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Review Article

Drug Metabolism by Intestinal Microorganisms

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STUDIES DEALING with the metabolic fate of drugs are of considerable importance as they may often elucidate significant aspects of the biological action of these compounds. The literature in this field is extensive and many excellent reviews are available including those by Williams (1), Gillette (2, 3), Ariëns and Simonis (4), Maynert (5), Boyland and Booth (6), Shideman and Mannering (7), Williams and Parke (8), Hayes (9), Remmer (10), and Bush and Sanders (11). Interest has hitherto been nearly exclusively devoted to the metabolic processes which take place in the tissues. However, drugs will often come into contact with the microorganisms which comprise the normal gastrointestinal flora. This situation is encountered when drugs are administered orally and especially when their absorption is delayed because of low solubility or other factors. Also, diffusion or secretion of drugs into the intestinal lumen or, more commonly, excretion of them or their metabolites in the bile may occur. These situations may lead to alteration of the compounds as a result of metabolic activity of the microorganisms in the gastrointestinal tract.

Several examples of the intestinal metabolism of drugs have been discussed by Smith (12), primarily in connection with the fate of biliary conjugates in the intestine. However, most of the information presently available is scattered throughout the literature and often stems from incidental observations made in the course of other studies. The incidence of such reports has increased recently and it is becoming more widely recognized that the gastrointestinal flora may be of great significance in determining the metabolic fate of drugs. This review therefore aims to summarize and assess the present knowledge of the metabolism of drugs by the gastrointestinal microorganisms. For this purpose the definition of a drug will be interpreted liberally. However, emphasis will be placed on compounds which can be considered as foreign to the animal organism.

GASTROINTESTINAL FLORA

Nature of Flora—The normal gastrointestinal flora in man and animals has not been extensively studied in the past although interest in this field of microbiology has increased considerably in recent years. The reasons for neglect are partly historical in that emphasis has been placed on pathogenic microorganisms and partly technical in that many members of the normal flora are difficult to cultivate. The complex nature of the gastrointestinal flora is emphasized by the fact that more than 60 bacterial species have been isolated from the in-

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testinal tracts or feces of healthy animals, including man (13). Many studies have dealt with the coliforms and enterococci and this reflects the ease with which these organisms can be cultivated. However, recent evidence suggests that more typical representatives of the flora are to be found among the lactobacilli, the anaerobic nonsporulating bacilli (*Bacteroides*), and the fusiform organisms (14). Spears and Freter (15) have recently shown that the predominant, strictly anaerobic bacteria of the flora are sensitive to even transient contact with atmospheric oxygen. Thus it must be emphasized that our conception of the flora is greatly dependent on the techniques used and also that our present knowledge may be both limited and somewhat misleading.

In spite of these reservations it is worthwhile to briefly summarize the findings now available. Table I lists the main groups of gastrointestinal microorganisms in mammals. Further information on the subject is available in the works of Rosebury (16), Wilson and Miles (17), and Dubos (18). Donaldson (13, 19, 20) and Raibaud *et al.* (21–24) have also published valuable reviews and other pertinent information is available (14, 25–34).

Species Differences—Smith (27) investigated the gastrointestinal flora in 15 homothermic species including the common laboratory animals when fed on conventional diets. Although great quantitative differences were noted, the florae were found to consist of the same types of organisms in all species except the rabbit. The major groups or species were bacteroides, lactobacilli, *E. coli*, streptococci, *Clostridium welchii*, veillonellae, and yeasts. Except for bacteroides which were confined to the large intestine, these organisms were distributed throughout the gastrointestinal tract. The rabbit differed greatly in that the flora of the large intestine consisted nearly entirely of bacteroides whereas the stomach and small intestine were usually sterile. This was probably due to the low pH of the stomach contents in rabbits. However, Bornside and Cohn (31) have reported a high incidence of bacteroides and bacilli in the small intestine of rabbits.

The human jejunum has been found to be sparsely populated and the microorganisms have generally been considered to be transients (13, 30, 31). The ileum has also often been reported to be sterile (13, 17), however this belief will no doubt have to be altered in view of recent findings (30, 31, 33). In general, it appears that the predominant bacterial groups in the intestinal tract of man are those found in many other species including dogs (31) and mice (14).

Effect of Diet—It is well known that the diet may influence the nature of the gastrointestinal flora. The earlier literature has been reviewed by Donaldson (13). The effects of high and low protein diets and starvation (27) and of high fat diets (29) on the flora in rats have been reported. Some of the findings are conflicting but it appears that a diet high in meat protein or casein will increase the numbers of clostridia and coliforms. Yeasts appear to be very sensitive to the nature of the diet and are numerous in animals fed mainly cereals but largely absent when meat or protein is fed (27). Lactobacilli also appear to be present in greater

TABLE I—MICROORGANISMS FOUND IN THE GASTROINTESTINAL TRACT

Group, Genus or Species	Description	Remarks
Bacteroides	Gram-negative, strictly anaerobic, nonsporulating rods	Found mainly in lower intestine
Lactobacilli	Gram-positive, microaerophilic or anaerobic rods	Numerous in all parts
Enterobacteria	Gram-negative, aerobic or facultatively anaerobic rods	Found in all parts but more numerous in lower intestine
E. coli		
Aerobacter		
Klebsiella		
Proteus		
Pseudomonas	Gram-negative, aerobic rods	
Ps. aeruginosa		
Streptococci		
Enterococci	Gram-positive, aerobic or facultatively anaerobic cocci	Found in all parts but more numerous in lower intestine
S. faecalis		
Anaerobic	Gram-positive, anaerobic cocci	Numerous in all parts
Clostridia	Gram-positive, anaerobic sporulating rods	Sometimes found in all parts but mainly in lower intestine
Bacilli	Gram-positive, aerobic or facultatively anaerobic sporulating rods	
Staphylococci	Gram-positive, aerobic or facultatively anaerobic cocci	Found in all parts
Fusobacteria	Gram-negative, anaerobic rods	
Veillonellae	Gram-negative, anaerobic cocci	Found in all parts but more numerous in lower intestine
Yeasts		Numerous in all parts

numbers in animals fed on cereals. High fat diets seem to have little effect on the flora in rats (29).

While radical changes in the nature of the diet may therefore alter the composition of the intestinal flora, it does not seem likely that appreciable variations will be encountered with ordinary diets. However, it is not uncommon to use fasted animals in metabolic studies and the effects of starvation on the flora may be extensive. Thus, Smith (27) found large reductions in the numbers of yeasts, moderate reductions in the numbers of *E. coli*, *Cl. welchii*, and lactobacilli and no change in the numbers of streptococci and bacteroides when rats maintained on a normal diet were starved for 24 hr.

Effect of Age—Appreciable variations in the types and numbers of microorganisms are observed in animals of different ages (13, 17, 22, 25, 28) with the greatest changes occurring during early life.

Effect of Drugs—The nature of the flora may be profoundly altered when drugs having an antibacterial effect are fed. Such substances are often added to animal feedstuffs. This subject will not be discussed here as it has been reviewed by Luckey (35). Further information is found in the articles by Donaldson (13), Evenson *et al.* (36), and Dubos *et al.* (37).

REACTIONS

Hydrolysis of Glucuronides—It is well known that glucuronides may undergo hydrolysis in the intestine and it seems likely that this is one of the most important of the intestinal reactions. Its importance arises from the fact that many drugs and foreign compounds are excreted in the bile as glucuronide conjugates (12, 38). These then pass along the intestine where they may undergo hydrolysis. The liberated aglycone may be absorbed from the intestine to varying degrees and thereby establish an enterohepatic circulation of the compound. It is also possible for the aglycone to undergo further intestinal metabolism and for these metabolites to be absorbed into the body or excreted in the feces. This interplay between tissue and intestinal metabolism can thus lead to a fairly complex metabolic picture for some compounds. On the other hand, some glucuronides may be stable to β-glucuronidase and thus be excreted unchanged in the feces.

