, P., B. Golden, E. Britui, and S. L. Gorbach. 1978. Metabolism of androgens and estrogens by human fecal microorganisms. *J. Steroid Biochem.* **9:** 795–801.
209. Schubert, K., J. Schlegel, K. H. Böhme, and C. Hörhold. 1967. Mikrobielle hydrierungs- und dehydrierungs-Reaktionen bei Δ^4-3-Ketosteroiden mit einer 5-Hydroxygruppe. *Biochem. Biophys. Acta.* **144:** 132–138.
210. Shackleton, C. H. L., J. W. Honour, J. Winter, and V. D. Bokkenheuser. 1979. Urinary metabolites of 18-hydroxylated corticosteroids: microbial preparation of reference compounds. *J. Steroid Biochem.* **11:** 1141–1144.
211. Bokkenheuser, V. D., J. Winter, J. W. Honour, and C. H. L. Shackleton. 1979. Reduction of aldosterone by anaerobic bacteria: origin of urinary 21-deoxymetabolites in man. *J. Steroid. Biochem.* **11:** 1145–1149.
212. Cook, B. A., and D. K. Vallance. 1965. Metabolism of megestrol acetate and related progesterone analogues by liver preparation in vitro. *Biochem. J.* **97:** 672–677.
213. Schubert, K., J. Schlegel, and C. Hörhold. 1963. Stereospezifische Hydrierung von Δ^{14}-Androstadiendion-(3.17) zu Δ^1-5β-Androstendion-(3.17) und 5β-Androstanol-(3α)-on-(17) mit *Clostridium paraputrificum* unter anaeroben Bedingrungen. *Hoppe Seylers S. Physiol. Chem.* **332:** 310–313.
214. Schubert, K., J. Schlegel, and C. Hörhold. 1965. Stereospecific hydrogenation of Δ^4-,$\Delta^{1,4}$-,$\Delta^{4,6}$-, and $\Delta^{1,4,6}$-3-ketosteroids by *Clostridium paraputrificum*. *Steroids.* **Suppl. 1:** 175–184.
215. Goddard, P., F. Fernandez, B. West, M. J. Hill, and P. Barnes. 1975. The nuclear dehydrogenation of steroids by intestinal bacteria. *J. Med. Microbiol.* **8:** 429–435.
216. Wade, A. P., J. D. M. Slater, A. E. Kellie, and M. E. Holliday. 1959. Urinary excretion of 17-ketosteroids following rectal infusion of cortisol. *J. Clin. Endocrinol. Metab.* **19:** 444–453.
217. Gustafsson, J. A. 168. Steroids in germ-free and conventional rats. 7. Identification of C_{19} and C_{21} steroids in feces from conventional rats. *Eur. J. Biochem.* **6:** 248–255.
218. Eriksson, H., and J. A. Gustafsson. 1971. Excretion of steroid hormones in adults. Steroids in feces from adults. *Eur. J. Biochem.* **18:** 146–150.
219. Cerone-McLernon, A. M., J. Winter, E. H. Mosbach, and V. D. Bokkenheuser. 1981. Side chain cleavage of cortisol by fecal flora. *Biochim. Biophys. Acta.* **666:** 341–347.
220. Winter, J., A. Cerone-McLernon, S. O'Rourke, and V. D. Bokkenheuser. 1983. Formation of 20β-dehydrosteroids by anaerobic bacteria. *J. Steroid Biochem.* **17:** 661–667.
221. Bokkenheuser, V. D., J. Winter, P. B. Hylemon, N. K. N. Ayengar, and E. H. Mosbach. 1981. Dehydroxylation of 16α-hydroxyprogesterone by fecal flora of man and rat. *J. Lipid Res.* **22:** 95–102.
222. Eriksson, H., J. A. Gustafsson, and J. Sjövall. 1968. Steroids in germ-free and conventional rats. 4. Identification and bacterial formation in 17α-pregnane derivatives. *Eur. J. Biochem.* **6:** 219–226.
223. Calvin, H. I., and S. Lieberman. 1962. Studies on the metabolism of 16α-hydroxy progesterone in humans: conversion to urinary 17-isopregnanolone. *Biochemistry.* **1:** 639–645.
224. Diczfalusy, E., and M. Levitz. 1970. Formation, metabolism and transport of estrogen conjugates. *In* Chemical and Biological Aspects of Steroid Conjugates. S. Bernstein and S. Solomon, editors. Springer Verlag, Berlin. 231–320.
225. Adlercreutz, M., F. Martin, M. Pulkkinen, M. Dencker, V. Rimér, N. O. Sjöberg, and M. S. Tikkanen. 1976. Intestinal metabolism of estrogens. *J. Clin. Endocrinol. Metab.* **43:** 497–505.