PHARMACOLOGICAL REVIEWS
Vol. 25, No. 4

Printed in U.S.A.

Metabolism of Foreign Compounds by Gastrointestinal Microorganisms

RONALD R. SCHELINE

Department of Pharmacology, School of Medicine, University of Bergen, MFH-Bygget, 5000-Bergen, Norway

I. Introduction

THE study of the metabolic fate of foreign compounds has a history which can be traced back to the time of some of the earliest discoveries in organic chemistry and biochemistry. During most of the subsequent period, investigations in the whole animal have dominated although the use of various tissues and tissue preparations, especially of the liver, has been widespread in recent decades. The emphasis in these studies has been placed nearly exclusively on the metabolism of compounds in the animal tissues and little interest has been shown in the metabolic activities of the microorganisms harbored in the gastrointestinal tract in relation to foreign compound metabolism. However, the metabolic capacity of these microorganisms can be very large and foreign compounds may be exposed quite easily to these effects. This may occur after oral administration and especially when absorption is retarded or it may take place as a result of diffusion or secretion of the compound or its metabolites into the gastrointestinal tract. Until recently there appears to have been a reluctance to accept these possibilities and to investigate the microbial metabolism of foreign compounds. While this hesitancy and therefore the neglect of one of the many facets of the discipline of foreign compound metabolism is sometimes still to be seen, we have nonetheless witnessed a surge of interest in this area during the past decade. During this period an impressive variety of reactions has been reported and the description of these metabolic pathways formed the basis of the subject when microbial drug metabolism was initially reviewed (348). However, the intervening period of nearly 5 years, in addition to furnishing examples of new types of metabolic reactions, has seen a rapid growth in the awareness that foreign compound metabolism by gastrointestinal microorganisms may have important implications. It has become increasingly obvious that the gastrointestinal metabolism of compounds

may have appreciable effects on their absorption, distribution, and excretion as well as on their biological properties. The latter point has received an increasing share of interest due to its toxicological significance (350, 381, 443a). The present review reflects this recent expansion of the subject although the central theme concerns the types of metabolic reactions which occur with drugs and other foreign compounds by gastrointestinal microorganisms. While the purely microbiological aspects are obviously an integral part of this subject, this area has purposely been treated in less detail than the biochemical and pharmacological features.

II. Gastrointestinal Microflora

A. Nature of the Gastrointestinal Microflora

Although studies of the normal microbial flora of the gastrointestinal tract date back to the early part of this century, this area of microbiology has historically received less attention than that dealing with pathogenic microorganisms. Although a major contributing factor to this difference has been the lack of suitable methods for cultivating many members of the normal microflora, advances made in recent years have overcome many of these problems. As a consequence, a significant expansion in this field has resulted and a fairly extensive literature is now available. It is felt, however, that no essential purpose would be achieved in the present review by covering this topic in detail. Instead, brief mention will be made of some of the important characteristics of the gastrointestinal microflora. In addition, sufficient literature references are included so that the interested reader may pursue this area in greater depth.

Any discussion of the microbial inhabitants of the gastrointestinal tract must, at the outset, note the great variety of species normally present. In fact, more than 60 different species have been isolated from the intestinal tract or feces of animals or man (115). While many of these are normally only minor inhabitants, the list of those microorganisms which are found commonly in moderate to high numbers is still fairly extensive. Nonetheless, much recent evidence has made it clear that the dominant intestinal bacteria in most animals are members of the genera *Bacteroides* and *Bifidobacterium* (anaerobic lactobacilli) (117, 124, 127, 165, 184, 375, 397). Recent studies of the mouse cecal flora have indicated that members of genus *Fusobacterium*, which are extremely sensitive to oxygen, are also abundant (243, 397). However, in spite of the use of improved techniques for maintaining anaerobic conditions, a considerable portion of the cecal microflora has still not been cultivated (6, 387). In any case it should be noted that the common Gram-negative enterobacteria, the enterococci, and the clostridia represent only a small part of the microflora. The aerobic flora of human feces consists mainly of coliforms, streptococci, and lactobacilli which account for 5% or less of the total bacterial population whereas clostridia, staphylococci, aerobic spore formers, proteus, and yeasts account, when demonstrable, for less than 0.001% (185). The types of microorganisms which are invariably or often found in the gastrointestinal tracts of animals are listed in table 1. Further information on the nature of the gastrointestinal microflora is available in the following articles: general—56a, 115, 124, 127, 164, 184, 320, 445; numerous animal species—48, 119, 375, 376, 378; human—49, 122, 149, 165, 166, 222, 223; rat—313, 314; mouse—126, 167, 243, 397; hamster—186; piglets—104; rumen—57.

B. Distribution of Microorganisms in the Gastrointestinal Tract

In general, the upper parts of the human gastrointestinal tract including the stomach, duodenum, jejunum, and upper ileum are sparsely populated with microorganisms. Gastric acidity seems to be an important factor in determining the nature of the stomach flora; acid-resistant microorganisms including streptococci, aerobic lactobacilli, and fungi are the main inhabitants (164). The acidic stomach contents of man often

TABLE 1
Typical representatives of the gastrointestinal microflora

Microorganisms	Description
Bacteriodes	Gram-negative, strictly anaerobic, non-spore forming rods
Lactobacilli	Gram-positive rods
1. Anaerobic (*Bifidobacterium*)	
2. Aerobic	
Fusobacterium	Gram-negative, strictly anaerobic, spindle-shaped rods
Enterobacteria	Gram-negative, aerobic or facultatively anaerobic, non-spore forming rods
1. *Escherichia coli*	
2. *Aerobacter*	
3. *Klebsiella*	
4. *Proteus*	
5. Providence group	
Clostridia	Gram-positive, anaerobic, spore-forming rods
Streptococci	
1. Enterococci	Gram-positive, aerobic or facultatively anaerobic cocci
2. Anaerobic	Gram-positive, anaerobic cocci
Pseudomonads	Gram-negative, aerobic motile rods
1. *Pseudomonas*	
2. *Alcaligenes faecalis*	
Staphylococci	Gram-positive, aerobic or facultatively anaerobic cocci
Veillonella	Gram negative, anaerobic cocci
Yeasts	

have been reported to be sterile but the above-mentioned microorganisms may be carried down from the oral cavity during meals (122). However, small numbers of them have been reported even in fasting individuals (163). The numbers of microorganisms in the anterior part of the stomachs of numerous animal species are higher than those in the posterior part (375). It was also pointed out that the rabbit, unlike other homothermic animals, has a stomach that is usually sterile or populated only by bacteroides. This genus and the bifidobacteria were later reported to be the sole members of the stomach microflora in rabbits (119).

The upper small intestine in man is sparsely populated with a flora consisting largely of Gram-positive, facultative microorganisms (164). Other investigations have found coliforms (48) and sometimes bacteroides (119) but they have also stressed the lack of a large microbial population in this region of the intestine. Of the common laboratory animals, the rabbit is more comparable to man in that relatively few types of microorganisms are present (119). These are mainly bacteroides and bifidobacteria (119, 375). In contrast, these investigations showed that a much more varied flora similar to that shown in table 1 is present in the proximal intestine of other species including the common domestic and laboratory animals.

Although the lower small intestine in man may be sparsely populated with microorganisms, this region generally shows an abundant flora which is similar to that found in the common laboratory animals (119). The concentrations of organisms in the distal ileum, with its larger numbers of anaerobic forms, are intermediate between those found in the upper gastrointestinal tract and the large intestine and this region can be considered as a transitional zone (164, 166). A pattern of increasing numbers of intestinal bacteria as the lower small intestine is reached was found to be general in many animal species although the rabbit still shows a flora made up of fewer types and numbers of microorganisms (119, 375).

A significant change in the intestinal microflora in man is seen below the ileocecal valve. This is manifested by an increase in the anaerobic microorganisms which outnumber the aerobic and facultative flora by a factor of 10^3 to 10^4 (164). Thus the microflora of the human large intestine consists

predominantly of bacteroides and bifidobacteria (119) with lesser contributions being made by other types, mainly those listed in table 1. The large intestine is, of course, the site of the most abundant microbial flora in the gastrointestinal tracts of most other animals. In general, the types of microorganisms are similar to those found in man. Ruminants are a notable exception to this general picture with a large fermenting bulk located in the ruminoreticulum which is found between the esophagus and stomach (abomasum).

While the above remarks deal with the longitudinal distribution of microorganisms in the intestinal tract, it should also be noted that variations in the cross-sectional distribution occur. Thus, it has been demonstrated recently that many bacteria are associated with the mucosa of the gastrointestinal tract (128, 280, 305, 339). These associations, which appear to be quite stable, have been observed in several mammalian species including man. The microorganisms have not been found in the gastrointestinal wall itself.

C. Variations in the Gastrointestinal Microflora among Different Animal Species

In general the gastrointestinal microfloras in mammals and other homothermic animals are remarkably similar (375). Nevertheless, both qualitative and quantitative differences have been reported with the most striking examples being between man and other animals rather than between members of the latter group. As pointed out in section II B, many of these dissimilarities are related to differences in the types and numbers of microorganisms found in different parts of the gastrointestinal tract. Man has a microflora mainly in the lower part of the tract whereas some animals and especially rats and mice have large microbial populations also in the upper parts. Here feeding habits including coprophagy are important although, in the case of rabbits, the latter practice does not appear to influence the nature of the microflora (375). Further information is available in the articles listed at the end of section II A.

D. Influence of Diet on the Gastrointestinal Microflora

While it is well known that the gastrointestinal microflora in animals and man remains approximately constant under stable conditions, it is also recognized that considerable variations, both quantitative and qualitative, can result from environmental and physiological changes (127). It has long been realized that the nature of the diet may be important in this regard (115). However, many of the available results have been obtained from studies in which gross alterations in the composition of the diet have been made. For this reason these findings may have relatively little significance for many investigations with animals maintained on a standard and stable diet. On the other hand, switching to a simpler type of diet, *e.g.*, one based upon casein, sugar, oil, salts, and vitamins, has been a fairly common practice in many investigations when it is necessary to reduce the amounts and numbers of urinary metabolites arising from dietary sources. The present state of knowledge of the influence of dietary factors on the intestinal microflora, as covered briefly below, does not permit the development of a system of guidelines which could be of use when studying the intestinal metabolism of foreign compounds. There is obviously a need for more information in this area.

A decrease in the number of lactobacilli and an increase in the numbers of coliforms and enterococci were shown to occur when diets containing a high proportion of meat protein (307) or casein (125) were fed. Similar findings have been reported in rats fed raw pork or casein compared with an ordinary pellet diet and it was also found that the meat diet greatly increased the numbers of *Clostridium welchii* throughout the alimentary tract (375). The latter investigation also demonstrated that the lactobacilli population was greatly decreased

when diets lacking cereals were fed. In addition, yeasts were numerous when cereals were given whereas they were practically absent in rats fed high protein diets. On the other hand, the fecal flora of rats maintained on diets containing 40% corn oil or butter did not differ appreciably from that found in animals given a normal pellet diet (168). The only noteworthy change was with *Clostridium perfringens* which was often seen when high fat diets were fed whereas it was usually absent from the feces of rats on the pellet diet. Interestingly, changes in the diet of rabbits have not produced appreciable changes in the microflora (375). Animals fed *ad libitum* on raw cabbage or dried milk showed similar fecal bacterial counts to those from animals maintained on an ordinary commercial ration, except that the rabbits fed dried milk developed a considerable population of lactobacilli. The composition of the diet markedly influences the nature of the microflora in the chick (376). Large differences have been noted in the gastrointestinal flora of suckling and early-weaned piglets (104). The major differences were the reduction in the numbers of lactobacilli, the increase in coliforms and the predominance of strict anaerobes in the upper intestine of the piglets fed an artificial liquid diet. A related point of interest concerns the spacing of feedings and it has been suggested that much greater fluctuations in the upper intestinal microflora must occur when well spaced meals are given compared with *ad libitum* feeding (375).

Studies in man indicate that, except for breast feeding which promotes an intestinal microflora consisting nearly entirely of the anaerobic *Lactobacillus bifidus*, drastic changes in the microflora do not take place after nutritional alterations (185). It was shown, for example, that the type of diet among four groups of subjects maintained on meat and eggs, milk and vegetables, vegetables, or raw vegetables could not be deduced from the resulting fecal microflora. The effects on the fecal microflora of individuals placed on highly defined, highly digestible, and highly absorbable diets were found to be small and the dietary changes could not account for other variabilities seen in the flora during the study (388). Individual variations which were sometimes quite pronounced were also noted. In this regard it has been found that a standard diet containing a high proportion of carbohydrate did not produce a standardization of the microflora (165). The variations in fecal bacterial counts in individuals eating the standard diet were similar to those on random, unsupervised diets.

1. Effects of starvation. The use of fasted animals in metabolic studies is a fairly common practice and it should therefore be noted that changes in the gastrointestinal flora may occur as a result of this treatment. Results obtained from studies with rats starved for 24 hr have indicated a variable but sometimes extensive alteration in the numbers of some gastrointestinal microorganisms (375).

2. Effects of coprophagy. The effects of the prevention of coprophagy on the microflora in guinea pigs, rabbits, and rats for periods of up to 14 days have been investigated (375). The practice was found to have little influence on the qualitative and quantitative composition of the microflora in rabbits and rats, although the numbers of lactobacilli in the upper parts of the gastrointestinal tract in the latter animals were slightly lower. However, prevention of coprophagy in rats for 4 weeks was earlier reported to result in a large decrease in the numbers of these organisms in cecal contents and feces (181). It was shown subsequently that the marked decrease in the numbers of fecal lactobacilli began very soon after coprophagy was prevented (141). On the other hand, the counts of enterococci and coliform organisms were increased. With the exception of the disappearance of anaerobic lactobacilli within a few days, the fecal bacterial counts were unchanged in guinea pigs when coprophagy was prevented (375).

E. Influence of Age on the Gastrointestinal Microflora

The lumen of the gastrointestinal tract, which is sterile in the fetus, begins to be colonized shortly after birth. The development of the microflora in breast-fed infants and in infants after weaning has long been a topic of interest (see 115, 445), especially in regard to the anaerobic lactobacilli. *L. bifidus* constitutes the bulk of the microflora in breast-fed infants whereas bottle-feeding promotes a more varied microflora which includes *Lactobacillus acidophilus*, enterococci, coliform organisms, and non-spore forming anaerobes. Studies on the fecal microflora of some domestic animals and man after birth showed a general similarity during early life (378). Subsequently, larger differences, especially of a quantitative nature, were noted. The initial colonization was carried out by *Escherichia coli*, *Cl. welchii*, and certain types of streptococci which later decreased in numbers as lactobacilli and bacteroides emerged as the dominant bacterial inhabitants. This investigation was extended subsequently to include the development of the microflora of different parts of the gastrointestinal tract among a wider variety of domestic and laboratory animals (376). It was found that lactobacilli were generally the most common inhabitants of the stomach and small intestine whereas bacteroides, after a longer period of colonization, were mainly restricted to the large intestine where they were the principal inhabitants. Other studies on the development of the gastrointestinal microflora in mice (127) and rats (314) have appeared.

The nature of the human fecal microbial flora was investigated in groups of individuals ranging from 20 to 100 years of age (165). Elderly persons were found to harbor fewer anaerobic lactobacilli and more coliform organisms and fungi than younger individuals.

F. Influence of Disease on the Gastrointestinal Microflora

Mention has been made above of the relatively sparsely populated upper gastrointestinal tract in man. However, a proliferation of bacterial growth occurs in certain disease conditions and the microflora will then come to resemble that seen, for example, in the rodent. Conditions which give rise to intestinal stasis and thus encourage the growth of the bacterial flora in the region proximal to the affected area may be caused by blind loops, abnormal intestinal motility or strictures arising from Crohn's disease, tuberculosis, or congenital conditions (399, 400). In addition, abnormalities in gastric function may also encourage bacterial growth in the intestine.

G. Influence of Foreign Compounds on the Gastrointestinal Microflora

It is common knowledge that treatment with antimicrobial drugs may also affect the gastrointestinal microflora, sometimes producing gastrointestinal disorders. Likewise, these compounds have been used widely in animal feedstuffs for their growth promoting effects (253, 377). The effects of antimicrobial substances on the gastrointestinal microorganisms have been briefly summarized (115, 136) and it was concluded that the interrelationships between the compounds and the microflora may be very complex. However, the main effects on the human intestinal flora produced by a large number of antimicrobial substances have been conveniently summarized (136). These results indicate that selective modification of the microflora is feasible. Anaerobic bacteria can, for example, be largely eliminated with little to no change in the aerobes with lincomycin whereas the opposite result can be largely achieved with kanamycin and related aminoglycosides. However, the results from this type of experiment are not always uniform and the latter group of antibiotics may also reduce the numbers of

Bacteroides. This genus is not affected by neomycin *in vitro* but the oral treatment with this compound of rats with blind loop significantly reduced the numbers of *Bacteroides* (270). This was thought to be brought about by a change in the environmental conditions in the intestinal lumen as a result of the reduction of aerobic bacteria. In mice reared under conditions that promote a simpler flora containing large numbers of lactobacilli, the floral pattern was very sensitive to the effects of orally administered penicillin, oxytetracycline, or chloramphenicol (129). These antibiotics brought about the disappearance of the lactobacilli whereas the numbers of enterococci and Gram-negative bacilli were greatly increased. Similar but smaller changes were found in normally maintained mice which have a more complex flora.

Opportunities also exist for altering the composition of the gastrointestinal flora with compounds other than the antibiotics and related drugs mentioned above. Oral administration of lactulose, a sugar that is very poorly absorbed from the intestine, has been shown to have this effect (200–202). The total numbers of fecal bacteria were reduced and this decrease was seen with the populations of *E. coli* and *Bacteroides*. In contrast, the numbers of lactobacilli and bifidobacteria showed a pronounced increase. However, a lactulose feeding study by other investigators has failed to show any alteration in the pattern of the fecal microflora (75). The chronic feeding of the carcinogen *N*-hydroxy-*N*-2-fluorenylacetamide resulted in the alteration of the cecal microflora in rats (440) (see section III G 2). The main findings were a proliferation of yeasts and the nearly complete absence of coliform organisms. Daily administration of cyclamate to rats was shown to greatly increase the numbers of clostridia found in the feces and this organism is apparently responsible for the conversion of cyclamate to cyclohexylamine in this animal species (120) (see section III E).

III. Metabolic Reactions

A. Hydrolysis of Glycosides

1. Glucuronides. Perhaps the best known example of the bacterial metabolism of foreign organic compounds in the intestine is that observed with glucuronide conjugates. Many investigations have directly or indirectly shown that these compounds are hydrolyzed by the intestinal bacteria. It is of interest to note that this reaction distinguishes itself from most of the others described in section III in that the compound metabolized usually is produced endogenously. While the aglycones themselves may have diverse origins and include synthetic, plant, or endogenous substances or metabolites of these, the deconjugation involves the loss of a moiety which, in most cases, has been furnished by the animal itself. As the formation of glucuronides are often major metabolic reactions which occur with many types of compounds, it can be readily appreciated that their hydrolysis by the intestinal bacteria is likely to be one of the most common and most important of the gastrointestinal reactions. However, for this to be so the glucuronide must first be transported from its site of formation to the intestinal lumen. In many cases this may be accomplished by the biliary excretion of the conjugate; extensive studies, mainly during the past decade, have demonstrated the significance of this excretory route. After the hydrolysis of the conjugate the liberated aglycone may be reabsorbed from the intestinal lumen and thereby establish an enterohepatic circulation of the compound. Thus, the intestinal microorganisms often play a vital role in this phenomenon. While glucuronide hydrolysis is usually the reaction involved, other metabolic pathways may also be encountered. In view of this, the subject of the intestinal metabolism of compounds in relation to enterohepatic circulation will be treated separately (section VI). Many examples of the intestinal hydrolysis of glucuronides which serve primarily to illustrate this phenomenon there-

fore are covered only in section VI. The present section is devoted mainly to results which show the hydrolysis of glucuronides by mixed or pure cultures of intestinal bacteria and to a discussion of the bacteria known to carry out this reaction.