The most common situation appears to be the one in which glucuronide hydrolysis is followed by absorption of the aglycone to a varying extent. Several such examples are listed in Table II. The first three deal with glucuronides administered orally as these compounds are not important biliary metabolites following the administration of phenol, *p*-hydroxybenzoic acid, or salicylic acid. The urinary recoveries are often considerably reduced following administration of the glucuronides and accounted for only 35 and 50% of the dose with salicylic acid glucuronide and phenyl glucuronide, respectively. A more pronounced example of this occurs with iopanoic acid [3-(3-amino-2,4,6-triiodophenyl)-2-ethylpropionic acid] ester glucuronide in cats (48, 49). Oral administration of this glucuronide resulted in about 10% of the dose being excreted in the urine and the remainder in the feces. As the nature of the material in the feces was not determined, it is not known if the high fecal excretion is due to the stability of the glucuronide to intestinal hydrolysis or the limited ability of the liberated iopanoic acid to be absorbed.

The other examples listed in Table II are of glucuronides which are biliary metabolites. Further examples have been reported although the fate of the aglycones is not so well understood. Thus the biliary metabolites of ethynylestradiol-3-cyclopentyl ether, which are predominately glucuronides, are absorbed to a

TABLE II—GLUCURONIDES WHICH ARE HYDROLYZED IN THE INTESTINE

Conjugate	Species	Fate of Aglycone	Reference
Phenyl glucuronide	Rabbit	Liberated phenol absorbed, metabolized to sulfate and glucuronide conjugates and polyphenols which are excreted in urine	(39)
p-Hydroxybenzoic acid diglucuronide	Man	Liberated *p*-hydroxybenzoic acid absorbed and excreted in urine free and combined with glycine	(40)
Salicylic acid ether glucuronide	Rat	Liberated salicylic acid absorbed and excreted in urine free and combined with glycine and glucuronic acid	(41)
Indomethacin [1-*p*-(chlorobenzyl)-5-methoxy-2-methylindole-3-acetic acid] glucuronide	Dog	Liberated indomethacin undergoes enterohepatic circulation and excretion in feces	(42)
Desacetylbisacodyl (4,4'-dihydroxydiphenyl-2-pyridylmethane) glucuronide	Rat	Liberated desacetylbisacodyl partly absorbed but mainly excreted in feces	(43)
Morphine glucuronide	Dog	Liberated morphine both absorbed and excreted in feces	(44, 45)
Thyroxine glucuronide	Rat, dog	Liberated thyroxine nearly completely absorbed	(46, 47)

small extent following their infusion into the small intestine (50). Extensive enterohepatic circulation of diethylstilbestrol has been shown to take place in the rat (51). The main biliary metabolite of this compound is the monoglucuronide (52) and it is likely that it is hydrolyzed in the intestine prior to reabsorption. Glutethimide (2-ethyl-2-phenyl-glutarimide) is extensively excreted in the bile as glucuronide conjugates when administered to rats (53). The slow reabsorption of the glutethimide metabolites from the intestine suggests that they are first hydrolyzed. A similar picture is shown by griseofulvin which is extensively excreted in the bile as a conjugate of 4-demethylgriseofulvin (54). A major biliary metabolite of butylated hydroxytoluene (3,5-di-*tert*-butyl-4-hydroxytoluene) in the rat is the ester glucuronide of 3,5-di-*tert*-butyl-4-hydroxybenzoic acid which is believed to be partly responsible for the enterohepatic circulation seen in this species (55). However, the free acid is the main fecal metabolite and it seems likely that intestinal hydrolysis occurs prior to its absorption. Naphthaleneacetic acid is excreted in the bile as a glucuronide which has been postulated to undergo intestinal hydrolysis and absorption (56).

Table III lists glucuronides which have been shown to be hydrolyzed and further metabolized in the intestine. These intestinal metabolites may be absorbed and appear in the urine as major metabolites. Ferulic acid glucuronide provides an interesting example in that it undergoes a sequence of intestinal reactions including glucuronide hydrolysis, reduction of a double bond, demethylation, and dehydroxylation before being absorbed and excreted in the urine as *m*-hydroxyphenylpropionic acid.

The above findings showing that glucuronides are very susceptible to hydrolysis in the intestine are not unexpected in view of the high β-glucuronidase activity found in the intestinal contents from many species including fowl, ruminants, and common laboratory species (59). Intestinal contents or feces have been found to hydrolyze chloramphenicol glucuronide (57), estriol glucuronide (60), and 4-methylumbelliferone glucuronide (61). High activity is always noted in the large bowel, low or no activity is seen in the stomach while activity in the small intestine is variable. These findings suggest that the β-glucuronidase activity is derived from one or more microorganisms of widespread occurrence which are able to populate a fairly large portion of the gastrointestinal tract. These ideas are in agreement with the fact that *E. coli* is an intestinal inhabitant in many species and is known to possess β-glucuronidase activity (57, 62). The numbers of this organism are nearly always greatest in the cecum or large intestine and this could very well explain the greater β-glucuronidase activity found there.

Hydrolysis of Glycosides—A common example of the intestinal hydrolysis of glycosides is that seen with the anthraquinone cathartics. The active constituents of these agents, which include cascara sagrada and senna, are related to emodin (1,3,8-trihydroxy-6-methylanthraquinone). These active substances are released from the glycosides by bacterial action in the large intestine (63).

Glycoside hydrolysis is important in the metabolism of the cardiac glycosides. These substances exist in plants as precursors which are called native, natural, or genuine glycosides. Examples of these are the lanatosides (digilanids) found in *Digitalis lanata* leaf and the desacetyldigilanids found in *D. purpurea* leaf. All of these contain one glucose and three digitoxose moieties attached to the aglycone. Incubation of lanatoside A with rat feces suspensions was shown to result in the rapid hydrolysis of the terminal glucose unit to give acetyldigitoxin which was then deacetylated to digitoxin (64). Hydrolysis of the native glycoside *k*-strophanthoside occurred under these conditions and resulted in the loss of two glucose units to give the glycoside cymarin. The latter finding has also been reported by Engler *et al.* (65).

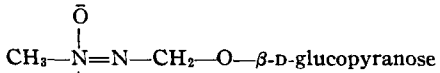
Studies on the intestinal 6-bromo-2-naphthyl glycosidases in rats showed that the cecal enzymes have higher pH optima than the corresponding enzymes from the small intestine (66). The cecal 6-bromo-2-naphthyl-α-glucosidase activity was found to be dependent on bacterial enzymes. However, the origin of the cecal 6-bromo-2-naphthyl-β-glucosidase and -β-galactosidase activities was not determined.

TABLE III—GLUCURONIDES WHICH ARE HYDROLYZED AND FURTHER METABOLIZED IN THE INTESTINE

Conjugate	Species	Fate of Aglycone	Reference
Chloramphenicol-3-glucuronide	Rat	Liberated chloramphenicol reduced to arylamines which are partly absorbed and excreted in urine	(57)
Ferulic acid glucuronide	Rat	Liberated ferulic acid metabolized to *m*-hydroxyphenylpropionic acid which is absorbed and excreted in urine	(58)

Much of the information presently available on the hydrolysis of glycosides in the intestine deals with the flavonoid compounds. It has been shown in several studies that the ingestion of flavonoid glycosides results in the urinary excretion of the aglycones and other metabolites which are primarily ring fission products. These results have been obtained with hesperidin (hesperetin-7-rhamnoglucoside) (67), diosmin (diosmetin-7-rhamnoglucoside) (67), and naringin (naringenin-7-rhamnoglucoside) (68). Similar studies with rutin (quercetin-3-rutinoside) show that it is converted to several metabolites lacking the sugar residue (69). It was subsequently shown that this degradation occurred when rutin was incubated with intestinal contents (70). This finding has been confirmed using rat cecal microorganisms and both the aglycone quercetin, as well as the subsequent ring fission metabolites, were detected (61). Also, hesperidin was metabolized to hesperetin and phenyl-β-D-glucoside to phenol under these conditions.