The ability of mixed or pure cultures of intestinal bacteria to hydrolyze glucuronides has been known for many years. Some early reports indicated that some strains of *Staphylococcus albus* could hydrolyze pregnanediol glucuronide (19) and that some strains of *E. coli* contained β-glucuronidase activity (58). The glucuronide conjugate of thyroxine was found to be hydrolyzed by contents of the lower small intestine or large intestine of rats (404). Estriol glucuronide was converted to free estriol when incubated with human feces (394). Experiments with chloramphenicol glucuronide injected into isolated segments of rat intestine showed that hydrolysis occurred in the cecum and large intestine but not in the jejunum (157). Liberation of chloramphenicol from the glucuronide was shown in incubates containing rat cecal contents, human feces, or suspensions of *E. coli* or *Aerobacter aerogenes*. A survey of β-glucuronidase activity in the digestive tracts of numerous animal species showed that high activity was invariably present in the colon and cecum from sheep, cow, horse, rabbit, rat, pig, cat, and fowl (256). While activity was low or lacking in the stomach except in ruminants, appreciable activity was sometimes noted in the small intestine.

As noted above, *E. coli* has been shown to produce β-glucuronidase. However, it has been pointed out that this microorganism normally comprises only a small proportion of the gastrointestinal flora (192). Accordingly, the contributions of other intestinal inhabitants to the β-glucuronidase activity were assessed. It was concluded that, in the rat, glucuronides are hydrolyzed mainly by the non-spore forming anaerobes (bacteroides and bifidobacterium) and the lactobacilli. Little contribution to this activity is made by *E. coli* in the lower parts of the intestine although some contribution may be made in the proximal small intestine. However, very striking differences in the patterns of intestinal β-glucuronidase activity were noted for the rat and mouse compared with rabbits, guinea pigs, and man. The former two species showed very high values in the small intestine and this is a reflection of the relatively large numbers of microorganisms which are found in the upper parts of their gastrointestinal tract. Glucuronide hydrolysis by a *Bacteroides* species has also been reported in experiments with 4-methylumbelliferone glucuronide (383), a conjugate which previously had been shown to undergo hydrolysis when incubated with mixed cultures of rat cecal microorganisms (347). β-Glucuronidase activity was studied in several genera of intestinal bacteria and, in addition to bacteroides, hydrolysis was also found when *E. coli* was used.

Studies in germ-free rats have indicated that the β-glucuronidase activity of the cecum is not entirely of microbial origin (133, 433, 440). The optimum activity of the bacterial enzyme was found to be at pH 6 whereas the activity of the cecal enzyme in germ-free rats was much lower, *i.e.*, at pH 5. The latter value is characteristic of mammalian β-glucuronidase and its presence in the cecum has been suggested to be due to sloughing off of mucosal cells into the intestinal lumen (132). The development of intestinal β-glucuronidase activity in young animals is discussed in section IV C.

Further relevant findings have been obtained from investigations in which the metabolism of glucuronides administered orally to animals was studied. The diglucuronide of *p*-hydroxybenzoic acid was found to undergo hydrolysis in the human intestine after which the acid was absorbed and then excreted in the free form and as the glycine conjugate in the urine (311). Similar results were obtained with phenyl glucuronide in rabbits (150) and salicylic acid ether glucuronide in rats (247).

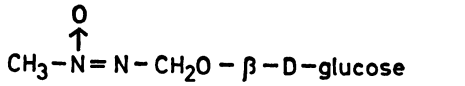
2. Other glycosides. Numerous glycosides have been shown to be hydrolyzed by the

intestinal bacteria. This reaction occurs with compounds containing either the α- or β-glycosidic linkage.

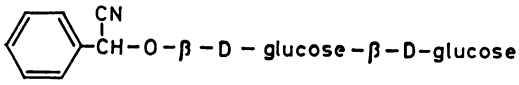
One of the most widely studied classes of glycosides in this regard is that of the flavonoids. Much of this interest arises incidentally since flavonoids, which are widely occurring plant compounds and therefore dietary constituents, undergo extensive metabolic change both by the intestinal microflora (section III L) and the tissue enzymes. Hydrolysis of the flavonoid glycosides by the intestinal bacteria is thus an early if not initial step in some interesting metabolic sequences. Several early feeding studies with flavonoid glycosides including rutin (quercetin-3-rhamnoglucoside) (41), hesperidin (hesperetin-7-rhamnoglucoside) (36), diosmin (diosmetin-7-rhamnoglucoside) (36), and naringin (naringenin-7-rhamnoglucoside) (37) showed that these compounds were excreted in the urine partly as their aglycones or as other metabolites lacking the sugar moiety. The ability of intestinal bacteria to carry out the hydrolysis of rutin was subsequently demonstrated with mixed cultures of rat fecal microorganisms (45). Although the aglycone itself was not detected in this study, hydrolysis of the rutinose residue would be required for the formation of the *m*-hydroxyphenylpropionic acid detected. However, in a similar study (347) the aglycone quercetin was detected in the incubates and hesperidin was hydrolyzed to its aglycone hesperetin. Other investigators have found that myricitrin (myricetin-3-rhamnoside), apiin (apigenin-7-apioglucoside), robinin (kaempferol-3-robinobioside-7-rhamnoside), pelargonin (pelargonidin-3,5-diglucoside), phloridizin (phloretin-2′-β-glucoside), and naringin undergo hydrolysis of the glycosidic linkage when incubated anaerobically with rat cecal microorganisms (177, 178, 371).

Flavonoid glycosides are hydrolyzed not only by rat intestinal microorganisms but also by bovine rumen microorganisms. Rutin, naringin, hesperidin, and quercitrin (quercetin-3-rhamnoside) were degraded by the microorganisms in rumen fluid upon anaerobic incubation and the products appeared to be similar to those produced by rat intestinal microorganisms (363). Subsequent reports have described several strains of rumen bacteria which can cleave the glycosidic bonds of rutin and quercitrin (67, 233).

An example of glycoside hydrolysis by intestinal bacteria which has received considerable attention is that found with cycasin (I). This compound is a constituent of cycad plants, which are indigenous to tropical and subtropical areas of the world and which are utilized as food, primarily because of their starch content (450). Much of the interest in cycasin stems from the fact that its ingestion by normal rats results in hepatotoxicity and the production of tumors in the liver, kidneys, and intestine whereas these effects are not observed when the glycoside is given to germ-free rats (239). It is also known that similar toxic effects are produced when methylazoxymethanol, the aglycone of cycasin, is administered orally to rats (258). The toxic properties were found to be associated with the aglycone rather than the glycoside itself, which is apparently without toxicity after intraperitoneal injection to rats (230). Moreover the glycoside is excreted almost quantitatively in the urine after administration intraperitoneally to normal rats or orally to germ-free rats (385). As these findings suggested that the hydrolysis must be brought about by intestinal microorganisms, germ-free rats were monocontaminated with several strains of bacteria with different levels of β-glucosidase activity (386). It was found that the degree of toxicity after feeding cycasin showed a positive correlation with the β-glucosidase levels of the particular bacterial strain used and that excretion of unchanged cycasin was reduced in those rats contaminated with bacteria having high enzyme levels.



I



II

Studies on several 6-bromo-2-naphthyl glycosidases in the digestive tract of conventional and germ-free rats have shown that these may originate from either the intestinal mucosa or the intestinal bacteria (85). However, the cecal α-glucosidase activity in conventional rats was found to be of bacterial origin.

A number of cyanogenic glucosides are found in plants, many of which are important foodstuffs (447). Studies on the release of glucosidal cyanide from lima beans indicated that hydrolysis occurs first in the colon (448). Incubation of cooked beans with feces extract or with *E. coli* resulted in the liberation of significant amounts of cyanide. Amygdalin (II), the β-gentiobioside of mandelonitrile, occurs in the seeds of bitter almonds. A recent report has shown that the toxicity of this compound after oral administration is due to its conversion to cyanide by the intestinal bacteria (381). The glycoside has a low degree of toxicity when given by injection or when given orally to animals pretreated with antibiotics and fasted to inhibit the intestinal microflora. It was also found that incubation of amygdalin with strains of enterobacteria and enterococci isolated from mouse intestine resulted in the liberation of cyanide.

Rat cecal microorganisms were shown to hydrolyze phenyl-β-D-glucoside to phenol upon anaerobic incubation (347). This reaction also occurred when the glycoside was incubated with a strain of *Lactobacillus* isolated from rat cecum or with strains of *Proteus vulgaris* and *A. aerogenes* (383).

Intestinal bacteria have been shown to be able to hydrolyze cardiac glycosides. Incubation of k-strophanthin-γ (k-strophanthidin-cymarose-glucose-glucose) with rat feces resulted in the loss of two glucose moieties to give cymarin (131). A subsequent investigation confirmed this result and it was also found that the glucose units of cerberoside and lanatoside A were removed in these incubates (240). It was suggested that this reaction can be carried out by clostridia. The loss of the terminal glucose unit in lanatoside C to give acetyldigoxin is carried out by strains of clostridia and by several other groups of intestinal bacteria (192). However, this cardiac glycoside was more often hydrolyzed by enterococci than by the other groups of bacteria.

The aforementioned investigation (192) used other glycosides including *p*-nitrophenyl glycosides and esculin (4,5-dihydroxycoumarin-β-D-glucoside). β-Glucosidase activity was shown with all of the groups of bacteria which included *E. coli*, enterococci, lactobacilli, clostridia, bacteroides, and bifidobacteria. While the enterococci showed the highest activity, the far larger numbers of lactobacilli, bacteroides, and bifidobacteria present in the intestine indicate that the intestinal hydrolysis of β-glucosides is carried out principally by these genera. Most of the hydrolysis of β-glycosides and α- and β-galactosides also was carried out by the lactobacilli and the non-spore forming anaerobes.

B. Hydrolysis of Sulfate Esters

Few studies on the hydrolysis of sulfate esters by intestinal microorganisms have been reported. Some of these studies deal with compounds of endogenous origin. Sodium estrone sulfate was hydrolyzed when it was incubated with human feces (394) and several steroid sulfates were hydrolyzed when incubated with rat cecal contents (132). In the latter study the sulfatase activity was shown to be of microbial origin as no hydrolysis occurred when cecal contents or intestinal mucosa from germ-free

rats was used. It is not known which strains of intestinal bacteria were responsible for the hydrolytic activity. The possible significance of the intestinal metabolism of thyroid hormones has been mentioned in a study which showed that 3,5,3′-triiodo-L-thyronine sulfate was hydrolyzed when incubated with rat intestinal bacteria (71). The intestinal bacteria have been implicated in the metabolism of chondroitin sulfate after its oral administration (113). When the ^{35}S-labeled compound was given to rats the urinary radioactivity was due to inorganic sulfate and the excretion peak was not reached until after 36 hr. These findings were shown to be due to the bacterial hydrolysis of the compound in the large intestine as pretreatment of the animals with oxytetracycline and phthalylsulfathiazole nearly abolished the urinary excretion of radioactivity. Also, incubation of chondroitin sulfate with rat feces resulted in the formation of inorganic sulfate.

Relatively few reports have appeared which indicate that xenobiotic compounds containing sulfate groups are hydrolyzed by the intestinal bacteria. A study of the metabolism of sodium amylopectin sulfate, a pepsin inhibitor and anti-ulcer compound, in the rat showed that the compound was not absorbed after oral dosage (316). However, a small amount (0.3%) of the radioactivity appeared in the urine in 4 days. This was due to the activity of bacterial sulfatases as rats pretreated with oxytetracycline and sulfaguanidine excreted the ^{35}S quantitatively in the feces. A similar study with cyclohexylphenyl 2-sulfate showed that the amount of inorganic sulfate excreted in the urine was decreased from roughly 20% in normal rats to only 1 to 2% in rats treated with antibiotics (194). An investigation of the nature and amounts of the metabolites of the carcinogen *N*-2-fluorenylacetamide indicated that a bacterial sulfatase is present in the cecum of rats (170).

The intestinal hydrolysis of some phenol sulfate derivatives has been investigated in relationship to their laxative properties (292). One compound which showed high laxative activity is the sulfate ester analogue of bisacodyl (X) in which both of the phenolic hydroxyl groups are attached to sulfate rather than to acetate groups. It was reported that this compound was not hydrolyzed in the intestine as no free phenol or metabolites could be detected in the urine or feces of rats given the test compound orally. However, in a reinvestigation, it was found that hydrolysis of the sulfate ester by intestinal microorganisms does occur and that liberation of the free diphenol is a requisite for the laxative action (143). Hydrolysis was also shown to occur when the sulfate ester was incubated with rat cecal contents. However, less than 4% of the added ester was converted to the diphenol under the conditions employed. This low figure again points to the lack of appreciable sulfatase activity among the intestinal microorganisms. In this particular case, however, a biological response is elicited by very small amounts of the free diphenol owing to its potency in inhibiting the absorption of water and electrolytes in the intestine.

Microbiological studies suggest that the sulfatase activity found in the intestine may be due to some enterobacteria. *p*-Nitrocatechol sulfate is hydrolyzed by *Pr. vulgaris* (112), *Proteus rettgeri* (269), and *A. aerogenes* (383). Some of the properties of an enzyme from a strain of *A. aerogenes* that catalyzes the hydrolysis of *p*-nitrophenyl sulfate have been described (146, 315).

Although the investigations cited above illustrate that hydrolysis of sulfate esters by the intestinal microorganisms may occur in some cases, other studies have demonstrated that compounds of this type are stable in the intestine or when incubated with intestinal bacteria. In fact, several studies on the metabolism of sodium estrone sulfate suggest that its metabolism *in vivo* is mainly by mammalian enzymes rather than by bacterial systems (394). When this compound labeled with ^{35}S was given orally to rats over 75% of the radioactivity was recovered in the urine and feces in 36 hr (188). A similar value was obtained when the

compound was given by subcutaneous or intravenous injection and biliary excretion was less than 15% of the ^{35}S. These results point to the tissues as the source of hydrolytic activity and this was demonstrated with liver homogenates. This conclusion was reached also in a recent study of the metabolism of sodium estrone sulfate (114) and of the mono- and disulfate esters of diethylstilbestrol (172) in rats.

Metabolism studies with other sulfate esters have similarly indicated that intestinal hydrolysis is not a significant reaction. Phenylsulfate is excreted almost quantitatively unchanged after oral administration to rabbits (150). In rats it is hydrolyzed to an extent of 4 to 6% but this is seen after both oral or intraperitoneal administration (191). The latter investigation gave similar results when 1- and 2-naphthylsulfate were used. As pointed out above, the desulfuration of cyclohexylphenyl 2-sulfate can be attributed almost completely to the intestinal microorganisms. However, the closely related cyclohexylphenyl 4-sulfate and biphenylyl 4-sulfate show only moderate or no loss of sulfate, respectively, as a result of the metabolic activities of the microflora (194). *p*-Nitrocatechol sulfate was not hydrolyzed when incubated with rat cecal microorganisms (347) or with strains of representative intestinal bacteria except *A. aerogenes* which showed minor activity (383).

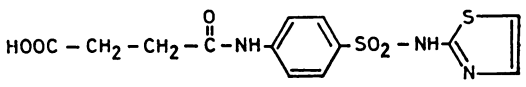
Thus, the information presently available does not appear to necessitate any major alteration of the viewpoint expressed earlier (348) that the intestinal hydrolysis of sulfate esters is a reaction of limited occurrence and importance. Another point of interest is the fact that biliary conjugates are generally glucuronides rather than sulfates (382). As a result, endogenously formed sulfate esters are unlikely to come into contact with the intestinal flora as a result of biliary excretion.

C. Hydrolysis of Amides

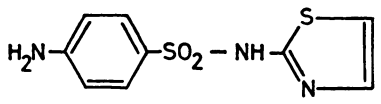
Compounds containing the amide linkage include glycine conjugates and *N*-acetyl derivatives; these are among the several forms of amides which are known to be hydrolyzed by the gastrointestinal bacteria. In view of the paucity of information available on the specificity of these amidases, it is felt that no real purpose is served by rigidly dividing this section on the basis of superficial chemical features of the various amides.

Several examples of the hydrolysis of amides by intestinal bacteria deal with N^4-acyl derivatives of sulfonamides. Perhaps the most well known examples of this reaction are those of phthalylsulfathiazole and succinylsulfathiazole (III), intestinal antiseptics which are activated in the large intestine by bacterial hydrolysis (431). Sulfathiazole (IV) was formed when succinylsulfathiazole was incubated anaerobically with mixed cultures of rat cecal microorganisms (347). However, this activity was not detected in isolated strains of some common intestinal bacteria (383). Deacylation of a series of N^4-acyl derivatives of sulfadiazine in the gastrointestinal tract was found to occur to the greatest extent with the propionyl and butyryl derivatives whereas negligible hydrolysis was found with very short or with long aliphatic chains in the acyl group (295). N^4-Acetylsulfanilamide was not hydrolyzed upon incubation with rat cecal microorganisms (347) or with numerous intestinal bacteria (383). However, further studies are needed to determine whether the apparent requirement of a moderately long acyl group for bacterial hydrolysis of sulfonamide derivatives, which has been indicated so far, is merely fortuitous.

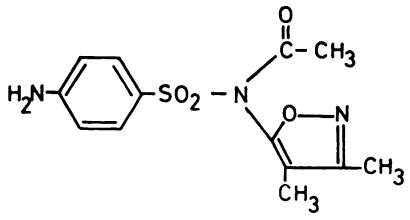
In contrast to the lack of hydrolysis of the N^4-acetyl sulfonamides, deacetylation of an N^1-acetyl derivative, N^1-acetylsulfisoxazole (V), has been shown with several species of intestinal bacteria (410). Activity



III



IV



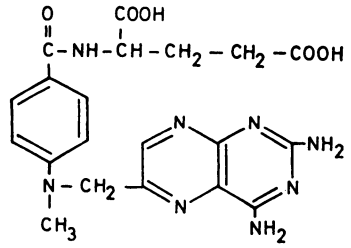
V

was found with *Streptococcus faecalis*, *L. acidophilus*, and *E. coli* with the highest activity occurring with *E. coli*.

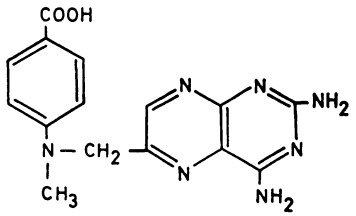
N-Acetylhistamine and *p*-acetamidobenzoic acid are also deacetylated. The former compound was shown to be hydrolyzed to histamine in the intestinal lumen (366) and upon anaerobic incubation with human fecal suspensions (365). *p*-Acetamidobenzoic acid was hydrolyzed when incubated anaerobically with mixed cultures of rat cecal microorganisms (347) and this reaction can also be carried out by *A. aerogenes* (383).

Another group of amides which has been shown to be hydrolyzed by the intestinal microflora consists of the *N*-acyl derivatives of amino acids. The usual amino acid in this group is glycine but examples with glutamic acid and taurine are also known. It should be noted that most of the research in this area has been devoted to studies of the deconjugation of glycocholic and taurocholic acids. Although this subject falls outside the scope of the present review, pertinent information is available in several articles (8, 118, 180, 197, 198, 267, 277, 285, 286, 321). A simple member of this general group of amides, hippuric acid, has been reported to be hydrolyzed by enterococci (285). The related *p*-aminohippuric acid is converted to *p*-aminobenzoic acid when incubated with rat cecal microorganisms (347) or with pure cultures of *Str. faecalis* or *A. aerogenes* (383). Both *p*-aminohippuric acid and *p*-acetylaminohippuric acid have been shown to be hydrolyzed at the amide linkage in the gastrointestinal tract of man (209).

The metabolic fate of the folic acid antagonist and anti-tumor agent methotrexate (VI) in rats and mice was found to be significantly altered when the animals received antibiotic pretreatment or when germ-free animals were used (453, 454). The nature of the metabolites was not determined but it was suggested that they arose in the intestine as a result of bacterial metabolism. This hypothesis has been confirmed recently as incubation of methotrexate with mouse cecal contents resulted in conversion to several metabolites including 4-amino-4-deoxy-N^{10}-methylpteroic acid (VII), the major fecal metabolite of mice given methotrexate (411). Although the particular microorganisms responsible for the amidase activity in these experiments have not been identified, it is known that this reaction can be carried out by *Alcaligenes faecalis* (261) as well as by several species of *Pseudomonas* (31, 245).



VI

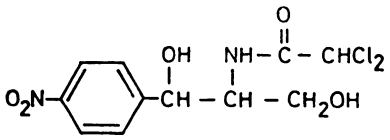


VII

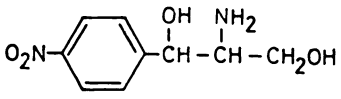
A recent study of the deamination of nicotinamide in the gastrointestinal tract of rats showed that this reaction occurs in the preventricular portion of the stomach in normal but not in germ-free rats (403). Furthermore, a bacillus with high deamidase activity was isolated from this area. A subsequent investigation showed that deamidase activity, in decreasing order, was present in *Flavobacterium peregrinum*, *E. coli*, *Str.*

faecalis, and *L. acidophilus* isolated from the preventricular region of the rat stomach (362).

An early example of amide hydrolysis by intestinal bacteria is that of chloramphenicol (VIII). The antibiotic was found to be hydrolyzed to 1-*p*-nitrophenyl-2-amino-1,3-propanediol (IX) and dichloroacetic acid by *E. coli*, *Bacillus mycoides*, *Pr. vulgaris*, and *Bacillus subtilis* (374). Another investigation indicated that incubation of chloramphenicol with *E. coli* resulted in the cleavage of the amide linkage (204). The extensive degradation of chloramphenicol by these four microorganisms was subsequently elucidated and, among the 18 or so products identified, both 1-*p*-nitrophenyl-2-amino-1,3-propanediol (IX) and its 1-*p*-aminophenyl reduction product were detected in large amounts (373). These incubations were carried out in aerated nutrient medium broth and they may therefore be somewhat unrepresentative of the changes likely to take place in the intestinal tract of animals. However, this remark probably applies more to some of the terminal metabolic steps than to the amide hydrolysis itself as both 1-*p*-nitrophenyl-2-amino-1,3-propanediol (IX) and its *p*-amino reduction product have been detected in the urine of rats after oral administration of chloramphenicol glucuronide, a major biliary metabolite of the antibitiotic (157).



VIII



IX

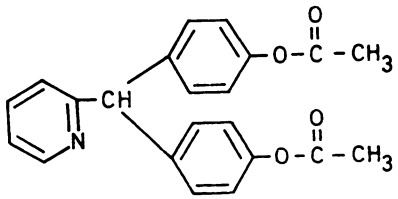
Pencillin acylase or amidase catalyzes the hydrolysis of the side chain of penicillins to produce 6-aminopenicillanic acid. This enzyme therefore has considerable commercial significance in regard to the preparation of the semisynthetic penicillins, but it is not an important factor in the bacterial resistance to penicillin (187). Penicillin acylase activity is present in strains of many genera of intestinal bacteria including *Aerobacter*, *Micrococcus*, *Proteus*, and *Pseudomonas* and also in *E. coli* (73, 187). A further study on the penicillin acylase of *E. coli* has shown that amides and *N*-acylglycines unrelated to penicillin are also hydrolyzed (74).

The other bacterial enzyme which catalyzes the hydrolysis of an amide linkage in penicillins and other β-lactam antibiotics is a β-lactamase. Since the β-lactam ring is essential for the antibacterial activity of these compounds, hydrolysis results in their inactivation. β-Lactamase activity is found in both Gram-negative and Gram-positive bacteria although more common in the former (393). Activity has been reported in many enterobacteria, including *E. coli*, *A. aerogenes*, *Aerobacter cloacae* and several species of the genus *Proteus* (195, 393).

The arylamidase activity of 18 Gram-negative and Gram-positive bacteria, including some common intestinal inhabitants, has been studied with amino-acid β-naphthylamines as substrates (28). Hydrolytic activity was found most frequently in the Gram-negative bacteria.

D. Hydrolysis of Esters

When considering the intestinal hydrolysis of esters it should be kept in mind that esterase activity may be associated with enzymes in the intestinal secretions or in the mucosa. An example of the former situation appears to be found with the laxative drug bisacodyl (X) which underwent hydrolysis when incubated with small intestine juice from the rat (134). Esterases have been shown to be present in the mucosa from all parts of the human gastrointestinal tract (101). It is these mucosal enzymes that have been implicated in the intestinal hydrolysis of some foreign compounds including reserpine (158), aspirin (246), and the hypolipidemic agent halofenate (207).

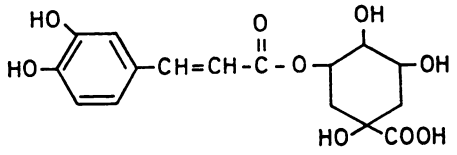


X

Other compounds subject to intestinal ester hydrolysis include the ester of citraurin, a constituent of orange peel (46), chloramphenicol mono- and di-succinate (156), erythromycin propionate (244), the diesters of taraxanthin and zeazanthol (47), penamecillin, the acetoxymethyl ester of penicillin (91) and 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (13). In these cases the available data do not indicate the source of the hydrolytic activity.

In addition, a third possibility exists in that esterases of the intestinal bacteria are now known to carry out this reaction with a number of foreign compounds. The ester linkage of acetylated cardiac glycosides was found to be hydrolyzed after incubation with intestinal microorganisms (240). Lanatoside A (digitoxigenin-bis-digitoxosido-acetyl-digitoxosido-glucoside) and acetyldigitoxin were both converted to digitoxin (digitoxigenin-tridigitoxide) under these conditions. Some enterococcal strains recently have been found to hydrolyze acetyldigoxin to digoxin (192).

Gallic acid methyl ester was hydrolyzed when incubated anaerobically with mixed cultures of rat cecal microorganisms (347). However, a subsequent study was unable to correlate this reaction with specific representatives of nine genera of intestinal bacteria (383). Chlorogenic acid (XI) underwent hydrolysis and further metabolism to *m*-hydroxyphenyl-propionic acid when given orally to rats (35). This metabolite was formed also when chlorogenic acid was incubated with mixed cultures of rat cecal bacteria (352) and bacterial metabolism can therefore fully account for this reaction sequence.

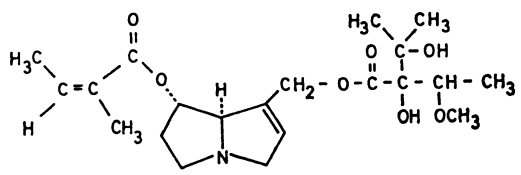


XI

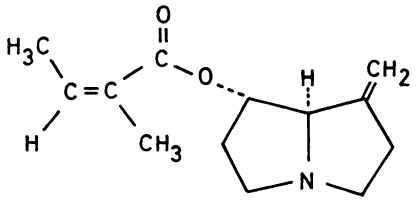
Studies on the metabolic disposition of methylatropine iodide, a quaternary anticholinergic drug, showed that oral administration to several animal species resulted in the urinary excretion of two metabolites not observed after parenteral dosage (4). These metabolites had the same chromatographic properties as the hydrolysis products of methylatropine and it was felt that substantial hydrolysis of the drug occurred in the gastrointestinal tract. Since some bacteria possess esterases that hydrolyze atropine, it was concluded that some of the observed hydrolysis was carried out by the intestinal bacteria.

While other aspects of the metabolism of the hepatotoxic pyrrolizidine alkaloids by intestinal bacteria are discussed in sections III M, S, and Y, the degradation of lasiocarpine (XII) will be presented now as ester hydrolysis is involved. Incubation *in vitro* with sheep rumen contents resulted in the conversion of lasiocarpine (XII) to 7α-angeloxy-1-methylene-8α-pyrrolizidine (XIII) which was then followed by hydrolysis to 7-α-hydroxy-1α-methyl-8α-pyrrolizidine (XIV) (238).

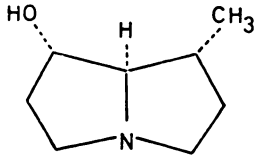
Several pentacyclic triterpenoid compounds have shown therapeutic usefulness because of their wound-healing properties. Two of these compounds, carbenoxolone (β-glycyrrhetic acid hemisuccinate, XV), and asiaticoside (XVI) contain ester groups and the hydrolysis of these groups which



XII

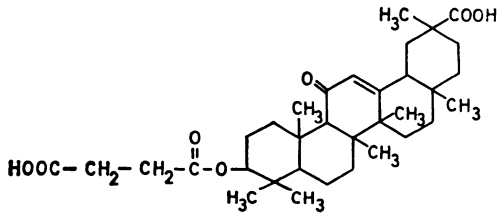


XIII



XIV

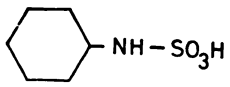
occurs when these compounds are administered to rats is carried out by bacteria in the gastrointestinal tract (66, 214). Carbenoxolone, labeled with ^{14}C in the succinate moiety, was not hydrolyzed when incubated with blood, liver homogenates, or human gastric contents whereas extensive hydrolysis was detected when rat stomach or cecal contents were employed (214). Furthermore, inhibition of the gastrointestinal microflora with antibiotics completely inhibited the hydrolysis of orally administered carbenoxolone as none of it was metabolized to $^{14}CO_2$. In normal rats this conversion amounted to about 70% of the dose. Hydrolysis of asiaticoside to its aglycone, asiatic acid, was also demonstrated upon incubation of the compound with rat cecal contents (66). Also, asiatic acid was identified in the cecal contents of rats given the glycoside orally.



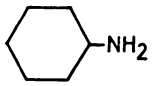
XV

E. Hydrolysis of Sulfamates

This section is presently limited to a single compound, cyclamate (XVII, cyclohexylsulfamic acid), the salts of which have been used extensively as sweetening agents. Partly because of recent events regarding the use of cyclamate, widespread interest has been given to the pharmacological, toxicological, and metabolic aspects of this compound. Current knowledge in these areas has been reviewed recently (5).

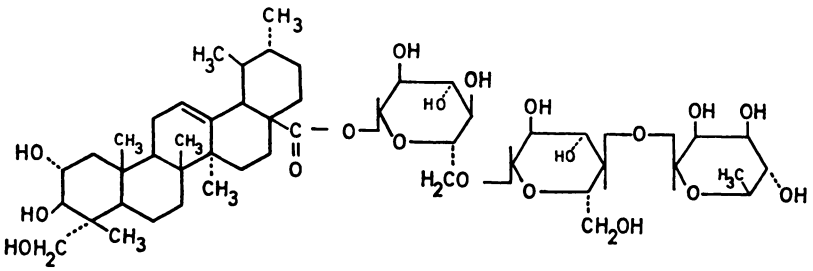


XVII



XVIII

Cyclamate was long considered to be a metabolically stable compound but it was eventually demonstrated that it could be metabolized to cyclohexylamine (XVIII) (232). It soon became evident that conversion to cyclohexylamine was variable, both with respect to the percentage of individuals showing the conversion and the amount of metabolite formed (10, 34, 87, 100, 162, 241,



XVI

242, 250, 289, 309, 318, 420). While the source of this activity was not immediately clear, it became increasingly likely that intestinal microorganisms were responsible for cyclohexylamine production (10, 87, 162, 289, 309, 384, 420). This was indicated by the findings that cyclohexylamine production was reduced when the animals receiving cyclamate were pretreated with antibiotics (384) and that rats which were non-converters would become converters after being caged with converters (87). Although some experiments *in vitro* in which cyclamate was incubated with fecal bacteria failed to show a conversion to cyclohexylamine (100, 241), another study reported a small conversion when intestinal contents or a strain of *Cl. perfringens* from dogs was used (162).

A subsequent investigation of the hydrolysis of cyclamate has demonstrated convincingly that this reaction is carried out by the intestinal microflora (120, 121, 317, 381). Incubation of cyclamate with the intestinal contents from converter rats (317) or with strains of intestinal bacteria from converters (120, 121) resulted in the formamation of cyclohexylamine. The implicated bacteria included enterococci from human feces, clostridia from rat feces, and enterobacteria from rabbit feces. A significant result of this investigation was the finding that the intestinal bacteria acquire the ability to produce cyclohexylamine after prolonged exposure to cyclamate. This type of induction phenomenon has important practical and theoretical consequences and these considerations, as well as some of the relevant data concerning cyclamate metabolism, are therefore dealt with in section IV D.

F. Hydrolysis of Nitrates

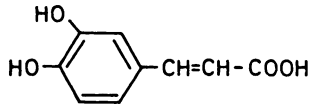
Studies of the absorption and metabolism of the antianginal drug pentaerythritol tetranitrate in rats have shown that degradation to pentaerythritol and the mono-, di-, and trinitrates takes place (108). Injection of the compound into the ligated large intestine resulted in the appearance of increasing amounts of the lower nitrates in this organ during the experimental period. It was proposed that this hydrolysis was carried out by the intestinal bacteria. Greatest absorption of the ^{14}C-labeled drug occurred initially from the small intestine but after 2 hr absorption of the radioactivity was faster from the large intestine. This was believed to be due to the formation of the lower nitrates which should have greater water and lipid solubility and therefore pass more readily through the intestinal wall than the original compound which is very insoluble.

G. Dehydroxylation

1. C-Hydroxyl compounds. Investigations of the metabolism of bile acids provided the first evidence of dehydroxylation by intestinal microorganisms. However, as the present review is devoted primarily to the metabolism of xenobiotic rather than endogenous compounds, no coverage of this topic will be made. The subject was discussed briefly in an earlier review (348) and further information has appeared (9, 183, 198, 268).

Many of the investigations dealing with metabolic dehydroxylation have their origin in a report published in 1955 which indicated that 3,4-dihydroxyphenylacetic acid (homoprotocatechuic acid) was metabolized partly to *m*-hydroxyphenylacetic acid in rats and rabbits (40). Subsequent experiments showed that metabolites containing the *m*-hydroxyphenyl group were also produced in rats, rabbits, and man from caffeic acid (3,4-dihydroxycinnamic acid, XIX) (35, 107). 3,4-Dihydroxyphenylalanine (dopa) was dehydroxylated to *m*-hydroxyphenylacetic acid when given to rats and rabbits (107). On the other hand, protocatechuic acid, the corresponding benzoic acid derivative, was not found to undergo dehydroxylation. Confirmation of the *para*-dehydroxylation of homoprotocatechuic acid was obtained with ^{14}C-labeled compound which, when given orally to rabbits at a dose level of 100 mg/kg,

was converted to the extent of 14% to the *m*-hydroxyphenyl derivative (357). In addition, slightly over 1% of the dose was found to be dehydroxylated in the *meta*-position. A subsequent study with ^{14}C-homoprotocatechuic acid in rats showed the same extent of *meta*-dehydroxylation but only half the amount of *para*-dehydroxylation (82). When the metabolism of protocatechuic acid was re-investigated with ^{14}C-labeled material, it was found that dehydroxylation occurred to a minor extent (83, 84). A total of about 5% of the dose of 100 mg/kg was metabolized in rats to *m*-hydroxy-, *p*-hydroxy-, and *m*-methoxybenzoic acid.



XIX

The first information on the site of formation of these *m*-hydroxyphenyl metabolites was obtained from feeding studies which showed that their excretion is virually abolished when plant materials are removed from the diet (361). In addition, treatment of man or rats for several days with neomycin reduces the urinary excretion of *m*-hydroxyphenyl compounds to insignificant levels, regardless of the nature of the diet. As a result of these findings it was postulated that dehydroxylation of the 3,4-dihydroxyphenyl nucleus to give *m*-hydroxyphenyl derivatives is a reaction peculiar to bacteria. This suggestion has been supported strongly by studies with conventional, antibiotic-treated and germ-free animals. As noted above, ^{14}C-labeled protocatechuic and homoprotocatechnic acids were dehydroxylated when administered orally to animals. It was also found that treatment of the animals with neomycin prior to dosage suppressed the formation of the dehydroxylated metabolites (82–84). Similar results have been obtained in man given caffeic acid (XIX) (103). It was shown recently that germ-free rats given the latter compound excreted no *m*-hydroxyphenylpropionic acid in the urine whereas this compound was the major urinary metabolite when conventional animals were used (297a, 354). Metabolism studies in rats with the closely related ferulic acid (4-hydroxy-3-methoxycinnamic acid) have shown that *m*-hydroxyphenylpropionic acid is a prominent urinary metabolite (35, 345, 406, 407).

In addition to the numerous studies in animals mentioned above, other investigations on the dehydroxylation of phenolic acids by intestinal contents and by isolated strains of intestinal bacteria have been carried out. Dehydroxylation of caffeic acid (XIX) and several other catechol derivatives was first shown to occur in incubates of intestinal contents from numerous animal species (44, 45). The reaction required anaerobic conditions and was inhibited by several antibiotic or antiseptic substances. Several other studies with mixed cultures of rat cecal microorganisms have demonstrated the dehydroxylation of catechol derivatives including caffeic acid (345), dihydrocaffeic acid (3,4-dihydroxyphenylpropionic acid) (345), and homoprotocatechuic acid (344, 345). Similar incubation of protocatechuic acid, the corresponding benzoic acid derivative, failed to reveal any dehydroxylation (45, 354) and catechol itself was not converted to phenol (354). These results appear to indicate that the degree of dehydroxylation is increased in compounds having a longer side chain. Moreover, the corresponding C_6-C_5 acid, 5-(3,4-dihydroxyphenyl)-valeric acid is reported to undergo *para*-dehydroxylation when incubated anaerobically with rat cecal microorganisms (349).