An interesting example of the role of the intestinal microorganisms in glycoside hydrolysis is found with cycasin, the β-glucoside of methylazoxymethanol (71). This compound is of in-



Cycasin

terest because it is present in cycad plants from which various products are used as foods in some parts of the world. Cycasin is not toxic when given by injection but is both hepatotoxic and carcinogenic when fed to small laboratory animals. This toxicity is also shown by the aglycone. Cycasin is neither absorbed nor toxic when given to germ-free rats. When these animals were contaminated with several strains of bacteria including *S. faecalis*, both the hydrolysis and toxicity of cycasin were observed. Intestinal microorganisms therefore convert cycasin to the toxic aglycone and variations in the intestinal flora probably have a role in determining the variations in toxicity found in animals given comparable doses of cycasin.

Hydrolysis of Ethereal Sulfates—Relatively little is known of the ability of the intestinal microflora to hydrolyze ethereal sulfates. Closon *et al.* (72) reported that 3,5,3′-triiodo-L-thyronine sulfuric acid ester was hydrolyzed to triiodothyronine when incubated with rat intestinal bacteria. However, *p*-nitrocatechol sulfate (2-hydroxy-5-nitrophenylsulfate) has been found not to be hydrolyzed by rat cecal microorganisms (61). This result is in agreement with earlier reports on the fate of ethereal sulfates in animals. Garton and Williams (39) found that phenylsulfate was excreted essentially unchanged after oral dosage in rabbits. The fate of phenylsulfate and 1- and 2-naphthylsulfate has been studied in rats (73). These compounds were well absorbed following oral dosage and only 4–12% of the dose underwent hydrolysis. However, the extent of hydrolysis was similar after injection and it was concluded that little hydrolysis of these arylsulfates occurred in the gastrointestinal tract. Sodium estrone sulfate has been shown to be hydrolyzed to the extent of about 75% of the dose when administered to female rats (74). This value was similar after gastric administration or injection which suggested that the hydrolysis occurred in the tissues. It was also found that rat liver homogenates hydrolyzed the ester. However, Stimmel (60) has reported that sodium estrone sulfate is hydrolyzed when incubated with human feces. A recent study has shown that sulfuric acid esters of phenols having laxative properties are not hydrolyzed in the intestine (75).

Based upon the above findings, it appears that the intestinal hydrolysis of ethereal sulfates is a reaction of limited occurrence and importance. Also of significance in this regard is the fact that biliary metabolites of phenols are generally glucuronides rather than sulfates (52, 76). The latter are mainly excreted in the urine and will therefore not come into contact with the intestinal contents to any significant extent. This has been shown in recent studies where it was found that only very small amounts of 3-aminophenyl sulfate (76) or dodecyl sulfate (77) were excreted in the bile following their injection in rats.

Hydrolysis of Glycine Conjugates—The metabolism of glycine conjugates in the intestine has been studied mainly with regard to the bile acids. Norman and Grubb (78) found that cultures of rat fecal suspensions could hydrolyze the peptide bond in glycocholic acid. Several strains of clostridia and enterococci were shown to carry out this reaction. They also reported that enterococci are able to hydrolyze hippurate to glycine and benzoic acid. A recent study by Hill and Drasar (79) dealt with the degradation of bile salts by pure cultures of intestinal bacteria. They found that taurocholate was readily deconjugated by many strains of the strictly anaerobic genera *Bacteroides*, *Veillonella*, *Bifidobacterium*, and *Clostridium* and also by about half of the tested strains of *S. faecalis* and a few strains of *Staphylococcus aureus*.

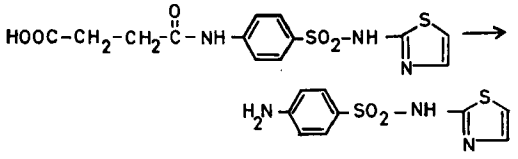
p-Aminohippuric acid and *p*-acetylaminohip-

puric acid undergo extensive hydrolysis in the alimentary tract following their oral administration to man (80). *p*-Aminohippuric acid is readily converted to *p*-aminobenzoic acid when it is incubated with rat cecal microorganisms (61).

N-Deacetylation—This reaction has been studied to only a limited extent and then largely in regard to the fate of *N*-acetyl derivatives of sulfonamides. Patki and Shirsat (81) found that N^4-acetylsulfadiazine was deacetylated to a negligible extent in the gastrointestinal tract in mice. No deacetylation was detected with N^4-acetylsulfanilamide when it was incubated with rat cecal microorganisms (61). However, *N*-deacetylation can occur under these conditions as *p*-aminobenzoic acid was formed from *p*-acetamidobenzoic acid. Also, N^1-acetylsulfisoxazole is known to undergo deacetylation when incubated with several intestinal bacteria including *E. coli*, *Lactobacillus acidophilus*, and *S. faecalis* (82).

N-Acetylhistamine was reported to be deacetylated when incubated with human fecal suspensions (83).

Hydrolysis of Amides—In addition to the examples in the two groups mentioned above, other compounds containing the amide bond have been found to undergo hydrolysis in the intestinal tract. This may often be of practical importance as several useful drugs are metabolized in this manner. Phthalylsulfathiazole and succinylsulfathiazole are intestinal antiseptics which owe their activity to the liberation of sulfathiazole in the large intestine as a result of bacterial hydrolysis. This reaction has been shown to occur with the latter compound when incubated with the rat cecal microflora (61) (Scheme I).



Scheme I—Metabolism of succinylsulfathiazole by rat intestinal microorganisms (61).

Hydrolysis of the amide group in other N^4-acyl derivatives of sulfonamides has been observed (81). The stability of the N^4-acetyl derivatives has been noted above but extensive gastrointestinal deacylation was found with N^4-propionyl- and N^4-butyrylsulfadiazine. Deacylation decreased with increasing chain length and was negligible with the N^4-lauryl and N^4-palmityl derivatives.

Another antibacterial substance known to undergo bacterial hydrolysis of the amide bond is chloramphenicol (84, 85). Some of the bacteria shown to carry out this reaction, including *E. coli* and *Proteus vulgaris*, are common intestinal inhabitants.

The bacterial amidases have been extensively studied in the case of penicillin where two types of hydrolysis are possible. One type, carried out by enzymes referred to as penicillinases or β-lactamases, opens the β-lactam ring and results in inactivation of the antibiotic. These enzymes are produced mainly by Gram-negative bacilli including coliforms, *Aerobacter aerogenes*, and *Proteus* present in the human intestine (86). In addition, β-lactamase is formed by many strains of certain Gram-positive organisms (*S. aureus*, *S. albus*, and *Bacillus cereus*) (86). The second type of hydrolysis removes the side chain and is carried out by an enzyme referred to as penicillin acylase or amidase. Acylase activity is found in many microorganisms belonging to the genera *Aerobacter*, *Micrococcus*, *Proteus*, and *Pseudomonas* and also in *E. coli* (87).

Hydrolysis of Esters—Studies on the absorption, metabolism, and excretion of the succinic acid esters of chloramphenicol have shown that they undergo intestinal hydrolysis prior to absorption (88). Similar findings have been made with erythromycin propionate (89).

Booth (90) found that the ester of citraurin, which occurs in orange peel, was hydrolyzed during passage through the alimentary tract of the rat. Similarly, the diesters of taraxanthan and zeaxanthol were found to be extensively hydrolyzed to free carotenol (91). The laxative bisacodyl [bis(*p*-acetoxyphenyl)-2-pyridylmethane] undergoes nearly complete hydrolysis in the intestine (92). Carbenoxolone, the hemisuccinate of β-glycyrrhetic acid is used in treating gastric ulcers. Studies using the ^{14}C-labeled compound have shown that it is principally hydrolyzed to β-glycyrrhetic acid in the gastrointestinal tract (93).

The origin of the esterase activity in the above examples was not determined but the splitting of carotenoid esters was assumed to be brought about by an intestinal lipase (91). Also, the hydrolysis of the antibiotic esters is sometimes described as being carried out by these enzymes. It is well known that the lipases of the pancreatic juice are capable of splitting esters and that this action takes place in the small intestine. Intestinal hydrolysis of esters at this site would be expected to result in appreciable absorption of the liberated compound. Chloramphenicol, which is itself extensively absorbed, has been shown to

give incomplete absorption when administered as the mono- and disuccinic acid esters (88). This may indicate that hydrolysis is carried out by bacterial esterases in the large intestine where absorption would be expected to be lower.