Studies with caffeic acid or dihydrocaffeic acid have shown that dehydroxylation can be carried out by isolated strains of intestinal bacteria. This was first shown in anaerobic incubates of a *Pseudomonas* species isolated from rat feces (299) and subsequently with *Ps. fluorescens*, *Ps. viburni*, *Ps. insolita*, *Ps. myxogenes*, and *Ps. chlororaphis* (298). Recently, several strains of bacteria isolated

from human feces were tested for their ability to degrade caffeic acid and its metabolites (296). It was found that a mixed culture of *E. coli* and *Str. faecalis* var. *liquifaciens* isolated with the help of a medium containing dihydrocaffeic acid would metabolize this compound to *m*-hydroxyphenylpropionic acid. In another investigation, however, 12 strains of common intestinal bacteria belonging to nine genera failed to demonstrate dehydroxylation of dihydrocaffeic acid (383). Interestingly, gnotobiotic rats, infected with two strains of lactobacilli, a strain of bacteroides and a strain of group N streptococci, excreted *m*-hydroxyphenylpropionic acid in the urine after ingestion of caffeic acid, whereas the metabolite was not detected in cultures of the four bacteria grown in the presence of caffeic acid (297a).

While the dehydroxylation of compounds containing an *ortho*-dihydroxyphenyl group has received the greatest amount of attention in this field, it has also been shown that the 3,4,5-trihydroxyphenyl group can undergo this reaction. Pyragallol was dehydroxylated to resorcinol but not to catechol when incubated with rat intestinal contents (343, 345). This situation therefore differs from that noted above with catechol which was not dehydroxylated under similar conditions. However, the results with the C_6-C_1, C_6-C_2, and C_6-C_3 acids are similar to those described above for the series of catechol acids. Thus, gallic acid (3,4,5-trihydroxybenzoic acid) was not found to be dehydroxylated by rat intestinal contents (343, 354), although this may perhaps be related to the relative ease of decarboxylation of this compound (see section III H).

The dehydroxylation of the higher homologues of these trihydric acids has received considerable attention recently. 3,4,5-Trihydroxyphenylacetic acid underwent *para*-dehydroxylation when incubated anaerobically with rat cecal microorganisms (179, 371). The same metabolite was excreted in the urine of rats given the trihydroxy compound orally and its formation was abolished when the animals were treated with neomycin. The same pattern of preferential *para*-dehydroxylation leading to 3,5-dihydroxyphenyl derivatives was noted in a similar study of the metabolism of 3,4,5-trihydroxyphenylpropionic acid (265, 266). Metabolites containing the 3,5-dihydroxyphenyl structure are also formed from numerous methyl ethers of trihydroxyphenyl compounds (see section III I 1).

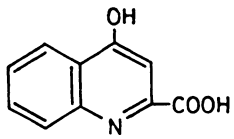
Dehydroxylation is an important reaction in the metabolism of several flavonoid compounds including quercetin, hesperetin, and (+)-catechin. Flavonoid metabolism by intestinal microorganisms is discussed in section III L and will therefore not be dealt with in detail here. However, many flavonoids are degraded to phenolic acids of the type discussed above and these products may then be dehydroxylated. Alternately, dehydroxylation may also occur in intermediate stages of flavonoid degradation. (+)-Catechin is metabolized by intestinal bacteria to δ-phenyl-γ-valerolactone derivatives and one of these lacks the *para*-hydroxyl group found in the B-ring of the original compound.

Increased interest in the metabolism of L-dihydroxyphenylalanine (L-dopa) has developed recently as a result of the use of this amino acid in the treatment of parkinsonism. As noted above, an early study showed that it was metabolized to *m*-hydroxyphenylacetic acid when given to rats and rabbits (107). On the other hand, an initial study *in vitro* with rat intestinal microorganisms showed that it was degraded to *m*-hydroxyphenylpropionic acid (45). Recent investigations in man have shown that L-dopa undergoes *para*-dehydroxylation (61) and that metabolite formation can be suppressed by inhibition of the intestinal microflora with neomycin (338). The degradation of L-dopa by rat cecal bacteria has been re-investigated and both *m*-hydroxyphenylacetic acid and *m*-hydroxyphenylpropionic acid are formed (17, 162a). Interestingly, dopamine is not converted to phenolic acid metabolites by the cecal bacteria. Both of the above *m*-hydroxy acids are excreted in the urine of

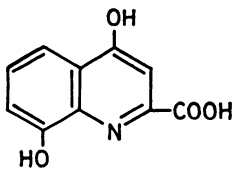
conventional rats given dopa but are absent when germ-free rats are used (162a). Another report has indicated that both dopa and dopamine can undergo *meta*-dehydroxylation to form *p*-tyramine (51). However, this reaction was found to be carried out not by intestinal microorganisms but by enzymes located in rat brain.

An unusual reaction sequence occurs in guinea pigs treated with tolbutamide, an oral hypoglycemic agent, which resulted in the degradation of dopamine, norepinephrine, normetanephrine, and 4-hydroxy-3-methoxyphenylglycol to *m*-hydroxyphenylacetic acid (369, 370). This metabolic sequence involves both aromatic and aliphatic dehydroxylations. These findings have been confirmed (51) but it would be of further interest to ascertain the sites of formation of these metabolites. An earlier study failed to demonstrate any metabolism to dehydroxylated acids when epinephrine or norepinephrine were incubated anaerobically with mixed cultures of rat intestinal bacteria (45).

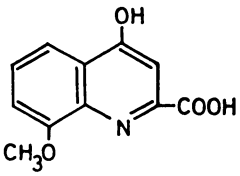
The examples described above indicate that numerous phenolic compounds possessing an *ortho*-dihydroxyl structure may undergo dehydroxylation as a result of metabolic activities of intestinal microorganisms. However, the requirement for the catechol structure is not absolute and several examples are now known in which removal of a solitary hydroxyl group takes place. This was noted with *p*-hydroxyphenylpropionic acid (phloretic acid) and L-tyrosine which were found to be converted to phenylpropionic acid when incubated with rumen microorganisms (359, 360). Several studies of the metabolism of kynurenic acid (4-hydroxyquinaldic acid, XX) and xanthurenic acid (4,8-dihydroxyquinaldic acid, XXI) have shown that these compounds undergo dehydroxylation. This reaction involves removal of the 4-hydroxyl group and occurs in rats, cats, and man (42, 218, 401, 402) and especially rabbits (219). In experiments with rabbits, oral administration of xanthurenic acid resulted in the urinary excretion of most of the dose as 8-hydroxyquinaldic acid whereas injection of the compound led to its excretion mainly unchanged. These results suggested that the reaction took place in the gastrointestinal tract and this interpretation was validated in a subsequent study with rabbits treated orally with neomycin (220). Dehydroxylation ability 3 weeks after antibiotic treatment had been terminated was again present at high levels. Similar results have been found with the 8-methyl ether of xanthurenic acid (XXII) as the urinary excretion of 8-methoxyquinaldic acid in rabbits was severely inhibited when the animals were treated with neomycin (252). Direct evidence of the dehydroxylation of 4-hydroxyquinoline-2-carboxylic acids by intestinal microorganisms from rats, rabbits, sheep, and man has also been obtained (42, 347). Xanthurenic acid and, to a lesser extent, kynurenic acid underwent loss of the 4-hydroxyl group upon incubation under anaerobic conditions.



XX



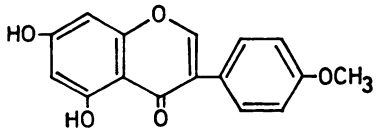
XXI



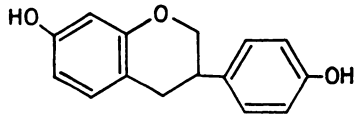
XXII

Examples are also known of the dehydroxylation of *meta*-dihydroxyl compounds.

This reaction recently was shown to occur with 3,5-dihydroxyphenylpropionic acid which, upon prolonged anaerobic incubation with rat cecal microorganisms, was converted to *m*-hydroxyphenylpropionic acid (179). Similar experiments with 3,4,5-trihydroxyphenylacetic acid resulted in substantial dehydroxylation to the 3,5-dihydroxy derivative but also to a trace of *m*-hydroxyphenylacetic acid. Further examples of the dehydroxylation of phenolic compounds having a *meta*-dihydroxyl structure have been found with the isoflavonoids as genistein (62) and its 4′-methyl ether biochanin A (XXIII) (63) are metabolized in the fowl to equol (XXIV). However, the site of the conversion was not determined and the possible role of the intestinal microorganisms in this reaction is a matter of speculation.

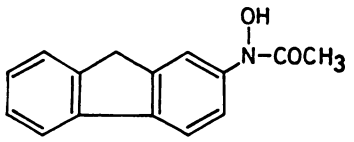


XXIII



XXIV

2. N-Hydroxyl compounds. Metabolic studies of the carcinogen *N*-hydroxy-*N*-2-fluorenylacetamide (XXV) showed that *N*-dehydroxylation occurred when the compound was incubated with rat liver or brain homogenates or with the soluble fraction of liver (171). Further studies on the metabolic fate of this compound with germ-free and conventional rats (170, 433) and intestinal microorganisms (440) have shown that this reaction is also carried out by enzymes of the intestinal bacteria. Although incubation of the *N*-hydroxyl compound with cecal contents from germ-free rats did not lead to disappearance of the substrate, a progressive loss of substrate together with the appearance of *N*-2-fluorenylacetamide was observed when cecal contents from conventional rats were used. The rate of dehydroxylation in these incubates increased progressively when additional substrate was added after 4 and 8 hr. Furthermore, dehydroxylation was also carried out by a strain of *E. coli*. The dual origin of *N*-dehydroxylating activity in both the tissues and the intestinal microflora is thus in contrast to the situation described above with phenolic xenobiotic compounds which are dehydroxylated only by the intestinal bacteria.



XXV

H. Decarboxylation

The decarboxylation of amino acids by the intestinal bacteria has received increased attention because of the possible significance of the urinary excretion of amines in certain disease states (300). It was found that several urinary amines previously considered to be of internal metabolic origin result instead largely or entirely from the activities of the intestinal bacteria. An earlier study (263) has listed several intestinal microorganisms including *E. coli*, *Str. faecalis*, *Clostridium* spp., *Lactobacillus* spp., and *Pr. vulgaris* which can decarboxylate amino acids. Other studies have demonstrated that amino acids can be extensively metabolized by intestinal microorganisms. Tyrosine and 3,4-dihydroxyphenylalanine (dopa) furnish good examples of this and the end products of degradation include simple phenols (15–17). The reaction sequence with these amino acids may be initiated with decarboxylation and it is known that this reaction can be carried out with a decarboxylase obtained from *Str. faecalis* (33, 135). However, the details of these pathways are not clear and this topic is therefore covered in section III Y. It has been found recently that most

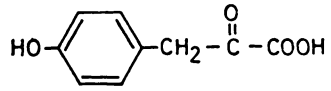
of the L-dopa administered orally to man is decarboxylated in the digestive tract prior to reaching the general circulation (29). However, it was not determined whether this was due to intestinal bacteria or to decarboxylases in the intestinal mucosa.

While much of the subject dealing with the decarboxylation of amino acids by intestinal bacteria falls outside the scope of this article, mention will be made of two relevant examples. After the administration of 3,5,3′-triiodothyronine by intravenous injection to rats its decarboxylation product, 3,5,3′-triiodothyronamine, was detected in the intestinal contents and feces (319). As the metabolite was not present in the bile it was suggested that the decarboxylation took place in the intestine after the biliary excretion of the original compound or its conjugates. A recent study of histidine decarboxylase in the rat stomach has shown that much of the activity present is due to a bacterial enzyme (205). This is derived from several strains of *L. acidophilus* which are located in a mucin-like material on the epithelium of the stomach. The histamine concentration in gastric juice of germ-free rats was very low and the values found in normal rats could be decreased by fasting or antibiotic administration which reduced the numbers of gastric lactobacilli.

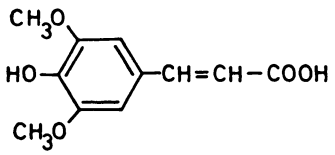
Considerable interest has been shown recently in the intestinal decarboxylation of phenolic acids. This reaction results in the formation of simple phenols and these compounds are often found as urinary metabolites. Resorcinol, as its monosulfate ester, has been detected in the urine of some individuals (80) and it was subsequently found to arise from a tea constituent or constituents (81). Catechol is excreted in conjugated form in human and cattle urine (133) and the presence of urinary pyrogallol has been reported (409). In the latter case it was postulated that the phenol arises from decarboxylation of gallic acid (3,4,5-trihydroxybenzoic acid) in the intestine. The metabolic fate of gallic acid in rats and rabbits has been investigated subsequently (39, 425) and pyrogallol was detected as a urinary metabolite. However, the finding that the decarboxylated compound was also excreted when gallic acid was given intraperitoneally was believed to preclude the possibility that the pyrogallol was of intestinal origin (39). As it had been noted that protocatechuic acid (3,4-dihydroxybenzoic acid) was decarboxylated to catechol when incubated with rat intestinal contents (45), a study of the metabolism of protocatechuic and gallic acids in the rat was carried out in order to assess the role of the intestinal microorganisms (343). It was found that decarboxylation of both of these phenolic acids occurred when they were incubated anaerobically with rat cecal or colon contents. Further metabolism to resorcinol was noted with gallic acid when rat feces were used as the source of the decarboxylating activity. Furthermore, the decarboxylation reaction was strongly inhibited when the antibiotic oxytetracycline was added to the incubates.

These initial findings which pointed to the significance of the intestinal microorganisms in the decarboxylation of phenolic acids prompted the study of the metabolism of a large number of these compounds by the rat cecal microflora (344, 345, 354). Phenolic benzoic, phenylacetic, phenylpropionic, and cinnamic acids were used and decarboxylation was observed in all types except the phenylpropionic acids. The results indicated that a free *p*-hydroxyl group is necessary for the decarboxylation reaction. However, a recent study with longer incubation times has shown that decarboxylation can also occur with a *m*-hydroxyphenyl compound (17). Nonetheless, present evidence leaves no doubt that the decarboxylation of phenolic acids by intestinal bacteria is primarily a reaction occurring with *p*-hydroxylated compounds. This has also been shown in a study of the metabolism of l-tyrosine, its degradation products and related compounds by rat cecal microorganisms (15). In the latter study, an α-keto acid, 4-hydroxyphenylpyruvic acid (XXVI), was also de-

carboxylated as 4-hydroxyphenylacetic acid was detected in most of the incubation samples. Other investigations with protocatechuic acid have shown that it is decarboxylated to catechol by bovine rumen microorganisms (363) and that the carboxy-^{14}C-labeled compound is metabolized to $^{14}CO_2$ when incubated with rat intestinal contents (84).



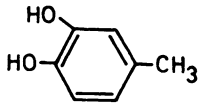
XXVI



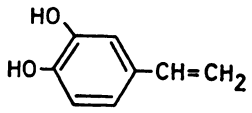
XXVII

While the presence of a *p*-hydroxyl group appears to be an important structural feature of the phenolic acids which undergo decarboxylation by intestinal bacteria, further ring substitution leads to varying degrees of inhibition of the reaction (345, 354). For example, strong inhibition is found among 2-hydroxy acids and 3,5-dimethoxy compounds. An example of the latter situation has been found with sinapic acid (XXVII) which is not decarboxylated when incubated with rat cecal microorganisms (265).

The significance of these *in vitro* microbial decarboxylations with regard to the metabolic fate of phenolic acids in animals has been demonstrated in a number of studies. The decarboxylation of protocatechuic and gallic acids to catechol and pyrogallol, respectively, was observed when the acids were given orally but not intraperitoneally to rats (343). Furthermore, inhibition of the intestinal microflora with antibiotics known to inhibit the reaction *in vitro* reduced or abolished the excretion of the decarboxylated metabolites. Some of the results obtained from metabolism studies with protocatehuic acid have been explained on the basis of this decarboxylation reaction (83, 84). When the ^{14}C-labeled compound was given orally to rats it was found that 72% of the radioactivity was excreted in the urine and feces in 7 days in normal rats whereas 99% was excreted in rats treated with neomycin to inhibit intestinal bacteria. It was concluded that at least part of this 20 to 30% difference in recovery of the ^{14}C is probably due to formation of $^{14}CO_2$ by the intestinal bacteria in the normal animals. The same general picture has been shown with homoprotocatechuic acid (3,4-dihydroxyphenylacetic acid) which was partly decarboxylated to 4-methylcatechol (XXVIII) (344) and with caffeic acid (3,4-dihydroxycinnamic acid, XIX) which was converted to a small extent to 4-vinylcatechol (XXIX) and its reduction product 4-ethylcatechol (345). Experiments with carboxy-^{14}C-labeled homoprotocatechuic acid in rats showed that the ^{14}C was quantitatively excreted in the urine and feces in neomycin-treated rats whereas 93% was accounted for in the normal animals (82). It was suggested that this difference of 7% reflects the degree of metabolism of the compound to $^{14}CO_2$. The lack of decarboxylation of sinapic acid (XXVII) by rat cecal microorganisms has been noted above and this compound was not converted to decarboxylated metabolites when administered orally to rats (175, 266).



XXVIII



XXIX

Numerous studies of the decarboxylation of phenolic acids by particular bacteria have been reported. The microorganism most commonly associated with decarboxylation has been *A. aerogenes* which

forms catechol when incubated with protocatechuic acid (303). In a study of the ability of this species to decarboxylate cinnamic acid derivatives those compounds containing a *p*-hydroxyl group were metabolized whereas related derivatives lacking this group did not undergo decarboxylation (138). The reaction rate was greatest with a single *p*-hydroxyl group and the rate decreased when adjacent substituents were present. Some recent studies with *A. aerogenes* have shown that *p*-hydroxybenzoic acid is converted to phenol (294) and that this phenolic acid as well as protocatechuic acid and gallic acid undergo nonoxidative decarboxylation (169). Gentisic acid was also decarboxylated to a lesser extent and, in this regard, the results differ from those found with mixed cultures of cecal bacteria. The metabolism of several classes of phenolic acids previously shown to undergo decarboxylation by mixed cultures of rat cecal bacteria was investigated with *A. aerogenes* (383). The benzoic acid and cinnamic acid derivatives which contained a *p*-hydroxyl group were decarboxylated. However, no activity was seen with phenylacetic acid derivatives and the decarboxylation of these compounds which has been shown previously in rats (344) and with mixed cecal cultures (345) cannot presently be associated with particular bacteria.

The ability of several strains of *Bacillus* isolated from rat intestine to decarboxylate different classes of phenolic acids has been investigated (211, 383). It was found that the reaction took place only with cinnamic acid derivatives containing a *p*-hydroxyl group. A recent report (296) has dealt with the metabolism of caffeic acid (XIX) by 12 bacteria isolated from human feces. Neither *A. aerogenes* nor *Bacillus* sp. was among these but it was found that *Streptococcus fecium* metabolized caffeic acid to 4-vinylcatechol (XXIX) under anaerobic conditions.

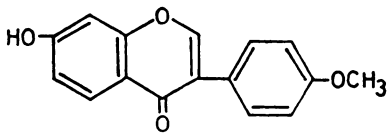
Another example of decarboxylation has been reported with the 8-methyl ether of xanthurenic acid (XXII) (251) although the site of this reaction was not identified. Administration of the compound to mice resulted in only minor conversion to 4-hydroxy-8-methoxyquinoline although more was produced after oral than after subcutaneous dosage. Other reactions including dehydroxylation (section III G 1) and *O*-demethylation (section III I 1) have been shown to be carried out by intestinal microorganisms with this and related compounds. It would therefore be of interest to see whether this is the case with the decarboxylation reaction as well.