There is some evidence available which suggests that the bacterial esterases may play an important role in the intestinal hydrolysis of esters. Lanatoside A (digitoxigenin-bis-digitoxosido-acetyldigitoxosido-glucoside) and acetyldigitoxin were shown to be converted to digitoxin (digitoxigenin-tridigitoxoside) when incubated with intestinal microorganisms (64). Methyl gallate is readily hydrolyzed to gallic acid by rat cecal microorganisms (61). Chlorogenic acid, the quinic acid ester of caffeic acid, is hydrolyzed and further metabolized to *m*-hydroxyphenylpropionic acid when fed to rats (94). This reaction sequence is also seen when chlorogenic acid is incubated with rat intestinal microorganisms (95).

Dehydroxylation—The reaction of removal of hydroxyl groups was first shown to occur with the bile acids. Cholic acid was found to be transformed in rats and by rat fecal suspensions into a number of metabolites including some which lack a hydroxyl group at the 7-position (96, 97). These metabolites are not formed in germ-free rats (98). Several strictly anaerobic strains of microorganisms from rat and human feces have been isolated which are capable of dehydroxylating chenodeoxycholate at the 7-position (99). These were subsequently found to be strains of anaerobic Gram-positive, nonsporeforming rods belonging to the tribe *Lactobacilleae* (100). Recently, Hill and Drasar (79) reported that many strains of the strictly anaerobic genera *Bacteroides*, *Clostridium*, and *Veillonella* and also *S. faecalis* are able to remove the 7-hydroxyl group from cholate to yield deoxycholate. They also isolated strains which dehydroxylated cholate and deoxycholate at the 12-position.

The dehydroxylation reaction has also been observed in feeding experiments with other classes of compounds. Thus, 3,4-dihydroxyphenylacetic (homoprotocatechuic) acid was found to be metabolized partly to *m*-hydroxyphenylacetic acid when administered to rats and rabbits (101). This finding was subsequently confirmed using ^{14}C-homoprotocatechuic acid (102, 103). Nearly 7% of the dose (100 mg./kg.) was converted to *m*-hydroxyphenylacetic acid in rats while the amount was twice this in rabbits. In addition, a small amount of *meta*-dehydroxylation which yielded *p*-hydroxyphenylacetic acid was observed.

The metabolism in animals of 3,4-dihydroxybenzoic (protocatechuic) acid, 3,4-dihydroxycinnamic (caffeic) acid, and 3,4-dihydroxyphenylalanine (DOPA) has been studied (94, 104). Dehydroxylation occurred with the latter compounds yielding *m*-hydroxyphenylpropionic acid and *m*-hydroxyphenylacetic acid, respectively. No evidence was obtained for dehydroxylation of the benzoic acid derivative. However, Dacre and Williams (105, 106) were later able to demonstrate with the use of ^{14}C-protocatechuic acid that this compound was dehydroxylated to a minor extent in rats.

The site of formation of the *m*-hydroxyphenyl compounds was indicated by the findings of Shaw *et al.* (107) who reported that inhibition of the intestinal microorganisms with neomycin virtually abolished the excretion of these substances. Similar results have been obtained with neomycin-treated animals given ^{14}C-labeled protocatechuic and homoprotocatechuic acids (103, 106).

Further confirmation that the intestine is the site of dehydroxylation of phenolic acids has been made in studies of the metabolism of these compounds by intestinal contents. Microorganisms from intestinal contents or feces from many animal species were found to dehydroxylate caffeic acid, homoprotocatechuic acid and dihydroxyphenylalanine (70, 108). This reaction was inhibited by aerobic conditions and by several antibiotic substances. Recent studies using rat cecal microorganisms have confirmed the dehydroxylation of caffeic and homoprotocatechuic acids and shown that 3,4-dihydroxyphenylpropionic (hydrocaffeic) acid also undergoes this reaction (58, 109). The extent of dehydroxylation appears to be dependent upon side-chain length. Thus, caffeic and hydrocaffeic acids are extensively converted whereas only small amounts of *m*- and *p*-hydroxybenzoic acids are formed from protocatechuic acid (106). However, the carboxyl group is not essential for activity as pyrogallol was readily dehydroxylated to resorcinol by rat intestinal microorganisms (61, 110). Similar experiments using rabbit fecal extracts resulted in only traces of resorcinol being produced. Little has been reported on the characterization of the microorganisms responsible for the dehydroxylation of these phenolic compounds although Perez-Silva *et al.* (111) isolated a strain of *Pseudomonas* from rat feces which could dehydroxylate caffeic acid.

Dehydroxylation has also been shown to occur with quinaldic acid derivatives. Kynurenic (4-hydroxyquinaldic) acid and xanthurenic (4,8-dihydroxyquinaldic) acid are converted to quinaldic acid and 8-hydroxyquinaldic acid, respec-

tively, when given to animals (112, 113). It was subsequently shown that treatment of rabbits with neomycin in doses sufficient to depress the intestinal microflora prevented the dehydroxylation of xanthurenic acid (114). However, this ability was regained when neomycin administration was stopped. The role of the intestinal microorganisms in the conversion of xanthurenic acid has been confirmed in experiments using rabbit fecal and rat cecal extracts (61, 115).

Several other examples of dehydroxylation have been reported. Tyrosine and phloretic acid are converted to 3-phenylpropionic acid when incubated with sheep rumen liquor (116). An unusual reaction sequence has been reported to occur in the guinea pig treated with tolbutamide which resulted in the conversion of dopamine, norepinephrine, normetanephrine, and 4-hydroxy-3-methoxyphenylglycol to *m*-hydroxyphenylacetic acid (117). Thus, both aromatic and aliphatic dehydroxylations were observed.

Decarboxylation—Roche *et al.* (118) found 3,5,3′-triiodothyronamine in the intestinal contents and feces of rats after administration of 3,5,3′-triiodo-L-thyronine. As the decarboxylated metabolite was absent from the bile, it was concluded that it was formed in the intestine from the free hormone or its conjugates by bacterial action.

Orotic acid (1,2,3,6-tetrahydro-2,6-dioxo-4-pyrimidine carboxylic acid) is extensively decarboxylated in rats by the intestinal microflora (119). Germ-free rats fed a diet containing orotic acid excreted about one third of the compound unchanged in the feces. However, it was absent from the feces of conventional rats after the first few days of feeding.

Much of our present knowledge concerning intestinal decarboxylation has been obtained from experiments with phenolic acids. Tompsett (120) reported the presence of pyrogallol in human urine and suggested that it is probably derived from 3,4,5-trihydroxybenzoic (gallic) acid by decarboxylation in the alimentary tract. Gallic acid was subsequently shown to be metabolized partly to pyrogallol in rats and rabbits (121, 122) although the findings did not point to the gastrointestinal tract as the site of the reaction.

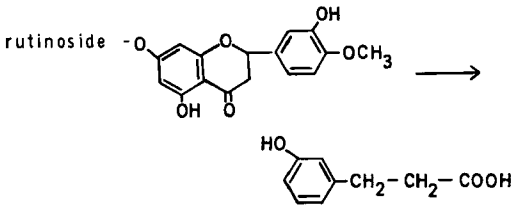
The metabolism of a related compound, 3,4-dihydroxybenzoic acid, by rat fecal and cecal extracts was reported by Booth and Williams (70) who found that it was decarboxylated to catechol. Subsequently, the metabolism of a large number of phenolic acids by the rat intestinal microflora was investigated (58, 109, 110, 123) and it was found that decarboxylation by the intestinal microorganisms is an important reaction with these compounds.

The phenolic benzoic acid derivatives have been most fully studied in regard to their metabolism by rat cecal microorganisms (110, 123). It was found that a free *p*-hydroxyl group is essential for the decarboxylation reaction. Thus *p*-hydroxybenzoic acid was extensively metabolized to phenol while *o*- and *m*-hydroxybenzoic acids were unaffected. Protocatechuic acid and gallic acid also underwent decarboxylation by the cecal microorganisms. The decarboxylation of protocatechuic acid by the rat microflora has been confirmed using ^{14}C-labeled compound (106). The reaction was reduced to varying degrees with substituents in the *meta* position and was abolished with *ortho* substitution. Decarboxylation was also observed with the phenolic phenylacetic and cinnamic acids but did not occur with the corresponding phenylpropionic acids (58, 109). In all cases, a *p*-hydroxyl group is essential for the reaction.