The decarboxylation of *o*- and *p*-aminobenzoic acids by cell-free preparations from *E. coli* has been reported (259). However, this reaction is unlikely to have significance in animals as aniline has not been reported to be a metabolite of *p*-aminobenzoic acid (443) and this acid was not found to be metabolized when subjected to the decarboxylating activity of *A. aerogenes* (169) or mixed cultures of rat cecal bacteria (352).

I. Dealkylation

1. *O-Alkyl compounds.* Studies on the metabolism of some naturally occurring plant estrogens by sheep rumen fluid first demonstrated that intestinal bacteria are capable of demethylating foreign compounds (282–284). Biochanin A (4′-*O*-methylgenistein, XXIII) and formononetin (4′-*O*-methyldaidzein, XXX) were demethylated to genistein and daidzein, respectively, when incubated with rumen fluid. These results have been confirmed (25) and they indicate that the methoxylated isoflavones can undergo demethylation while still in the rumen. However, the reaction has also been shown to be carried out in the liver (281). Demethylation of these compounds in the domestic fowl has also been reported (63). However, it was found recently that they are largely resistant to degradation when incubated with rat cecal microorganisms (178).

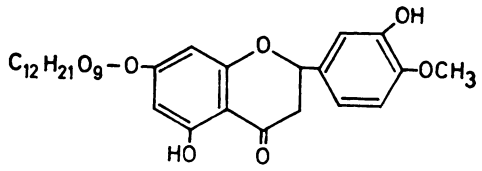
A study of the metabolic fate of the flavonoid hesperidin (XXXI) in rabbits



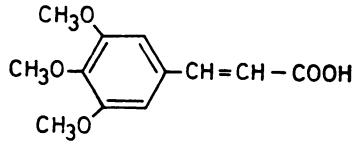
XXX

showed that 3,4-dihydroxyphenylpropionic acid and *m*-hydroxyphenylpropionic acid were among the metabolic products (36). The *O*-demethylation that is required for the formation of these products was suggested to occur in the intestine as a result of bacterial metabolism (345) and it was subsequently shown to take place *in vitro* when hesperidin was incubated with rat cecal contents (347).

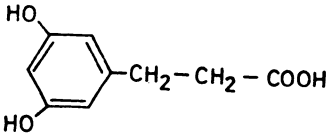
The ability of the intestinal microorganisms to *O*-demethylate compounds was first investigated in a systematic manner with a variety of mono-, di-, and trihydric aromatic acids and their derivatives (345, 354). Many methoxy derivatives of benzoic, phenylacetic, phenylpropionic, and cinnamic acids were found to be demethylated when incubated with rat cecal microorganisms. The few acids studied which contained a methoxyl group as the sole ring substituent were found to be resistent to demethylation (354). Similar results have been noted recently with the corresponding benzaldehyde and benzyl alcohol derivatives (351). However, the metabolism of biochanin A (XXIII) and formononetin (XXX) described above indicates that some methoxyl compounds lacking further ring substitution can be demethylated by the intestinal microorganisms. Di- and trihydric derivatives are generally demethylated fairly readily by mixed cultures of rat cecal microorganisms and a *m*-methoxyl group appears to be more labile than a *p*-methoxyl group (345, 354). This has been confirmed recently in a study of the sequential *O*-demethylation of 3,4,5-trimethoxycinnamic acid (XXXII) by rat cecal microorganisms (265). Several other reports dealing with the metabolism of methyl ethers of trihydric cinnamic and propionic acids in the rat have appeared which emphasize the important role of the intestinal microorganisms in determining the metabolic fate of these compounds (175, 176, 266). These studies showed that the trimethyl ethers were fully demethylated by the intestinal bacteria and, after dehydroxxylation, were excreted in the urine partly as



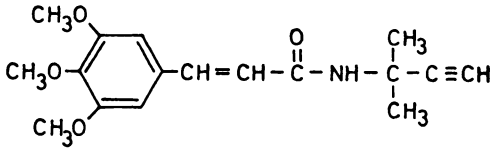
XXXI



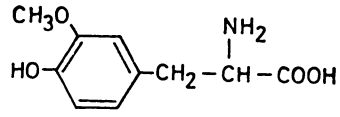
XXXII



XXXIII



XXXIV



XXXV

3,5-dihydroxyphenylpropionic acid (XXXIII). A similar reaction of the intestinal microflora has been described recently with *N* - (3,3 - dimethylpropynyl) - 3,4,5 - trimethoxycinnamide (XXXIV) which is excreted in the urine partly as a 3,5-dihydroxycinnamido derivative after its oral administration to rats (210).

The significance of intestinal *O*-demethyla-

tion in determining the metabolic fate of a compound has been illustrated recently in a study of the demethylation of 3-*O*-methyldopa (XXXV) in the rat (64, 65). This compound, labeled with ^{14}C in the methyl group, was given intraperitoneally and 15 to 20% of the injected radioactivity appeared in the expired air as $^{14}CO_2$ during the next 3 to 4 days. Rats with cannulated bile ducts excreted about 20% of the radioactivity in the bile, probably as a conjugate of the original compound, but $^{14}CO_2$ was no longer present in the expired air. These results show that the demethylation of 3-*O*-methyldopa (XXXV) occurred for all practical purposes exclusively in the intestine after its biliary excretion. The metabolism of the closely related ferulic acid (4-hydroxy-3-methoxycinnamic acid) appears to follow a similar pathway. A major metabolite of this compound when administered orally or intraperitoneally to rats is *m*-hydroxyphenylpropionic acid (35, 345, 407). Ferulic acid is excreted readily in the bile as its glucuronide conjugate (345) and the dehydroxylation reaction leading to the *m*-hydroxy metabolite is known to be due solely to the metabolic activities of the intestinal microorganisms (354). It is therefore clear that the entire sequence of reactions including deconjugation, hydrogenation of the double bond, *O*-demethylation, and dehydroxylation occurs in the intestine.

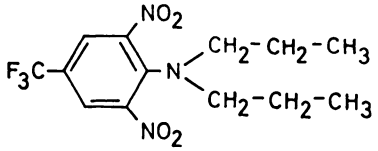
The metabolism of the 8-methyl ether of xanthurenic acid (XXII), which is a urinary metabolite of tryptophan shown to have carcinogenic properties, has been studied in rabbits (252). It is converted to *O*-demethylated metabolites after oral administration and this reaction was inhibited severely when the intestinal microflora was suppressed with neomycin sulfate. These results therefore furnish a further example showing that intestinal demethylation may be quantitatively far more important than that which occurs in the tissues.

The above examples of *O*-dealkylation are restricted to methoxyl compounds and no reports of the dealkylation of higher homologues have been published. However, a few preliminary studies of the metabolism of some ethyl ethers of phenolic acids and aldehydes by mixed cultures of rat cecal microorganisms under conditions which readily demethylated the corresponding methyl ethers failed to show any evidence of loss of the ethyl group (352).

Further studies on intestinal *O*-dealkylation are certainly warranted, especially in regard to the nature of the reaction involved. Although it is possible that the methyl group is lost as methane, the experiments with 3-*O*-methyldopa described above (64, 65) which detected the radioactivity as $^{14}CO_2$ in the expired air may point to another entity.

2. N-Alkyl compounds. Examples of *N*-dealkylation by intestinal microorganisms have appeared only recently. The herbicide trifluralin (XXXVI) is degraded to numerous products by rumen fluid (161) and by two strains of rumen bacteria (442). Several of these metabolites were characterized as compounds lacking one of the *N*-propyl groups and it was postulated that both of these groups can be removed by the rumen microorganisms. This seems likely in view of the finding of a fully dealkylated metabolite in the feces of a goat after oral administration of trifluralin (161). The distribution of radioactivity in the urine and feces of ruminants given ^{14}C-trifluralin is similar to that seen in rats (130) although dealkylated products are urinary metabolites in the latter case. It has been suggested (348) that dealkylation of trifluralin in rats is a tissue reaction but the findings now available indicate that the intestine may be the site of this reaction.

A recent study has dealt with the extrahepatic metabolism of some psychotropic drugs (271). Imipramine is converted to desmethylimipramine by gastric and in-



XXXVI

testinal contents of rats and by lower intestinal contents of man. It was calculated that the extent of imipramine metabolism in the rat is divided about equally between the liver and the gastrointestinal contents.

Methamphetamine is *N*-demethylated when incubated anaerobically with cecal or rectal contents of guinea pigs (60). The demethylation of methamphetamine and 4'-hydroxymethamphetamine was examined with representative strains of the main bacterial genera in the mammalian intestine. Demethylation activity with both substrates was highest among the enterococci and lactobacilli with some activity being shown by clostridia. Little, if any, *N*-demethylation occurred in the incubates with enterobacteria, bacteroides, or bifidobacteria.

3. Other alkyl derivatives. Studies on the metabolic fate of some organotin compounds have shown that ethyltin trichloride is metabolically inert when administered to rats whereas diethyltin dichloride is dealkylated to ethyltin to an appreciable extent (55, 56, 98). Diethyltin containing a ^{14}C ethyl group was not metabolized to radioactive CO_2 and it was suggested that the ethyl group is lost as ethane. The results obtained indicated that the reaction takes place in both the tissues and the intestinal tract. Dealkylation at the latter site is undoubtedly of microbiological origin as diethyltin was converted to ethyltin when it was incubated anaerobically with rat cecal contents.

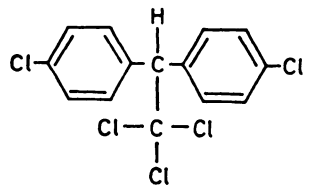
The metabolism of methyl mercuric chloride in germ-free and control rats has been investigated recently in order to determine whether the cleavage of the carbon-mercury bond which is observed with this compound is due to the intestinal microorganisms (287). The results obtained failed to show any differences between the two groups with regard to the amounts of inorganic mercury found in the cecal contents or feces and it was concluded that the microflora is not involved in this reaction.

The metabolism of triphenyl-lead acetate has been studied in rats (439) and may be included conveniently in this section. Oral administration of the ^{14}C-labeled compound resulted in excretion of 20% of the dose in the expired air in 7 days. This radioactivity was found to be present nearly entirely as benzene. In addition, the known metabolites of benzene were excreted in the urine. It was suggested that triphenyl-lead acetate is not absorbed as such from the intestine but is decomposed in the intestine, possibly by the intestinal microorganisms, to give benzene which is then absorbed.

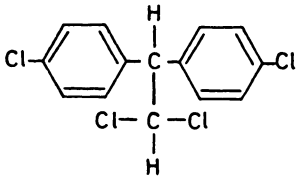
J. Dehalogenation

While metabolic dehalogenation is known to occur with organic chlorine, bromine, and iodine compounds, evidence for dehalogenation by the intestinal microorganisms has been presented only for chlorinated compounds. An early example of dechlorination which was explained by the action of intestinal bacteria was the finding that rabbits given tetrachlorobenzenes orally excreted di- and trichlorobenzenes in the expired air and di- and trichlorophenols in the urine during the first day or two (215). However, intraperitoneal administration of the tetrachlorobenzenes resulted in the urinary excretion of only the corresponding tetrachlorophenols during this initial period. The di- and trichlorophenols were excreted in the urine in increasing amounts 4 to 8 days after administration of the dose and this was presumed to result from slow excretion of tetrachloro compounds in the bile followed by bacterial dehalogenation. In a subsequent investigation with 1,3,5-trichlorobenzene and pentachlorobenzene, these compounds were similarly dechlorinated when given to rabbits and the reaction was again suggested to be carried out by the intestinal bacteria (293).

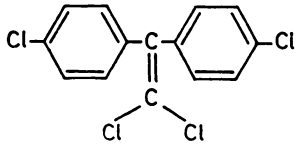
The compound which has received most attention in regard to dehalogenation by intestinal microorganisms is DDT (1,1,1-trichloro - 2,2 - bis (*p* - chlorophenyl) - ethane, XXXVII). Reductive dechlorination of DDT gives rise to DDD (1,1-dichloro-2,2-bis (*p*-chlorophenyl)-ethane,



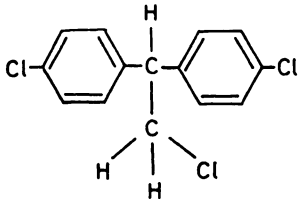
XXXVII



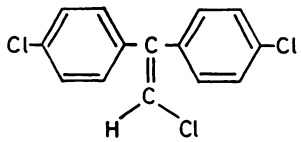
XXXVIII



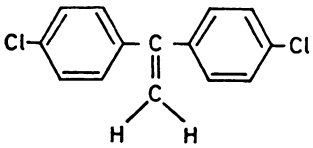
XXXIX



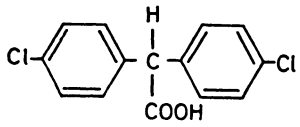
XL



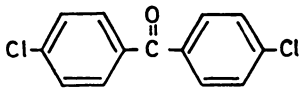
XLI



XLII



XLIII



XLIV

XXXVIII) and this compound was shown to be a metabolite of DDT in rats (301). Also of interest was the finding that DDD was formed by liver homogenates under conditions of putrefaction and it was proposed that, under these conditions, bacterial conversion of DDT may be partly responsible for the presence of DDD. Another report concluded that DDD is produced from DDT only in decomposing animal tissue and not in normal live tissues (20). Shortly thereafter, several investigations showed that this dechlorination reaction could be carried out by intestinal microorganisms. This was found with *Pr. vulgaris* isolated from mouse intestine (22), *E. coli*, and *A. aerogenes* isolated from rat feces (264) as well as several other representatives of intestinal bacteria (391). The latter investigation also showed that bacteria from the intestines of flies converted some of the DDT into DDE (1,1-dichloro-2,2-bis (*p*-chlorophenyl)-ethylene, XXXIX). Furthermore, the strain of *Pr. vulgaris* which was found to convert DDT to DDD was subsequently shown to metabolize the latter compound by reductive dechlorination to DDMS (1-chloro-2,2-bis-(*p*-chlorophenyl)-ethane, XL) and by dehydrochlorination to DDMV (1-chloro-2,2-bis (*p*-chlorophenyl)-ethylene, XLI) (21). The metabolic products formed from DDT when incubated with whole cells or cell-free extracts of several microorganisms including *A. aerogenes* and *E. coli* have been studied in detail (427–429). In the case of *A. aerogenes* at least seven metabolites were formed including those shown in chemical structures XXXVIII, XXXIX, XL, XLI, DDNV (unsym-bis (*p*-chlorophenyl)ethylene, XLII), DDA (2,2-bis (*p*-chlorophenyl) acetate, XLIII), and DBP (4,4′-dichlorobenzophenone, XLIV). It is noteworthy that

none of these reactions involve aromatic dechlorination.

Investigations of the metabolism of DDT by intestinal microorganisms in fish have shown that the microflora plays an important role in the detoxication of DDT in the rainbow trout, *Salmo gairdneri* (430) and in the northern anchovy, *Engraulis mordax* (255). Rumen microorganisms have been shown to be very effective in converting DDT to DDD (XXXVIII) (147, 235, 272) and a recent report has shown that DDE (XXXIX) and, tentatively, DDMV (XLI) are also formed (364).

K. Deamination

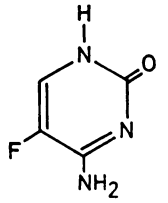
Deamination by intestinal microorganisms appears to be very largely a reaction of amino acids. Consequently much of the subject falls outside the scope of this review and and attention will be given only to amino acids employed as drugs or to compounds closely related to these.

The deamination of 3,5,3′-triiodo-L-thyronine was shown to be carried out by suspensions of rat intestinal bacteria (71). Similar experiments with DL-dopa showed that deamination and dehydroxylation occurred to produce *m*-hydroxyphenylpropionic acid (45). However, DL-dopa was earlier found to be metabolized to *m*-hydroxyphenylacetic acid when given to rats (107). A recent reinvestigation of this subject with L-dopa has shown that its anaerobic incubation with mixed cultures of rat cecal bacteria resulted in the formation of both the phenylacetic and phenylpropionic acid derivatives (17). In agreement with the earlier study, only phenylacetic acid derivatives were found in the urines of rats given dopa (16). *m*-Hydroxyphenylacetic acid has also been reported to be a urinary metabolite of L-dopa in man and its excretion was reduced when the intestinal microflora was suppressed by neomycin (338). Deamination of the closely related amino acid, tyrosine, has been reported to occur upon incubation with sheep rumen microorganisms (359) and rat cecal contents (15). Deamination of other amino acids including phenylalanine and tryptophan have been shown with microorganisms from sheep rumen (359) and from monkey feces (189).

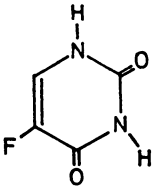
The oral administration of 5-fluorocytosine (XLV) to rats resulted in its deamination by the intestinal microflora to 5-fluorouracil (XLVI) (231).

L. Heterocyclic Ring Fission

The cleavage of heterocyclic ring systems of xenobiotic compounds by intestinal microorganisms often results in metabolic products quite unlike those which can be formed in the tissues. These reactions serve as excellent illustrations of the metabolic ingenuity which is possessed by the microflora. This factor undoubtedly has much to do with the growing interest in the metabolism of heterocyclic compounds, especially flavonoids, by intestinal bacteria. Heterocyclic ring fission has been demonstrated in chemically diverse ring systems containing either oxygen or nitrogen. However, relatively little systematic work aimed at assessing the scope of these microbial reactions has been carried out and this general area appears likely to be a profitable one for future investigations.

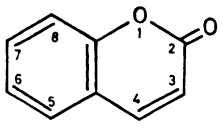


XLV

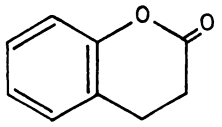


XLVI

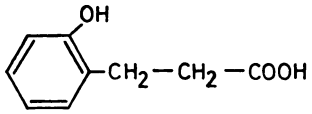
A class of oxygen ring compound of relatively simple type which has been studied in regard to its metabolism by intestinal microorganisms is that of the coumarins.



XLVII



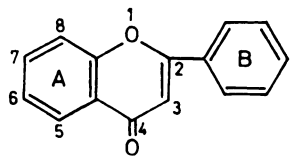
XLVIII



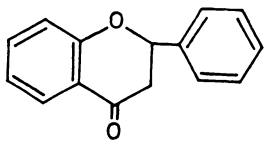
XLIX

Coumarin itself (XLVII) undergoes scission to melilotic acid (XLIX), presumably by way of the reduced intermediate dihydrocoumarin (XLVIII) when incubated anaerobically with mixed cultures of rat cecal microorganisms (346). The phenolic acid was found to be a urinary metabolite of coumarin-treated rats and the data supported the view that most, if not all, of it was of intestinal origin. This subject was studied subsequently more fully with 7-hydroxycoumarin (umbelliferone) and 7-methoxycoumarin (herniarin) (212). Again, phenylpropionic acid derivatives were formed from the coumarins upon incubation with rat cecal microorganisms and these metabolites were detected in the urine of rats given umbelliferone or herniarin. With the use of germ-free rats it was shown that the intestinal microorganisms were solely responsible for the formation of the phenylpropionic acids. An investigation of the scission of coumarin by members of 9 genera of intestinal bacteria was able to demonstrate only minor activity with one of these (*A. aerogenes*) (383).

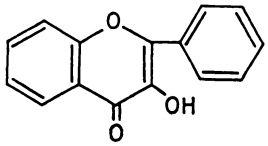
As mentioned above, studies on the metabolism of flavonoid compounds have furnished some of the most interesting examples of the metabolic capabilities of the intestinal microorganisms. In addition, a multiplicity of reactions is involved in the overall pathways of metabolism as hydrolysis of glycosidic linkages, dehydroxylation, demethylation, and reduction of double bonds may also be involved. Details of these reactions are found in sections III A 2, G, I 1, and M and the present section will deal instead with the ring fission reactions observed with flavonoid compounds and with some of the structural requirements for flavonoid degradation. Structural formulas of some of the major types of flavonoid compounds including the numbering and designation of the rings are shown below. The types depicted are flavone (L), flavanone (LI), flavonol (LII), flavanol (LIII), flavylium cation (LIV), isoflavone (LV), and dihydrochalcone (LVI).



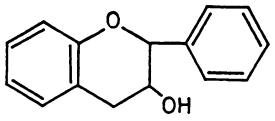
L



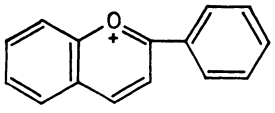
LI



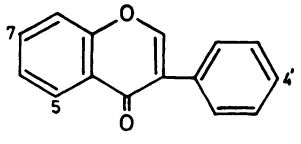
LII



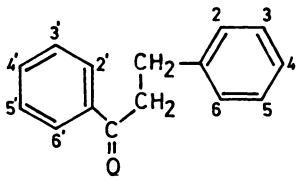
LIII



LIV



LV



LVI

One type of flavonoid which has received considerable attention from a metabolic point of view is that of the flavonols (LII). Quercetin (3, 4, 7, 3′, 4′-pentahydroxyflavone) and its glycoside rutin are the best known examples of flavonols and the existence of numerous reports on their fate in animals largely reflects their widespread occurrence in plants and their use as therapeutic agents (105, 148). An early study of the metabolism of rutin and some derivatives showed that their intestinal absorption was negligible but that they were not present in the feces (70). Incubation of rutin with fecal samples resulted in extensive degradation of the compound although no breakdown products were identified. Subsequently, several investigations of the metabolism of rutin and quercetin in animals were carried out. It was initially shown that the flavonols undergo extensive degradation and are ultimately excreted in the urine as 3-hydroxy-, 3,4-dihydroxy-(homoprotocatechuic acid) and 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid) (40, 41). These results were confirmed later with ^{14}C-quercetin given orally to rats (257, 302). Formation of these metabolites apparently arose from cleavage of the heterocyclic ring at the 1,2- and the 3,4-positions. Thus, they are derived from the catechol portion (B-ring) of the molecule and not from the phloroglucinol portion (A-ring). These findings make it clear that a major metabolic pathway in the metabolism of quercetin or its glycoside rutin leads to the formation of phenylacetic acid derivatives. However, several other reports have indicated that the overall metabolic picture is more complex as derivatives of benzoic, phenylpropionic, and cinnamic acids as well as a neutral compound characterized as an *o*-dihydroxy lactone have been detected in the urine of rats given quercetin (54, 278, 302).

Investigations on the site of formation of the quercetin metabolites have been carried out and it was reported that rat kidney homogenates were able to convert the flavonol to 3,4-dihydroxybenzoic acid under aerobic conditions (116). On the other hand, no degradation occurred when quercetin was placed in the ligated rat stomach (54). Although the degradation of rutin by various molds, streptomycetes, and bacteria had been reported (434), an interesting finding showed that it was converted to *m*-hydroxyphenylpropionic acid when incubated with rat intestinal microorganisms (45). This was confirmed subsequently with both quercetin and rutin and it was also found that *m*-hydroxyphenylacetic acid was formed (347). These results make it clear that several of the main urinary metabolites of these flavonols can be formed entirely by the intestinal microflora. Subsequent tissue reactions including β-oxidation, methylation, and dehydrogenation explain the presence of other urinary metabolites. Support for the key role played by the microflora in the metabolism of quercetin was obtained in experiments with neomycin-treated rats which no longer excreted the three characteristic hydroxyphenylacetic acids. Hydroxyethylation of the 5-, 7-, 3′-, and 4′-hydroxyl groups of quercetin results in a derivative which no longer undergoes ring fission by the intestinal bacteria (24a).

Several recent investigations have directed attention to the anaerobic degradation of some flavonoids including rutin by rumen microorganisms. Incubation with rumen fluid resulted in the degradation to phenolic compounds which appeared to be similar to those formed in the rat intestinal tract (363). Subsequently, numerous strains of

Butyrivibrio sp. isolated from bovine rumen contents were shown to degrade rutin anaerobically (67) and several phenolic compounds including phloroglucinol, 3,4-dihydroxybenzaldehyde, and 3,4-dihydroxyphenylacetic acid were identified as metabolic products (233). The results from the latter investigation indicate that the metabolic pattern is not identical to that observed with rat intestinal microorganisms. Also, the *Butyrivibrio sp.* metabolized quercetin glycosides whereas the aglycone was not degraded.

The finding cited above that rumen microorganisms degrade a quercetin glycoside to phloroglucinol (233) is of interest in relationship to the fate of the A-ring. An earlier study reported that phloroglucinol, phloroglucinol carboxylic acid, and 3,4-dihydroxybenzoic acid were formed when rats were given quercetin (221). However, it was suggested subsequently that, instead of being metabolic products, these compounds were artifacts arising from the chemical degradation of quercetin during sample preparation (257). Subsequently, the latter workers found that very small amounts of phloroglucinol could sometimes be detected in extracts of rat feces incubated with quercetin, especially when shorter incubation periods were used (106). The transient nature of this metabolite has also been noted in experiments with bovine rumen microorganisms (363). It is possible that quercetin degradation can proceed along more than one pathway. One of these would allow the A-ring to remain intact and thus give phloroglucinol carboxylic acid and phloroglucinol while the other would involve destruction of the A-ring and result in C_6—C_2 and C_6—C_3 phenolic acids. The relative significance of these routes may be a reflection of the types and relative numbers of microorganisms present in the intestinal microflora. However, it may also be significant that a dihydrochalcone derivative (see LVI) was cleaved to *p*-hydroxyphenylpropionic acid and phloroglucinol when incubated with rat cecal microorganisms (178). On the other hand, no process of simple fission of the flavonoid molecule will allow for the formation of both phloroglucinol carboxylic acid and the C_6—C_2 derivative and clarification of this subject will require further investigation.

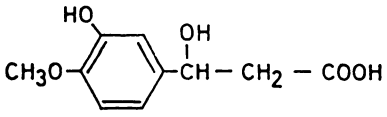
While the metabolism of other flavonols has been investigated, fewer reports are available than is the case with quercetin and its glycoside rutin. Nonetheless, experiments with normal and antibiotic-treated animals and with cultures of intestinal microorganisms have shown much the same pattern of metabolism with most of these related compounds (177–179, 371). Kaempferol (3, 5, 7, 4′-tetrahydroxyflavone) and its glycoside robinin (kaempferol 7-rhamnosido-3-galactorhamnoside) are degraded to the expected 4-hydroxyphenylacetic acid when incubated with rat cecal microorganisms and this phenolic acid is excreted in the urine of rats given these flavonols (178). Related flavonols having a 3, 5, 7-hydroxylation pattern are myricetin (3, 5, 7, 3′, 4′, 5′-hexahydroxyflavone) and its 3-rhamnoside, myricitrin. These compounds are metabolized in rats and by rat cecal microorganisms to phenylacetic acid derivatives (177, 179, 371). The conversion in rats was prevented when the animals were treated with neomycin before and during administration of the flavonoid. Robinetin (3, 7, 3′, 4′, 5′-pentahydroxyflavone) differs from myricetin only in the absence of the 5-hydroxyl group and it might seem reasonable to assume that the metabolic products would be identical to those from the hexahydroxy compound. However, in similar experiments only unchanged robinetin is present in the urines or incubates. These results suggest that hydroxylation of the A-ring at the 5-position is essential for ring fission. Other findings have been presented which show that the hydroxyl group at the 3, 5, and 7 positions must be free as the 3-, 5-, or 7-methyl ethers of quercetin were not metabolized when given orally to rats (106). Likewise, no urinary metabolites of 3, 5, 6, 7, 4′-pentamethoxyflavone (tangeretin) were detected.

Flavones (L) differ structurally from the flavonols in that they lack a hydroxyl group at the 3-position. That this deficiency is not decisive in regard to their ability to undergo metabolic degradation was shown in an early study of flavonoid metabolism which included diosmetin (5, 7, 3′-trihydroxy-4′-methoxyflavone) and its 7-rutinoside diosmin (36). The major urinary metabolite of these compounds after oral dosage to rats was *m*-hydroxyphenylpropionic acid and small amounts of the related cinnamic acid, *m*-coumaric acid, were also found. Thus, ring fission takes place in the absence of a 3-hydroxyl group but the major degradative product is a C_6—C_3 compound (phenylpropionic acid) rather than the C_6—C_2 compounds (phenylacetic acids) which are largely formed from the flavon-3-ols.

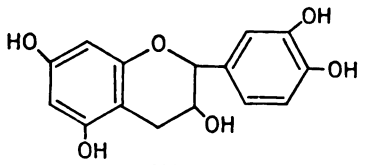
Recent reports on the metabolism of a large number of flavones in the rat and by intestinal microorganisms have clarified considerably the structural requirements for ring fission of these compounds (177–179, 371). Apigenin (5, 7, 4′-trihydroxyflavone) was metabolized to *p*-hydroxyphenylpropionic acid by rat cecal microorganisms and the latter compound, together with its metabolites *p*-hydroxycinnamic acid and *p*-hydroxybenzoic acid, was also a major urinary metabolite of the flavone in rats (178). Similar results were obtained with apiin (apigenin 7-apiosylglucoside). The general pattern of degradation was not altered as a result of increased substitution of the B-ring as both tricetin (5, 7, 3′, 4′ 5′-pentahydroxyflavone) and tricin (5, 7, 4′-trihydroxy-3′,5′-dimethoxyflavone) were partly converted to phenolic phenylpropionic acid derivatives when administered orally to rats or incubated with rat cecal microorganisms (179).

Experiments with 2 flavones lacking substitution in the B-ring [chrysin (5,7-dihydroxyflavone) and tectochrysin (5-hydroxy-7-methoxyflavone)] have indicated that the intestinal microorganisms are not capable of cleaving flavonoids of this type (178). Acidic metabolites therefore were not excreted in the urines of rats given these compounds although some hydroxylation of the B-ring occurred in the tissues. However, the nature of the B-ring substituents appears to be of importance and the degradation of the heterocyclic ring is impeded when methoxyl groups and not hydroxyl groups are present. This was shown with acacetin, the 4′-methyl ether of apigenin, and with the 3′,4′,5′-trimethyl ether of tricetin which underwent little or no fission to phenylpropionic acid derivatives under the usual *in vivo* or *in vitro* conditions (178, 179). While it has been postulated that flavonoids lacking a free 4′-hydroxyl group have a decreased susceptibility to ring fission (178), the results noted above with diosmetin and diosmin (36) indicate that extensive degradation of a 4′-methoxyl flavonoid may occur. However, it is also possible that the flavonoid may initially undergo *O*-demethylation of this group as many methoxyl compounds similar in structure to the B ring of flavonoids are cleaved readily by intestinal microorganisms (section III I 1). Another finding dealing with the structural requirements for ring fission points to the necessity of a 5-hydroxyl group in flavones as well as in flavonols as mentioned above. When 7,4′-dihydroxyflavone was incubated with rat cecal microorganisms no metabolites could be detected (178). Also, none of the expected *p*-hydroxyphenyl acids were excreted in the urine of rats given the flavone orally. It has been reported previously that the unsubstituted parent compound, flavone, is not degraded to ring fission products in the rat (94).

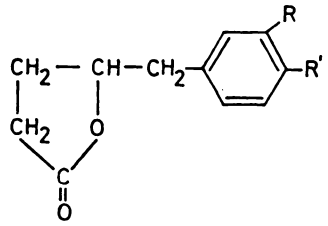
Flavanones (LI) make up another major type of flavonoid compound and differ from flavones only in the lack of a double bond at the 2,3-position. This chemical difference has not been found to alter their metabolic fate which is similar to that observed with the flavones. Hesperetin (5,7,3′-trihydroxy-4′-methoxyflavanone), its 7-rhamnoglucoside (hesperidin, XXXI), eriodictyol (5,7,3′,4′-tetrahydroxyflavanone), and homoeriodictyol (5,7,4′-trihydroxy-3′-



LVII



LVIII



LIX

methoxyflavanone) all underwent ring fission when given orally to rats (36). As with flavones, the degradation led to C_6—C_3 phenolic acids and *m*-hydroxyphenylpropionic acid was the principal urinary metabolite. Thus, dehydroxylation and/or *O*-demethylation are also involved in the metabolism of these flavonoids in the rat. Interestingly, these reactions appear to be of little importance in the human as oral administration of hesperetin or hesperidin led instead to the urinary excretion of a new metabolite which was identified as 3-hydroxy-4-methoxyphenylhydracrylic acid (LVII) (36). A similar study was made with naringenin (5,7,4′-trihydroxyflavanone) and its 7-rhamnoglucoside, naringin (37). Again, fission of the heterocyclic ring occurred and, in rats, the principal metabolic product was *p*-hydroxyphenylpropionic acid. In man, however, oral administration of naringin resulted only in the urinary excretion of naringenin and its glucuronide conjugate.

Studies which show that flavanones and their glycosides can be fully degraded to phenylpropionic acid derivatives by rat intestinal microorganisms have been carried out with hesperetin and hesperidin (347) and with naringin (178). The intestinal reactions are thus able to account fully for the major urinary metabolites formed from these flavonoids and subsequent dehydrogenation and β-oxidation of the phenylpropionic acids in the tissues give rise to the cinnamic and benzoic acid derivatives that are usually detected. Degradation of naringin and hesperidin to water-soluble phenolic products upon anaerobic incubation with bovine rumen microorganisms has been reported (363).

The flavanols differ structurally from the 3 previous types of flavonoids by their lack of a keto group at the 4-position. While metabolic investigations have been carried out with a limited number of flavanols, the number of studies and detail of information on one of these (+)-catechin (3,5,7,3′,4′-pentahydroxyflavan, LVIII), are greater than with any other flavonoid compound.

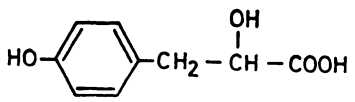
The initial metabolic studies with (+)-catechin showed that it was converted to several simple phenolic acids and to neutral compounds when given to rabbits (290, 291). The major acidic metabolites were identified as 3-hydroxy-, 3,4-dihydroxy-, and 4-hydroxy-3-methoxy-benzoic acid and the 3 neutral compounds excreted were postulated to be phenolic derivatives of phenylvalerolactone. A detailed investigation of the latter compounds showed that they were δ-(3-hydroxyphenyl)-γ-valerolactone (LIX, R=OH, R′=H) δ-(3,4-dihydroxyphenyl)-γ-valerolactone (LIX, R=R′=OH), and δ - (4 - hydroxy - 3 - methoxyphenyl) - γ - valerolactone (LIX, R=OCH_3, R′=OH) (421–424). A subsequent study in rats established that *m*-hydroxyphenylpropionic acid was formed from (+)-catechin and, on the basis of its excretion profile, it was suggested that its formation may be due to the action of the intestinal microflora (173). This reaction was soon shown to take place when the flavonoid was incubated anaerobically with rat intestinal microorganisms (45) and several subsequent investigations have confirmed these results (92, 347). It was also found that the phenylvalerolactones observed earlier as urinary metabolites were formed in the incubates (92).

Several further investigations of the meta-

bolic fate of (+)-catechin in rats (96, 97, 174), guinea pigs (95, 96), and man (93) have been carried out which both add considerably to our understanding of many of the details involved in catechin metabolism and substantiate the picture outlined above. The phenylvalerolactones have been shown to be intermediates in the formation of the phenolic acids as δ-(3-hydroxyphenyl)-γ-valerolactone given orally to guinea pigs is excreted in the urine partly as *m*-hydroxyphenylpropionic acid, *m*-hydroxybenzoic acid, and *m*-hydroxyhippuric acid (95). It seems reasonable to assume that the general pattern of (+)-catechin degradation is the same in the different animal species studied although the nature of the ultimate urinary metabolites probably reflects differences in the tissue metabolism of the terminal intestinal metabolites. Phenylpropionic acid derivatives are major urinary metabolites in rats and man whereas further metabolism of these in the tissues to benzoic and hippuric acid derivatives is seen in rabbits and guinea pigs. The microbial breakdown of (+)-catechin to higher homologues of the phenolic acids mentioned above has been reported (349) although this reaction is likely of limited importance when the flavonoid is given to animals. In this case (+)-catechin was converted to 5-(3-hydroxyphenyl)-valeric acid and 5-(3,4-dihydroxyphenyl)-valeric acid. This degradation to C_6—C_5 phenolic acids was the major reaction detected when (+)-catechin was incubated anaerobically with rabbit intestinal microorganisms whereas only small amounts were formed when rat cecal microorganisms were used.

Elucidation of the structures of the lactone metabolites of (+)-catechin has been an important advance in our understanding of the metabolic degradation of flavonoids. Unfortunately, the possible significance of related intermediates in the breakdown of other types of flavonoids remains largely a matter of speculation. Nonetheless, it may be significant that one of the urinary metabolites of quercetin in rats was found to be a lactone very similar to, but not identical with, LIX, R=R′=OH (54). Another finding which is of interest in this context is that (−)-epiafzelechin (3,5,7,4′-tetrahydroxyflavan), the only flavan-3-ol other than (+)-catechin that has been studied, was found to be metabolized to two neutral compounds when incubated anaerobically with rat cecal microorganisms (178). These were not identified but they were reported to be phenolic compounds. A third phenolic metabolite formed under these conditions was *p*-hydroxyphenylpropionic acid.

The flavylium cation (LIV) is a structure common to flavonoids of the anthocyanin type which are found as pigments in flowers, fruits, and leaves. An early investigation of the metabolic fate of the anthocyanin pigment from Concord grapes showed that no loss of color occurred as a result of incubation with extracts of human feces (206). Cyanidin chloride (3,5,7,3′,4′-pentahydroxyflavylium chloride) was not converted to phenolic metabolites when incubated anaerobically with rat cecal microorganisms (347). This finding has subsequently been confirmed (178). Cyanidin chloride has the same hydroxylation pattern as (+)-catechin and otherwise differs from the latter compound only by the presence of the cation. It has been postulated that this structural difference is the feature responsible for the metabolic stability of flavylium compounds (348). However, recent investigations (178, 179) have shown that some of these compounds can be metabolized when given orally to rats or incubated with intestinal microorganisms. Perlargonin (3,5,7,4′-tetrahydroxyflavylium chloride 3,5-diglucoside) was converted to a phenolic compound tentatively identified as *p*-hydroxyphenyllactic acid (LX) when incubated with rat cecal microorganisms (178). Similar experiments with delphinidin (3,5,7,3′,4′,5′-hexahydroxyflavylium chloride) resulted in the formation of two unidentified metabolites having, respectively, neutral and acidic characteristics whereas malvin (3,5,7,4′-tetrahydroxy-3′,5′-dimethoxyflavylium



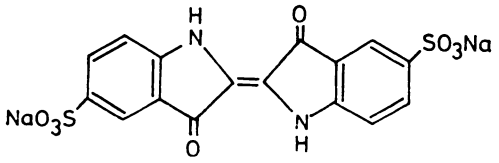
LX

chloride) did not undergo detectable metabolism (179). The acidic metabolite of delphinidin was also excreted in the urine of rats given this flavonoid orally. Malvin gave rise to three unidentified neutral metabolites under similar conditions. Thus, it appears that flavylium compounds may undergo metabolic alteration although to a much more limited extent than is the case with related flavonoids lacking the cationic group. Further information will be needed before the metabolic pathways involved are clarified.