More than one intestinal microorganism and decarboxylase is involved in these reactions. This was indicated by the finding that pretreatment of the cecal microorganisms to destroy vegetative forms but not spores abolished the decarboxylation reaction with all but the cinnamic acid derivatives (110). Subsequently, strains of *Bacillus* were isolated from rat intestine which specifically decarboxylated *p*-hydroxycinnamic acids (124).

The above investigations have also made it clear that these reactions take place following the administration of the phenolic acids to animals. The decarboxylated metabolites of protocatechuic acid and gallic acid were excreted in the urine when rats were given the acids by oral but not by intraperitoneal administration (110). This reaction was reduced or abolished when the animals were pretreated with antibiotics to reduce the numbers of intestinal microorganisms. 4-Methylcatechol was excreted in the urine when homoprotocatechuic acid was given to rats orally but not by injection (109). Similar results were obtained with caffeic acid which was decarboxylated to 4-vinylcatechol (58). Since normal diets contain a large number of phenolic acids or their precursors, it seems reasonable to assume that the bacterial decarboxylation of these compounds in the intestine is an important source of urinary phenols.

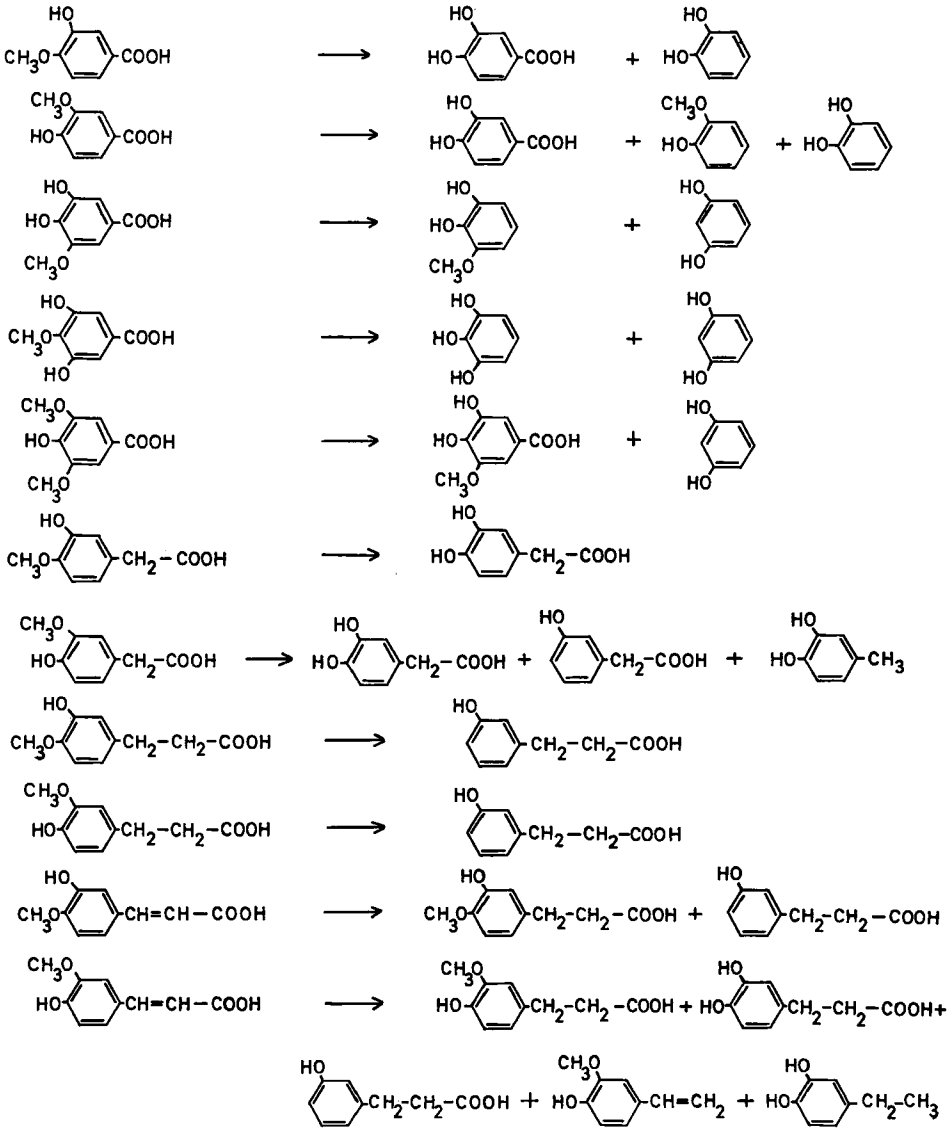
O-Demethylation—Until quite recently, the presence of an intestinal *O*-demethylation reaction appears to have gone unrecognized even



Scheme II—Metabolism of hesperidin by rat cecal microorganisms (61).

though some examples of demethylation could be more easily explained on this basis. An example is found with the flavonoid hesperidin which is partly metabolized in the rabbit to 3,4-dihydroxyphenylpropionic acid and *m*-hydroxyphenylpropionic acid (67). Demethylation of hesperidin is required for the formation of these metabolites and it has been suggested that this reaction occurs in the intestine (58). It was subsequently shown that rat cecal microorganisms are able to convert this flavonoid to *m*-hydroxyphenylpropionic acid (61) (Scheme II).

The metabolism of a large number of phenolic acids by the rat microflora has been investigated (58, 123) and it was found that many compounds containing methoxyl groups underwent demethylation. This reaction has been found to occur with methoxy derivatives of benzoic, phenylacetic, phenylpropionic, and cinnamic acids (see Scheme III).



Scheme III—Metabolism of methoxy compounds by rat cecal microorganisms (58, 123).

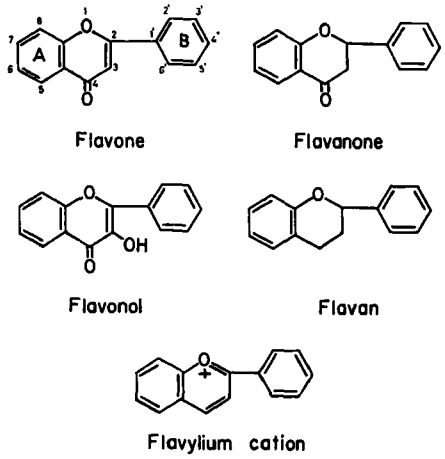


Fig. 1—Structural formulas of some of the major types of flavonoid compounds.

It is not unreasonable to assume that this reaction may be of significance in the metabolism of other classes of compounds including alkaloids, which often contain methoxyl groups. In this regard it is of interest to note that brucine is demethylated to strychnine derivatives in rabbits (125). However, the finding that the metabolites excreted in the urine were also excreted in the feces in nearly the same relative ratios, although in smaller amounts, suggests that the demethylation of brucine may have an intestinal origin.

Heterocyclic Ring Fission—Most studies of the fission of heterocyclic compounds by the intestinal microflora have been carried out with the flavonoids. These compounds make up a large and important group of phenolic substances which occur in all parts of higher plants. The flavone ring system and some of the major types of flavonoids are shown in Fig. 1.

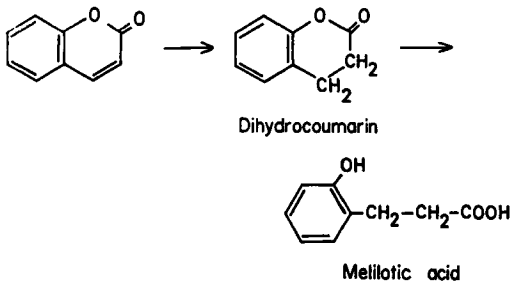
Flavonoids of the anthocyanin type occur widespread in nature as the pigments of fruits, flowers, and leaves. A cationic flavylium structure is common to these compounds and their aglycones, the anthocyanidins. Horwitt (126) found that the anthocyanin pigment from Concord grapes did not undergo loss of color when incubated with extracts of human feces. It appears that the intestinal microorganisms do not alter these flavylium compounds as cyanidin chloride (3,5,7,-3′,4′-pentahydroxyflavylium chloride) was not converted to phenolic metabolites when incubated with rat cecal microorganisms (61). The structural feature responsible for this stability appears to be the cation as (+)-catechin (3,5,7,-3′,4′-pentahydroxyflavan) is metabolized to *m*-hydroxyphenylpropionic acid by rat intestinal microorganisms (61, 70). Griffiths (127, 128) reported that the latter compound is a major urinary metabolite of (+)-catechin in rats and showed that its formation is dependent upon intestinal microorganisms.

Rutin, the glycoside of the flavonol quercetin (3,5,7,3′4′-pentahydroxyflavone), is perhaps the most frequently occurring flavonoid. It has been the subject of considerable investigation with regard to the ability of the flavonoids to modify capillary permeability (129). The metabolism of rutin by rat intestinal microorganisms was studied by Booth and Williams (70) who found that it was converted to *m*-hydroxyphenylpropionic acid. However, it was subsequently shown that *m*-hydroxyphenylacetic acid is also a product of rutin metabolism by rat intestinal microorganisms (61). Quercetin was metabolized similarly in these experiments. These findings are of interest because it is known that rutin and quercetin are excreted partly as phenylacetic acid and phenylpropionic acid derivatives when fed to rats (69, 130). The intestinal origin of these derivatives has also been demonstrated by indirect means as rats treated with neomycin to suppress the intestinal flora do not excrete them when fed quercetin (130).

Flavonoids of the flavanone type are illustrated by hesperidin and its aglycone hesperetin (5,7,3′-trihydroxy-4′-methoxyflavanone). Metabolism studies have shown that these compounds undergo ring fission when fed to rats and are excreted partly as phenylpropionic acid derivatives (67). It was recently reported that these two flavonoids are converted to *m*-hydroxyphenylpropionic acid by the rat intestinal flora (61).

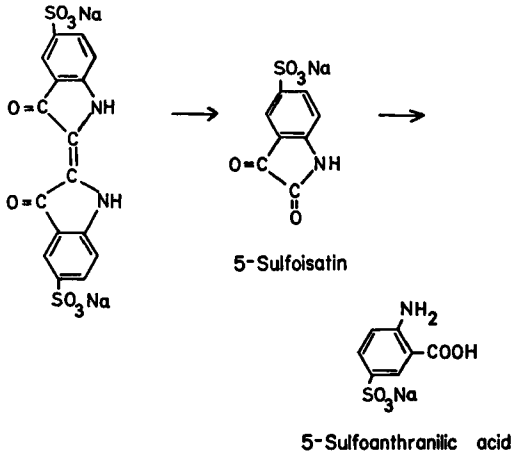
It therefore appears that ring fission by the intestinal microflora is a common reaction with heterocyclic oxygen compounds. With the flavonoids, it seems that a certain degree of hydroxylation of the heterocyclic ring system (A ring) is essential for fission as this reaction occurs to a very limited extent, if at all, with flavone itself (131). However, a hydroxylated aromatic ring may not be required in other classes and coumarin is converted to melilotic acid when incubated with rat cecal microorganisms (132) (Scheme IV).

Indigo carmine (sodium indigotin disulfonate) is metabolized to a small extent in rats to 5-sulfoisatin and further by heterocyclic ring fission to 5-sulfoanthranilic acid (133) (Scheme V). Incubation of this blue dye with rat intestinal contents resulted in fading of the color and in the appearance of the degradation products. It was suggested that these urinary metabolites may



Scheme IV—Metabolism of coumarin by rat cecal microorganisms (132).

arise partly from their absorption following the decomposition of the dye by intestinal bacteria.



Scheme V—Metabolism of indigo carmine by rat intestinal contents (133).

Heterocyclic ring fission by the intestinal bacteria has recently been shown to occur with tartrazine (134). When tartrazine labeled with ^{35}S in the *p*-sulfophenyl group of the pyrazolone moiety of the dye was given orally to rats, it was found that about 25% of the urinary sulfanilic acid was radioactive. Thus, this metabolite is produced partly by reduction of the 4-*p*-sulfophenylazo group and partly by degradation of the pyrazolone ring. The latter reaction was also found to occur when ^{35}S-tartrazine was incubated with rat intestinal contents. Small amounts of *p*-sulfophenylhydrazine were found in both experiments and it was suggested that the pyrazolone ring was cleaved to the hydrazine derivative which then underwent reductive fission to sulfanilic acid (Scheme VI).

Splitting of the β-lactam ring in penicillin has been mentioned in connection with the hydrolysis of amides.

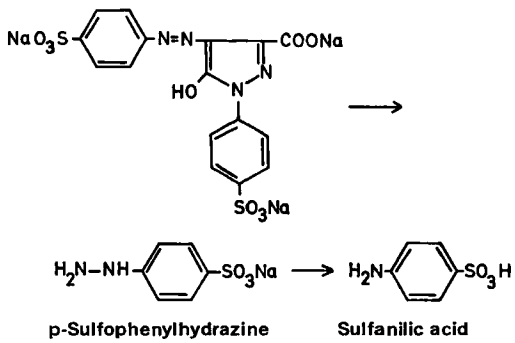
Reduction of Double Bonds—The reduction of double bonds by intestinal microorganisms has been most widely studied in connection with the metabolism of unsaturated fatty acids in the rumen and lower intestine. A recent report by Wilde and Dawson (135) described the hydrogenation of linolenic acid and oleic acid by sheep rumen microorganisms. Hydrogenation was carried out by a mixed population of microorganisms but not by several pure cultures of fecal bacteria including *Clostridium perfringens, S. faecalis, E. coli,* and a coliform organism.

Ricinoleic acid, a component of castor oil, is hydrogenated to hydroxystearic acid in both the rat and human intestine (136). This reaction is modified when neomycin is given with the castor oil so that much less hydroxystearic acid is found in the feces. This indicates that the conversion is a result of intestinal bacterial activity.

Booth and Williams (108) found that caffeic acid was partially reduced to 3,4-dihydroxyphenylpropionic acid when incubated with rat or rabbit cecal contents. A recent study of the metabolism of a number of phenolic acids by the rat cecal microorganisms showed that reduction of cinnamic acid derivatives was a major reaction of these compounds (58). This study included *o*-, *m*-, and *p*-coumaric acids, caffeic acid, ferulic acid, and isoferulic acid and reduction to the corresponding hydro derivatives was observed with all of the compounds.

Fecal microorganisms from monkeys have been shown to hydrogenate indolylacrylic acid and cinnamic acid to the corresponding propionic acid derivatives (137).

Nitro Reduction—The significance of the intestinal bacteria in the reduction of nitro groups has been recognized for some time. Studies on the metabolism of chloramphenicol in rats showed that parenteral administration resulted in the excretion of large amounts of inactive nitro compounds in the bile (138). These were partly reduced in the intestine to aryl amines which were excreted in the feces. Reduction of the nitro



Scheme VI—Metabolism of tartrazine by rat intestinal contents (134).

group in chloramphenicol has been shown to be carried out by several microorganisms including the common intestinal inhabitants *E. coli* and *P. vulgaris* (84, 139). It was subsequently found that rat cecum contents and *E. coli* hydrolyzed and reduced chloramphenicol glucuronide to aryl amines (57). The properties of an enzyme system from *E. coli* which will reduce the nitro groups in chloramphenicol and a number of simple organic nitro compounds have been reported (140, 141). This system differs in several respects from that occurring in rabbit liver and kidney which reduces the nitro groups of organic nitro compounds including chloramphenicol (142).

Bray *et al.* (143) studied the metabolism of tetrachloronitrobenzenes in the rabbit and found that some of the major metabolites were reduced in the intestine. They found that several nitro compounds including 2,3,4,5-tetrachloronitrobenzene, nitrobenzene, *m*-nitrophenol, *o*-, *m*-, and *p*-nitrobenzoic acid and their amides were readily reduced when incubated with rabbit intestine contents. *p*-Nitrophenol and *o*- and *p*-nitrotoluene were reduced very slowly. They concluded that nitro reduction by the intestinal bacteria is a factor which should be considered when the fate of aromatic nitro compounds is studied. This is especially important when the compounds are slowly absorbed as it is possible that virtually all the reduction observed may occur in the intestine prior to absorption.