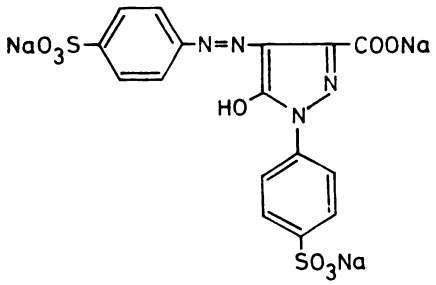
The dihydrochalcones (LVI) are related structurally to the flavanones (LI) and both types of compounds appear to share the same metabolic fate. This was first shown with pholoretin (2′,4,4′,6′-tetrahydroxydihydrochalcone) and its 2′-β-glucoside, phlorizin, which were metabolized in rats to *p*-hydroxyphenylpropionic acid, *p*-hydroxycinnamic acid, and *p*-hydroxybenzoic acid sulfate, the same metabolites seen after giving naringenin and naringin, the corresponding flavanones (37). It was later reported that this metabolic similarity occurred when the compounds were incubated with rat cecal microorganisms (106). Under these conditions phlorizin was converted to *p*-hydroxyphenylpropionic acid. These results have been confirmed recently (178).

The basic structure of isoflavonoid compounds differs from that of the types listed above in that the B-ring is attached to the heterocyclic ring at the 3-position instead of the 2-position. Four isoflavones (LV) have been investigated in regard to their metabolism in animals and by intestinal microorganisms. These are daidzein (7,4′-dihydroxyisoflavone), genistein (5,7,4′-trihydroxyisoflavone), formononetin (7-hydroxy-4′-methoxyisoflavone, XXX), and biochanin A (5,7-dihydroxy-4′-methoxyisoflavone, XXIII). However, it must be pointed out that the metabolic reactions so far described for these compounds do not always involve fission of the heterocyclic ring; for example, daidzein and formononetin are both converted to the isoflavan equol (XXIV) when incubated anaerobically with rat cecal microorganisms (178). Similar results were obtained previously with sheep rumen fluid (284). Equol is also a metabolite of genistein (62) and of biochanin A and formononetin (63) in the domestic fowl. However, the site of these conversions was not determined. On the other hand, rupture of the heterocyclic ring also occurs as genistein was metabolized readily by rat cecal microorganisms to *p*-ethylphenol (178), a urinary metabolite of this isoflavonoid in sheep (25). These findings suggest that hydroxylation in the 5-position of a A-ring may be as important for the heterocyclic ring fission of the isoflavonoids as it appears to be for the degradation of flavones and flavonols (178). The degradation of biochanin A by intestinal microorganisms is poorly understood although unidentified metabolites are formed (178, 284). However, intraruminal administration of the compound to sheep results in extensive metabolism to *p*-ethylphenol (25). Fission of the heterocyclic ring probably occurs subsequent to the demethylation of biochanin A. The latter reaction is carried out by intestinal microorganisms with both of the aforementioned 4′-methoxyisoflavones and is discussed in section III I 1.

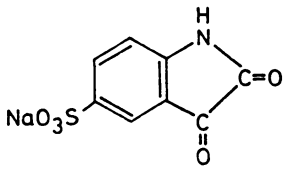
Several examples of the fission of nitrogen-containing ring systems by intestinal microorganisms are known. Interesting metabolic pathways have been demonstrated with the food colors indigo carmine (LXI) and tartrazine (LXII) which are derivatives of indole-3-one and pyrazole, respectively. Incubation of indigo carmine with rat intestinal contents resulted in the fading of the blue color of the dye and the appearance of the degradation products isatin-5-sulfonic acid (LXIII) and 5-sulfoanthranilic acid (LXIV) (249). Small amounts of these metabolites were excreted in the urine of rats given the dye and it was suggested that these may partly be due to



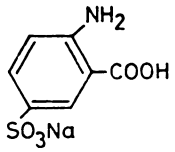
LXI



LXII



LXIII

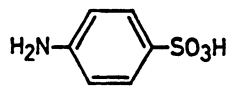


LXIV

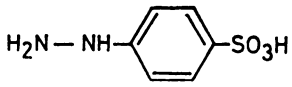
absorption of the degradation products from the intestine after their formation by the intestinal bacteria.

The metabolism of azo compounds by the intestinal microorganisms is discussed in section III O which underlines the importance of the reductive fission of the azo linkage. In the case of tartrazine (LXII) this process leads to the formation of sulfanilic acid (LXV) arising from cleavage of the *p*-sulfophenylazo portion of the molecule. However, experiments with tartrazine labeled with ^{35}S in the *p*-sulfophenyl moiety of the pyrazolone ring showed that radioactive sulfanilic acid was both excreted in the urine after oral dosage of the dye to rats and produced when the dye was incubated with rat intestinal contents (325). Thus, a further source of sulfanilic acid exists in the pyrazolone portion of the molecule. As the above experiments also showed the presence of small amounts of *p*-sulfophenylhydrazine (LXVI) it was believed that the ring is first cleaved to the hydrazine derivative and the latter metabolite then reduced to sulfanilic acid. A subsequent report (334) has confirmed this as the substituted aminopyrazolone formed from the reductive fission of the azo group of tartrazine was converted to aromatic amines when given orally to rats. Furthermore, *p*-sulfophenylhydrazine was readily metabolized to sulfanilic acid after oral, but not intraperitoneal, dosage. Additional evidence for the intestinal origin of this reaction sequence was obtained in experiments which showed that the hydrazine derivative was converted to sulfanilic acid when incubated with rat intestinal contents (333). Finally, the same metabolic picture was seen when a tartrazine analogue containing a methyl group in place of the carboxyl group in the pyrazolone ring was employed.

A recent investigation has shown that the imidazole ring of histamine is split by rumen microorganisms and that CO_2 is a degradation product (367). A subsequent study showed that this activity was also present in the feces of half of the human subjects studied (368).



LXV



LXVI

A further example of the rupture of a nitrogen-containing ring by intestinal bacteria is seen with the penicillins and related antibiotics containing a β-lactam ring. This topic is discussed briefly in section III C.

M. Reduction of Double Bonds

The bacterial reduction of double bonds in foreign compounds has very largely dealt

with various derivatives of cinnamic acid. However, it should also be mentioned that much work has been carried out on the hydrogenation of unsaturated fatty acids, especially with regard to their metabolism in the rumen. While this area falls somewhat outside the scope of the present view, brief mention of some of the pertinent findings may be worthwhile.

Mixed rumen bacteria hydrogenated linoleic and oleic acids under anaerobic conditions (275, 306). Of the numerous strains investigated, only *Butyrivibrio fibrisolvens*, a common rumen microorganism, was able to hydrogenate linoleic acid (306). A pathway for the probable hydrogenation of linoleic acid to stearic acid by rumen bacteria has been proposed (438). Again, the ability of pure cultures of intestinal bacteria to hydrogenate linoleic acid was limited to a few strains and in some cases it resulted in incomplete hydrogenation. Further reports have appeared on isolated strains of rumen bacteria capable of hydrogenating fatty acids (228, 436, 452). Hydrogenation of linoleic and oleic acids in the rumen is not dependent on the presence of ciliate protozoa (102). A study on the hydrogenation of ricinoleic acid, a constituent of castor oil, found that it was hydrogenated to hydroxystearic acid in both the rat and human intestine (426). The hydrogenation was inhibited when neomycin was administered orally and it was suggested that the reaction is due to intestinal bacterial activity.

Metabolism studies with rats and rabbits have shown that oral administration of caffeic acid (3,4-dihydroxycinnamic acid, XIX) results in the urinary excretion of numerous metabolic products, some of which possess a reduced side chain, *i.e.*, derivatives of phenylpropionic acid (35, 107, 297a, 345). Similar findings have been made with the closely related ferulic acid (35, 345, 407). It was also shown that the double bond in caffeic acid was hydrogenated upon incubation with the intestinal contents of several animal species (44, 45). A subsequent study of six hydroxylated cinnamic acid derivatives showed that reduction occurred with all of them upon anaerobic incubation with mixed cultures of rat cecal microorganisms (345). Recently, the reduction of *p*-hydroxycinnamic acid (*p*-coumaric acid) to the corresponding phenylpropionic acid derivative by the microflora has been confirmed (178). Cinnamic acid itself was found to be reduced to phenylpropionic acid when incubated with fecal bacteria from monkeys (189).

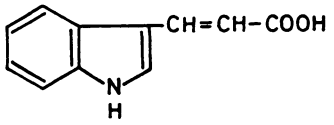
Further investigations of the metabolism of trihydric cinnamic acid derivatives including sinapic acid (XXVII) have been carried out (175, 176, 265, 266). Normal and antibiotic-treated rats as well as cultures of mixed cecal microorganisms were used and hydrogenation to phenylpropionic acid derivatives was a major metabolic pathway. The reduction observed was primarily a result of the metabolic activities of the intestinal bacteria rather than of tissue enzymes (266). No metabolites containing a reduced double bond were excreted in the urine of germ-free rats fed with diets containing caffeic acid or 4-vinylcatechol (XXIX) (297a).

The reduction of the double bond in cinnamic acid derivatives has also been studied with isolated strains of intestinal bacteria. A study of the dehydroxylation of caffeic acid by a strain of *Pseudomonas* sp. isolated from rat feces (see section III G 1) indicated that hydrogenation also occurred (299). A subsequent study with a number of species of *Pseudomonas* showed that this reaction took place in all cases (298). Cell-free extracts of *Ps. fluorescens* were also able to reduce caffeic acid. In an examination of 12 microorganisms isolated from human feces, caffeic acid was reduced to a small extent when incubated anaerobically with a strain of *Peptostreptococcus* sp. or of *Cl. perfringens* (296). This finding is of interest as, in another study with 12 strains of intestinal bacteria, only a strain of *Clostridium* sp. and a strain of *Lactobacillus* sp. were able to reduce 3-hydroxycinnamic acid to 3-hydroxyphenylpropionic acid (383). Re-

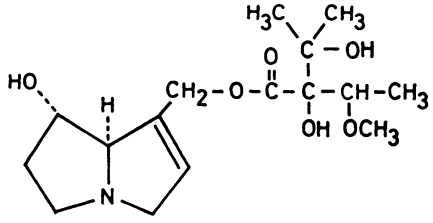
duction of cinnamic acid and some of its hydroxy derivatives previously has been shown to be carried out by a member of the genus *Lactobacillus* (437).

The hydrogenation of a few acrylic acid derivatives other than the aforementioned cinnamic acids (3-phenylacrylic acids) has been studied. Acrylate itself was reduced by a rumen microorganism (237) and indolylacrylic acid (LXVII) was reduced to indolylpropionic acid when incubated with monkey feces (189).

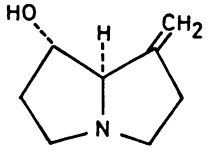
The reduction of the methylene group in some pyrrolizidine alkaloids by sheep rumen microorganisms has been reported (238). Heliotrine (LXVIII) and lasiocarpine (XII) are metabolized to 1-goreensine (7-α-hydroxy-1-methylene-8α-pyrrolizidine, LXIX)



LXVII



LXVIII



LXIX

which is reduced to the corresponding 1-methyl derivative (XIV).

N. Reduction of Nitro Groups

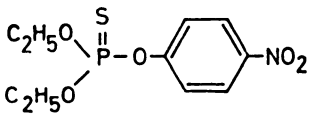
The reduction of nitro groups by the intestinal microorganisms was first reported over two decades ago and thereby provides us with one of the earliest examples in the field of microbial metabolism of foreign compounds (159). The then newly introduced antibiotic, chloramphenicol (VIII), was excreted to a limited extent in the urine of dogs and other animals, in contrast to the situation in man where about 90% of the dose was excreted in the urine as chloramphenicol and its derivatives. This difference was due to an extensive excretion of nitro compounds in the bile of lower animals. The concentration of nitro compounds in the intestinal tract of rats decreased with time with a concomitant increase in the amounts of aryl amines. In fact, the intestinal metabolites consisted nearly entirely of amino compounds after 17 hr and they were located largely in the cecum. The finding that incubation of the intestinal tract contents at 38°C overnight doubled the aryl amine content gave further support to the conclusion that the intestinal bacteria play an important role in the nitro reduction of chloramphenicol (159). In studies on the bacterial reduction of the nitro group in chloramphenicol, both *E. coli* and *Pr. vulgaris* carried out the reaction (372, 373). On further investigation of the origin and nature of the amines produced from chloramphenicol in the rat (157), only insignificant quantities of these metabolites were produced in isolated jejunal loops whereas large amounts were formed in similar preparations of the large intestine and cecum. Chloramphenicol glucuronide, a major biliary metabolite of chloramphenicol, was hydrolyzed and reduced to aromatic amines at the same sites and also by suspensions of *E. coli* and human feces. The reduction of chloramphenicol by cell-free extracts of *E. coli* has been studied (340–342) and the described properties are different than those subsequently found for the system in rabbit liver and kidney which reduces nitro groups of organic nitro compounds including chloramphenicol (145).

Another early report which clearly demonstrated the possible significance of the intestinal microorganisms in the reduction of nitro compounds dealt with the metabolism of 2,3,4,5- and 2,3,5,6-tetrachloronitrobenzene in the rabbit (52). These compounds have very low water solubility and are only partially absorbed from the intestine. It was pointed out that nearly all of the reduction observed with such nitro compounds may

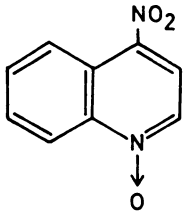
result from bacterial reduction in the intestine prior to absorption. The distribution and metabolism of the closely related pentachloronitrobenzene given chronically to dogs and rats have been studied recently (234) and the results obtained give a similar picture for this compound. Conversely, readily absorbed nitro compounds can largely avoid this fate and only a small amount of reduction may occur with these compounds. Examples of the latter type are seen with the nitrobenzoic acids and their amides which undergo a moderate degree of reduction to amino compounds in rabbits (53). As noted previously with chloramphenicol, however, biliary excretion of well absorbed nitro compounds can greatly alter this situation.

The finding that rabbit intestinal contents reduced the tetrachloronitrobenzenes prompted an investigation of this reaction with numerous other aromatic nitro compounds (52). Nitrobenzene, *m*-nitrophenol, *o*-, *m*-, and *p*-nitrobenzoic acids and their amides were reduced extensively whereas *p*-nitrophenol and *o*- and *p*-nitrotoluenes were reduced slowly.

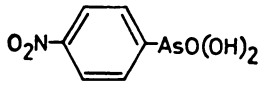
A study of the metabolism of the herbicide trifluralin (XXXVI) in rats and dogs showed that the compound was excreted to a large extent in the feces, also as amino products (130). On the basis of these results it was suggested that reduction of trifluralin occurred in the intestine (348). A subsequent investigation with microorganisms from rumen fluid has supported this view as the main route of metabolism was found to proceed *via* reduction of the nitro groups (161). These metabolites formed in the incubates with rumen fluid are the ultimate degradation products of trifluralin in the ruminant and two characterized strains of rumen bacteria recently have been shown to carry out these reactions (442). Another example of nitro reduction by rumen organisms is seen with parathion (LXX) which is reduced to the corresponding amino compound when incubated with bovine rumen fluid (77). This finding was suggested to



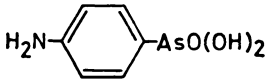
LXX



LXXI



LXXII



LXXIII

explain the relative lack of toxicity of this pesticide when it is given orally to cows.

Studies of nitro reduction with isolated strains of intestinal bacteria have been noted above with chloramphenicol and trifluralin. In addition, 4-nitroquinoline 1-oxide (LXXI) has been incubated with several microorganisms including *E. coli* which reduced the compound to 4-aminoquinoline 1-oxide *via* the corresponding hydroxylamino intermediate (288). The reduction of 4-nitrobenzoic acid *via* the hydroxylamino intermediate to 4-aminobenzoic acid by isolated strains of rat intestinal bacteria and other representatives of the intestinal flora recently has been shown to be carried out by *Str. faecalis*, *E. coli*, *Pr. vulgaris*, *A. aerogenes*, and strains of *Lactobacillus*, *Bacillus*, *Clostridium*, and *Bacteroides* (383).

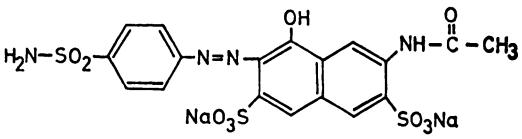
Oral administration of 4-nitrophenylarsonic acid (LXXII) to chickens was shown to lead to the excretion of about 20% of the dose as the corresponding amino compound (arsanilic acid, LXXIII) in 3 days (273). This reduction occurred mainly in the crop and was of microbiological origin. Similar results were obtained with the related 4-hydroxy-3-nitrophenylarsonic acid (274).

O. *Reduction of Azo Groups*

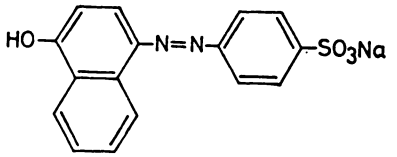
Azo compounds provide several of the most commonly used synthetic coloring agents in food and beverages and knowledge of their metabolic fate is therefore desirable. However, interest in this area remained low until about 10 years ago. Since then numerous investigations have been carried out, both with respect to the metabolism in the tissues and in the gastrointestinal tract (414). In fact, development within the latter area has been such that our knowledge of the metabolism of azo compounds by the intestinal bacteria is now more extensive than that with any other group of foreign organic compounds.

While most of these investigations are of fairly recent data, a few scattered reports dealing with the metabolism of azo compounds by various microorganisms appeared 30 to 35 years ago (414). In one of these early studies which is pertinent to the present discussion, prontosil soluble (neoprontosil, LXXIV) was both decolorized and converted to sulfanilamide to varying extents when incubated with various microorganisms including some intestinal inhabitants (389). An advance in our understanding of the metabolic fate of azo compounds resulted from the brief report which appeared in 1953 stating that sodium 4-*p*-sulfophenylazo-1-naphthol (LXXV) was converted rapidly to unidentified degradation products when incubated with dog intestinal contents (413).