The herbicide trifluralin (2,6-dinitro-*N*,*N*-di-*n*-propyl-α,α,α-trifluoro-*p*-toluidine) is poorly absorbed in rats following oral administration and nearly 80% of the dose is excreted in the feces (144). Both trifluralin and its monoamino derivative are prominent fecal metabolites. The latter compound was not detected in the urine although the corresponding compounds lacking one or both of the *N*-propyl groups were found. This suggests that trifluralin may be reduced in the intestine and the resulting amino metabolite absorbed and then dealkylated in the liver.

Azo Reduction—The reduction of azo compounds by the intestinal microflora has received considerable attention in recent years. Most investigations have dealt with water-soluble azo dyes containing sulfonic acid groups which are widely used as food coloring agents but the oil-soluble dyes have also been studied.

An early indication of the significance of the intestinal microflora in the metabolism of azo dyes was the finding that sodium 4-*p*-sulfophenylazo-1-naphthol was rapidly destroyed when incubated with dog intestinal contents (145). However, the nature of the breakdown products was not reported. Radomski and Mellinger (146) studied the fate of the disulfonated dyes amaranth [1-(4-sulfo-1-naphthylazo)-2-naphthol-3,6-disulfonic acid trisodium salt], Ponceau SX [2-(5-sulfo-2,4-xylylazo)-1-naphthol-4-sulfonic acid disodium salt], and sunset yellow (1-*p*-sulfophenylazo-2-naphthol-6-sulfonic acid disodium salt) in rats (see Fig. 2). They demonstrated that the liver azo reductase system is relatively unimportant in the metabolism of these compounds. However, the dyes were found to be nearly completely reduced to sulfonated amines by the intestinal bacteria. The reduction of amaranth is shown in Scheme VII. These metabolites were then absorbed from the intestine and excreted in the urine. Similar findings were obtained by Jones *et al.* (147) who studied the metabolism of tartrazine (3-carboxy-5-hydroxy-1-*p*-sulfophenylazopyrazole trisodium salt) in the rat, rabbit, and man (Scheme VII).

Acid yellow (4-aminoazobenzene-3,4′disulfonic acid disodium salt) has also been found to undergo fission of the azo linkage in rats following oral dosage (148) (Scheme VII). Azo reduction was not observed when the dye was given by injection. Acid yellow was shown to be readily reduced by extracts of rat feces and a strain of *S. faecalis* isolated from feces (61, 148).

Brown FK, which consists of a mixture of *p*-sulfophenylazo derivatives of 2,4-diaminotoluene and 1,3-diaminobenzene, underwent reductive fission to give sulfanilic acid, a phenazine-

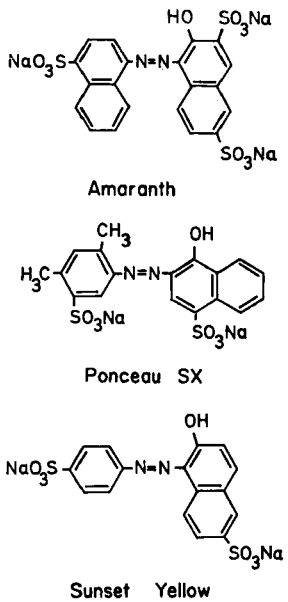
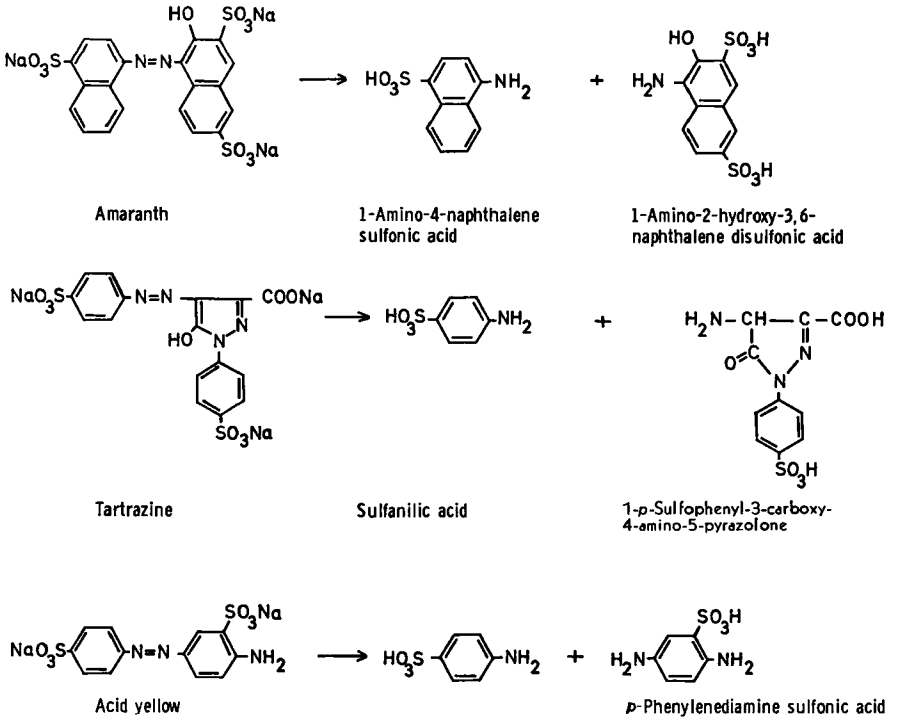


Fig. 2—Structural formulas of some water-soluble azo dyes.



Scheme VII—Reduction of azo dyes by rat intestinal microorganisms (146–148, 150).

like material and ill-defined products when incubated with rat intestinal contents (149). Several cecal microorganisms including *S. faecalis* were reported to be capable of decolorizing this dye.

Roxon *et al.* (150) reported that tartrazine was reduced when incubated with a *Proteus sp.* isolated from rat intestine. This organism was also able to reduce a number of other water-soluble azo dyes (151). Further studies on the mechanism of this reaction have shown that a soluble NADPH-dependent, FMN-flavoprotein is involved (152). It is probable that tartrazine and other azo dyes act as alternate electron acceptors for the respiratory chain under anaerobic conditions. It is of interest to note that the reduction of tartrazine occurs rapidly with cell extracts of *P. vulgaris* but not with freshly harvested microorganisms (150, 152). Reducing activity is present in aged cells and this is apparently due to permeability changes in the cell wall. However, it is known that intestinal microorganisms rapidly reduce azo dyes including tartrazine (148, 151) and it seems likely that much of the reduction that occurs when azo dyes are given orally to animals may be due to other intestinal microorganisms including *S. faecalis*.

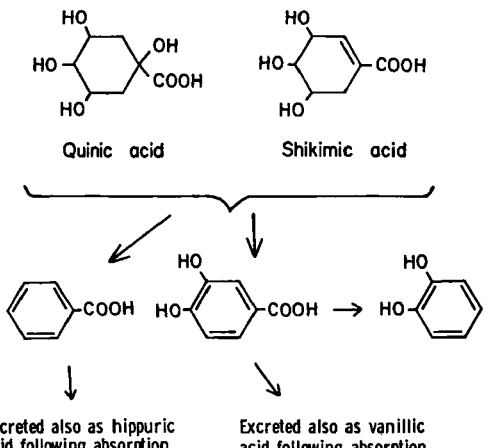
Oil-soluble azo dyes are also reduced by intestinal microorganisms. Childs *et al.* (153) found that 1-phenylazo-2-naphthol was reduced when incubated with rat intestinal contents or with *E. coli*, *A. aerogenes*, and *B. proteus* isolated from rat intestine. Similar results were obtained with methyl red (4-dimethylaminoazobenzene-2′-carboxylic acid) which was reduced to its component amines, *o*-aminobenzoic acid and *N,N*-dimethyl-*p*-phenylenediamine, when incubated with rat cecal microorganisms (61).

Aromatization—The reaction whereby nonaromatic cyclic compounds are converted to aromatic substances has been known for more than a century (154). Quinic acid (1,3,4,5-tetrahydroxycyclohexanecarboxylic acid), a common constituent of many fruits and vegetables, has been found by several investigators (154–159) to be metabolized to benzoic acid or hippuric acid. This is a major metabolic reaction of quinic acid in man and about 70% of the ingested dose is excreted as hippuric acid (158). Likewise, 30 to 60% of an oral dose is converted to hippuric acid in guinea pigs (157, 158). However, no conversion was found to occur when quinic acid was given to guinea pigs by subcutaneous injection (156, 158).

The differences in quinic acid aromatization following different routes of administration together with the knowledge that some strains of *A. aerogenes* could utilize quinic acid in aromatic biosynthesis (160) suggested that the intestinal bacteria might be involved in this reaction (158)

The role of the microflora was confirmed as inhibition of bacterial multiplication in the intestine with neomycin was found to prevent the conversion of quinic acid to hippuric acid in man (158). This finding was subsequently confirmed by Asatoor (159) using rats. An even greater rise in urinary hippuric acid excretion occurred when shikimic acid (3,4,5-trihydroxy-1-cyclohexene-1-carboxylic acid) was fed and this conversion was also prevented by neomycin (159).

Quinic acid and shikimic acid have also been found to increase the urinary catechol excretion when given orally to rats maintained on a purified diet (161). In addition, vanillic acid was detected in the urines. The conversion of quinic acid to catechol was subsequently shown to be carried out by rat intestinal microorganisms (70). It has been proposed that these polyhydroxy acids can be converted by the intestinal microflora to 3,4-dihydroxybenzoic acid which can be partly absorbed and methylated to give vanillic acid and partly decarboxylated in the intestine to give catechol which can then be absorbed (110). This suggestion is supported by the finding that shikimic acid is metabolized to protocatechuic acid when incubated with rat cecal microorganisms (61). These pathways are summarized in Scheme VIII.



Scheme VIII—Proposed pathways in the metabolism of quinic and shikimic acids.

Dehalogenation—The metabolism of DDT [1,1,1-trichloro-2,2-bis (*p*-chlorophenyl)-ethane] in higher animals is of considerable interest due to its widespread use as an insecticide. DDT is partly metabolized by reductive dechlorination to DDD [1,1-dichloro-2,2-bis (*p*-chlorophenyl)-ethane] when given orally to animals and it has been believed that this reaction occurs in the liver (162–164). However, Barker and Morrison (165) showed that DDD is produced from injected DDT in decomposing animal tissue but not in living tissues. It was subsequently found that a strain of *P. vulgaris* isolated from mouse intestinal contents could carry out this reaction (166). As this organism is one of the primary invaders of animal tissues after death, it was concluded that it, in part at least, was responsible for the conversion of DDT to DDD in aminals which have been killed by DDT.

The conversion of DDT to DDD was also studied by Mendel and Walton (167) who found that DDD was formed after oral but not intraperitoneal administration of DDT to rats. This indicated that DDT must pass through the gastrointestinal tract for the formation of DDD to occur. Furthermore, they showed that *A. aerogenes* and *E. coli* isolated from rat intestine readily carried out the dechlorination of DDT. These findings indicated that the gastrointestinal flora must be the main agent for the formation of DDD and that the conversion takes place during the life of the animal. The intestinal microflora has been shown to play a major role in DDT detoxication in the rainbow trout, *Salmo gairdneri* (168).

Recently, Wedemeyer (169–171) carried out an extensive investigation of the anaerobic dechlorination of DDT by *A. aerogenes*. He found that whole cells or cell-free extracts could catalyze the degradation of DDT to at least seven metabolites. The terminal steps of this sequence led to the formation of DDA [2,2-bis (*p*-chlorophenyl) acetic acid] and finally to 4,4′-dichlorobenzophenone.

Other Reactions—The reduction of ketones by the intestinal flora has been shown to occur. Stimmel (60) found that estrone was converted to a large extent to β-estradiol when incubated with human feces. Reduction of the 3-keto group in bile acids was reported to be carried out by mixed cultures of anaerobic fecal microorganisms (99).

Studies on the metabolic fate of diethyltin dichloride in rats have shown that it is dealkylated and that this reaction occurs both in the tissues and in the intestine (172). Also, it was shown that the conversion of diethyltin to ethyltin could take place when the former was incubated with rat cecal contents.

Diquat and paraquat are bipyridilium compounds which are used as herbicides. Their metabolic fate in rats has been investigated (173) and it was found that appreciable amounts were excreted in the feces in a degraded form. Small amounts of these products were absorbed from the intestine and excreted in the urine. Incu-

bation of diquat and paraquat with a rat fecal homogenate resulted in extensive degradation of the compounds. However, the nature of these products was not determined.

The metabolism of chloramphenicol by intestinal bacteria has been cited previously in connection with amide hydrolysis and nitro reduction. However, this substance has also been found to undergo oxidation of the secondary hydroxyl group and cleavage of the propanediol portion of the molecule when incubated with strains of several intestinal bacteria (84).

Asatoor and Simenhoff (174) have demonstrated that urinary dimethylamine is derived partly from ingested choline and lecithin and that this conversion is dependent on the breakdown of choline to trimethylamine by intestinal bacteria.

Deamination by the intestinal flora is known to occur with amino acids (175, 176). Rat intestinal bacteria can deaminate and decarboxylate triiodothyronine to triiodothyroacetic acid (72). Tyrosine, phenylalanine, and tryptophan undergo deamination by sheep rumen microorganisms (116) and dopa is deaminated by rat fecal and cecal extracts (70). The deamination of tryptophan, histidine, and phenylalanine to give the corresponding acrylic acid derivatives has been reported to be carried out by bacteria from monkey feces (137). Also, 5-fluorocytosine was deaminated to 5-fluorouracil by the intestinal flora in rats (177).

The number of reports dealing with synthetic intestinal reactions is limited. However, it appears that both acetylation and esterification reactions can be carried out by the intestinal bacteria. Histamine was found to be partly metabolized to *N*-acetylhistamine when incubated with human feces suspensions (83). There is also indirect evidence which suggests that sulfanilic acid and *p*-phenylenediamine sulfonic acid, the fission products of acid yellow, are partly acetylated in the intestine (148). Fecal bacteria have been reported to esterify coprostanol and cholesterol (178).

CONCLUSIONS

The findings presented above amply demonstrate that the gastrointestinal microorganisms can play a significant role in determining the metabolic fate of drugs and other organic compounds. The diversity of the intestinal reactions is impressive, although hardly surprising in view of the tremendous range of metabolic capabilities found in microorganisms. The intestinal reactions generally have a common characteristic in that they involve degradative processes. Thus, the various compounds usually undergo reduction in molecular size. These processes very often alter the physicochemical properties of the compound to a considerable extent. Examples of this are seen in the deconjugation of glucuronides and glycosides, reduction of nitro or azo compounds, and decarboxylation of phenolic acids. It is of great significance that these alterations may be accompanied by increases in biological activity or toxicity. The above examples may lead to the liberation of an active aglycone, production of a toxic arylamine, or formation of a more toxic phenol. These reactions often contrast markedly with those occurring in the tissues where the metabolic sequence leads ultimately to products of lower biological activity.

Many aspects of the study of the metabolism of drugs by the intestinal microflora have received only very limited attention. For example, relatively few reports have been published which correlate particular intestinal reactions with particular bacterial species. However, much of this work may depend on more satisfactory methods for the culture of some of the more fastidious intestinal inhabitants. Differences in the metabolic fate of some compounds may be a reflection of differences in the microflora. This may occur among different animal species and, in this regard, it is interesting to note the previously mentioned differences in the flora of the rabbit compared with other common species. Also, the differences may occur among a single species as a result of nutritional or environmental factors.

It is sincerely hoped that the present review may serve to stimulate interest in the study of drug metabolism by the gastrointestinal microorganisms as this has truly been a neglected aspect in the field of drug metabolism. However, its significance in the metabolism of many compounds demands that this factor must be given adequate attention in the future.

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Keyphrases

Drug metabolism—intestinal microorganisms
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