The fairly non-specific nature of the bacterial reduction of azo dyes was shown clearly with numerous compounds containing one or more azo linkages (110). This study employed 21 bacterial species including some representatives of the intestinal microflora. However, the investigation which appears to have had the greatest influence on the subsequent development in this area appeared in 1962 and dealt with the metabolism of some commonly used, water-soluble azo dyes (312). The three compounds studied were amaranth (LXXVI), ponceau SX (LXXVII), and sunset yellow (LXXVIII); most of the reduction of the azo linkage was carried out by intestinal bacteria whereas reduction in

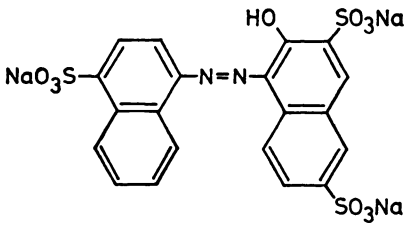


LXXIV

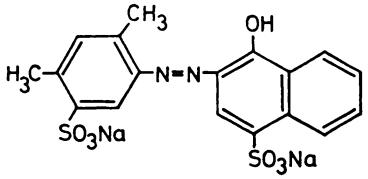


LXXV

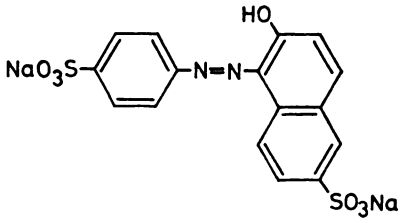
the liver was relatively unimportant. Furthermore, the sulfonated amino compounds formed from the reductive fission of the azo dyes were excreted in the urine after their absorption from the intestine. For example, 1-amino-4-naphthalene sulfonic acid (LXXIX) and 1-amino-2-hydroxy-3,6-naphthalene disulfonic acid (LXXX) were urinary metabolites of orally administered amaranth.



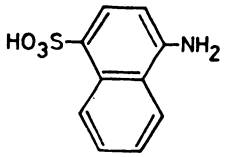
LXXVI



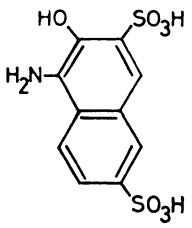
LXXVII



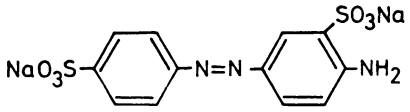
LXXVIII



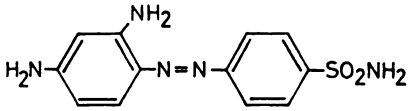
LXXIX



LXXX



LXXXI



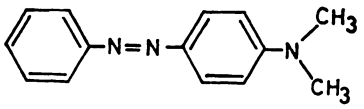
LXXXII

The finding that oral administration of azo dyes results in the urinary excretion of the component amines was also reported in a study of the metabolism of 11 azo food colors (88). Similar results were obtained in a number of subsequent investigations with water-soluble dyes including tartrazine (LXII) (216, 435), acid yellow (LXXXI) (353), and brown FK, a dye consisting of a mixture of *p*-sulfophenylazo derivatives of 2,4-diaminotoluene and 1,3-diaminobenzene (142). In contrast, intraperitoneal administration of the dyes resulted in no or relatively little fission of the azo linkage. For example, no fission products of tartrazine (216) or acid yellow (353) were detected in the urine after intraperitoneal injection of the dyes. Similar results have also been reported with the 4- and 5-sulfonated derivatives of 2-phenylazo-1-naphthol (23). On the other hand, in studies with brown FK (142) given by injection, fission products may be found in the urine but this probably stems from the intestinal metabolism of the dye and metabolites after their biliary excretion. In this regard it should be noted that the biliary excretion of azo compounds as well as of their hydroxylated derivatives and conjugates can be an important feature in the metabolic fate of these dyes (see 414). Nonetheless, reductive fission of the azo linkage by the body tissues may sometimes be encountered and prontosil (LXXXII), a lipid-soluble azo compound, and prontosil soluble (LXXIV) have been shown recently to be converted to sulfanilamide partly in the tissues and partly in the intestine as a result of bacterial metabolism (154). However, most of the reduction of prontosil soluble was found to occur in the intestine. Further indirect evidence showing the significance of the intestinal reduction was obtained from animals treated with antibiotics to suppress the intestinal microorganisms. The urinary excretion of sulfanilamide in these animals was greatly reduced. A recent report has stated that the total urinary sulfanilamide excretion in rats given 5 mg of prontosil intravenously was only about 10% of that found after intracecal administration (152).

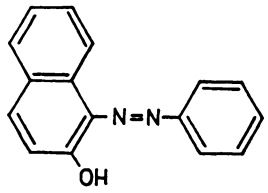
The above results obtained from animal studies clearly demonstrate the important role played by intestinal bacteria in the metabolism of azo compounds. This fact has been amply corroborated in numerous metabolic studies with mixed cultures or particular strains of intestinal bacteria. Acid yellow (LXXXI) undergoes reduction to to its component amines when incubated with extracts of rat feces or with a strain of *Str. faecalis* isolated from rat feces (347, 353). A subsequent study showed that this azo dye was reduced by 7 of 12 strains of intestinal bacteria belonging to 9 different genera (383). Reduction was extensive with *Str. faecalis*, moderate with *Lactobacillus and Bacteroides*, and minor with *Pseudomonas aeruginosa*, *Pr. vulgaris*, *A. aerogenes* and a strain of *Clostridium*. Tartrazine (LXII), the widely used yellow food color, has been studied extensively in regard to its reduction by intestinal bacteria. The reaction was carried out by suspensions of rat intestinal bacteria (327) and by *Pr. vulgaris* isolated from rat intestine (326, 328). Further examples of the reduction of water soluble azo compounds by mixed cultures of rat intestinal bacteria are seen with brown FK (142), with prontosil soluble (153, 154, 327), and with several other dyes belonging to the

aminoazobenzene, azopyrazolone, and azonaphthol groups (327).

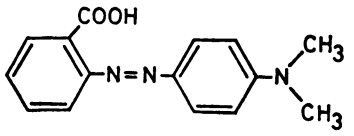
The role of the intestinal bacteria in the metabolism of lipid soluble azo compounds has also been investigated. While some conflicting results have been obtained, it is now evident that bacterial reduction of these dyes may be an important feature of their metabolism. Biliary excretion of this type of azo compound must be considered and the statement has been made that fat soluble dyes are reduced only if they are absorbed and excreted in the bile as water soluble conjugates (331). Evidence for this was obtained in experiments which showed that while conjugated metabolites of some 4-dimethylaminoazobenzene derivatives were reduced readily upon incubation with *Pr. vulgaris* or rat intestinal contents, this reaction was not observed in similar experiments with the dyes themselves. Nonetheless, several investigations have demonstrated the reduction of lipid soluble azo dyes by the intestinal microflora. One of the compounds studied was 4-dimethylaminoazobenzene (butter yellow, LXXXIII); the specific reductase activity in cecal contents of rats was considerably higher than that in liver (441). 1-Phenylazo-2-napththol (LXXXIV) was reduced when incubated with rat intestinal contents and with strains of *E. coli*, *A. aerogenes*, and *Proteus* sp. (68). Similar results have been obtained with methyl red (LXXXV) when incubated with rat cecal contents (347) and with all of the strains tested among 9 genera of common intestinal bacteria (383). Thus it seems likely that many lipid soluble azo dyes themselves may undergo reduction if, due to insufficient absorption after oral administration, they reach the appropriate part of the intestinal tract. In addition, water soluble conjugates of the dyes usually undergo biliary excretion and these metabolites then may be reduced by bacterial enzymes. However, quite different results may also be obtained as shown by the findings with bisazo dyes including sudan III (LXXXVI). This compound undergoes negligible biliary excretion



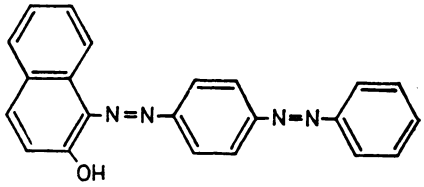
LXXXIII



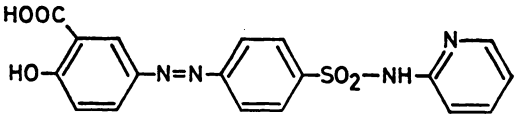
LXXXIV



LXXXV



LXXXVI



LXXXVII

and appears to be reduced in the body but not by the intestinal bacteria (331, 332).

A recent example of the intestinal reduction of a non-sulfonated azo compound is seen with salicylazosulfapyridine (LXXXVII). None of the drug was detected in the urine, feces, or cecal contents of conventional rats after oral administration (297). Instead, the excreta contained large amounts of metabolites resulting from the reductive fission of the azo bond. However, the unchanged compound was detected readily in the feces of rats treated with neomycin or of germ-free rats and in the latter case none of the metabolites found in the excreta of conventional rats was detected. Infection of germ-free rats with four typical intestinal bacteria resulted in the same pattern of metabolism of salicylazosulfapyridine as

found in conventional rats. These bacteria also carried out the azo reduction of the compound *in vitro*, as did 12 strains of typical human gastrointestinal bacteria. The fate of salicylazosulfapyridine in man has also been investigated and it was found that azo reduction by the intestinal bacteria was the most significant metabolic reaction which occurred (297b, 358).

From the evidence showing that azo reductase activity is present in many gastrointestinal microorganisms, it appears that this is a fairly general reaction rather than one carried out by merely one or a few of the intestinal inhabitants. *Str. faecalis* showed high reductase activity (383) but, on the other hand, other bacteria such as *Bacteroides* which are present in far greater numbers may make a greater contribution to the reduction of azo compounds in the intestine in spite of their having what appears to be lower levels of azo reductase activity (383). In experiments with whole cells the permeability of the bacterial cell wall may influence the rate of azo reduction. Tartrazine reduction was lower in fresh than in aged cultures of *Pr. vulgaris* (328) and the different rates of azo dye reduction by *Str. faecalis* and *E. coli* are due largely to differences in cell permeability (276).

The widespread occurrence of azo reductase activity is partly responsible for the fact that studies on the reaction mechanisms involved are more numerous here than they are with any other reaction carried out by the intestinal bacteria with foreign compounds. Cell-free preparations of *Pr. vulgaris* isolated from rat intestine have been shown to reduce tartrazine through the action of a soluble reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent flavoprotein (328). Similar experiments with acid yellow and cell-free extracts of *Str. faecalis* showed extensive reduction of the dye when NADPH- or NADH-generating systems were present (355). NADH was the more effective electron donor in these experiments. Addition of flavins to the incubates was found to result in marked stimulation of the reductase activity, both with tartrazine and acid yellow. The results of a comprehensive investigation of the mechanisms of azo reduction have appeared recently (155, 416, 417). Cell-free extracts of numerous bacteria were tested and those from *Str. faecalis*, which showed the highest specific azo reductase activity, were chosen for the study. Red 2 G, a sulfonated phenylazonaphthol, was used as the substrate and both reduced nicotinomide dinucleotide (NADH) and NADPH were active as electron donors. As found previously (355), the activity with this enzyme system was enhanced by additions of riboflavin, flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD). It was concluded that the reduction of azo dyes under anaerobic conditions results from their participation as electron acceptors from flavins which act as electron shuttles between the dyes and NADH-linked flavoproteins which normally are involved in cellular electron transport (155). The actual reduction of the dyes by the reduced flavin is probably non-enzymic and this correlated well with the wide substrate specificity and extensive distribution of the azo reductase systems. A recent report has indicated that this azo reductase system from *Str. faecalis* can reduce *p*-nitrobenzoic acid to *p*-hydroxylaminobenzoic acid but not further to *p*-aminobenzoic acid (151a). Furthermore, the system is not capable of reducing the double bond in cinnamic acids or the aldehyde group in benzaldehyde derivatives (see sections III M and P). The influence of structural factors on the rates of reduction of azo compounds by the intestinal microflora has also been investigated (419). The compounds studied were derivatives of 2-phenylazo-8-amino-1-naphthol-3,6-disulfonic acid (Red 2 G, Red 10 B and their analogues) and it was found that the electron density in the region of the azo group was the main factor in determining the reduction rate although stabilization through hydrogen bonding could also be involved.

P. Reduction of Aldehydes

The metabolism of a series of benzaldehyde derivatives by rat cecal microorganisms has been investigated (351) and the major reaction was the reduction to the corresponding benzyl alcohols.

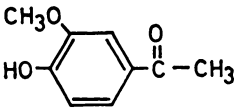
Q. Reduction of Ketones

The metabolism of ketones to carbinols by animal tissues is a well known reaction but only a few examples of this reduction by intestinal microorganisms have been reported. Appreciable reduction to 17 β-estradiol occurred when estrone was incubated with human feces (394) and the 3-keto group in bile acids was reduced to the 3 β-hydroxyl group when incubated anaerobically with mixed fecal microorganisms (182). Little is known regarding the intestinal metabolism of non-steroidal ketones although some preliminary results suggest that acetovanillone (LXXXVIII) and zingerone (LXXXIX) are not reduced to the corresponding carbinols when incubated anaerobically with mixed cultures of rat cecal microorganisms (352).

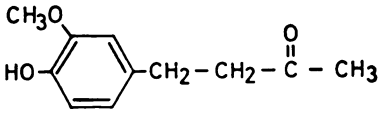
Reduction of the α-keto acid, 4-hydroxyphenylpyruvic acid (XXVI), to phloretic acid (4-hydroxyphenylpropionic acid) was detected when the compound was incubated anaerobically with rat cecal microorganisms (15). Another example of the complete reduction of a ketone group has been found with daidzein (7,4′-dihydroxyisoflavone, see LV) which was converted to equol (XXIV) when incubated with microorganisms from sheep rumen fluid (284) or rat cecal contents (178).

R. Reduction of Alcohols

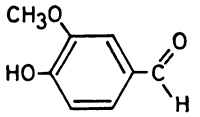
Investigation of the reduction by rat cecal microorganisms of some substituted benzaldehyde derivatives showed that the expected benzyl alcohols (see section III P) were absent or present only in small amounts when the compounds contained a *p*-hydroxyl group (351). This was found to be due to a further reduction of the benzyl



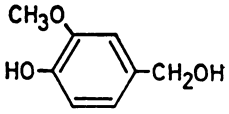
LXXXVIII



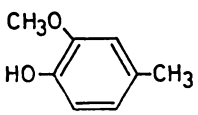
LXXXIX



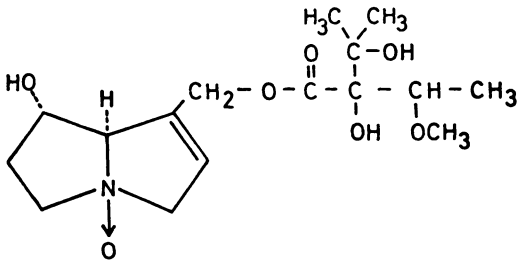
LXL



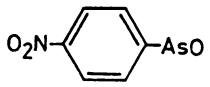
LXLI



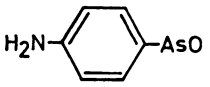
LXLII



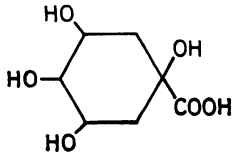
LXLIII



LXLIV



LXLV



LXLVI

alcohols to toluene derivatives. Thus, *p*-hydroxybenzaldehyde and *p*-hydroxybenzyl alcohol were both reduced to *p*-cresol whereas vanillin (LXL) and vanillyl alcohol (LXLI) were metabolized partly to 4-methylguaiacol (LXLII) and its demethylation product 4-methylcatechol (XXVIII). This reaction has significance in regard to the metabolic fate of vanillin (LXL) and vanillyl alcohol (LXLI) when administered to rats as small quantities of both LXLII and XXVIII were excreted in conjugated form in the urine (356).

S. Reduction of N-oxides

The metabolism of 4-nitroquinoline 1-oxide (LXXI) by several microorganisms has been investigated (288); reduction to 4-aminoquinoline occurred with several of these including *E. coli* and *Ps. aeruginosa*. The ability of the microorganisms from sheep rumen liquor to reduce *N*-oxides also has been demonstrated as heliotrine-*N*-oxide (LXLIII) was converted to heliotrine when incubated under anaerobic conditions (109).

An investigation of the metabolism of (−)-nicotine-1′-*N*-oxide in man showed that intravenous administration led to the quantitative excretion of the compound in the urine (26). However, considerable amounts of nicotine and cotinine were excreted also when the *N*-oxide was given orally or rectally. It was concluded that these differences can be explained by reduction by intestinal microorganisms of some of the *N*-oxide to nicotine which is then absorbed and partly metabolized in the liver to cotinine. A subsequent investigation has demonstrated that the *N*-oxide is reduced when incubated anaerobically with suspensions of rat intestinal contents (86).

T. Reduction of Arsonic Acids

Several organic arsenicals are employed to promote growth and control disease in poultry. One such compound, 4-nitrophenylarsonic acid (LXXII) is reduced to the corresponding arsenoxide (LXLIV) when incubated anaerobically with hen feces (273). Furthermore, the aromatic nitro group was reduced so that both arsanilic acid (4-aminophenylarsonic acid, LXXIII) and 4-aminoarsenoxide (LXLV) were detected in these incubates. Reduction of the arsonic acid group also occurred subsequent to the reduction of the nitro group as 4-aminophenylarsonic acid (LXXIII) was reduced to 4-aminoarsenoxide (LXLV) under the same conditions.

In addition to the above experiments *in vitro*, the metabolism of 4-nitrophenylarsonic acid (LXXII) labeled with ^{14}C after oral administration to hens was studied also (273). While no arsenoxides were found in the water-soluble fraction of the excreta, both 4-nitroarsenoxide (LXLIV) and 4-aminoarsenoxide (LXLV) were present in the residual fraction which contained roughly 10% of the radioactivity excreted during the experimental period. The results from various extraction procedures suggested that the ^{14}C and the arsenic were attached partly as arsenoxides through sulfhydryl groups in the fecal residue. Thus, the reduction of arsonic acids to arsenoxides which occurs in incubates with hen feces appears also to take place in the intestinal tract of hens given the compound orally.

U. Aromatization

Some non-aromatic cyclic compounds including quinic acid (1,3,4,5-tetrahydroxycyclohexanecarboxylic acid, LXLVI), a common plant constituent occurring in fruits, vegetables, coffee, and tea, are metabolized to aromatic substances when ingested by animals. The aromatization of quinic acid to benzoic acid, which is excreted in the urine as hippuric acid, occurs to an appreciable extent in man (3, 27, 30, 78, 310), guinea pigs (30, 78), and some species of monkeys (3). However, intraspecies differences have been reported as a recent study found that oral administration of quinic acid to guinea pigs did not give rise to aromatization (3). The latter investigation demonstrated that there are appreciable interspecies variations with man and Old

World monkeys showing extensive aromatization whereas New World monkeys and numerous lower animals including the common laboratory species showed little or no conversion. In the case of rats values of about 2 and 5% for two experimental groups were noted and the former figure is fairly close to the value reported earlier for this species (27). In a recent extensive study (32 rats) with a roughly similar oral dose (100 mg/animal), 12% of the dose of quinic acid was excreted in the urine as hippuric acid (213).

The above results refer to data obtained after oral administration of quinic acid and several investigations have shown clearly that the route of administration is critical in regard to the degree of conversion. Thus, no aromatization of quinic acid was observed when it was given by injection to guinea pigs (27, 78). Similar results have been reported in rats (405). The differences in aromatization after different routes of administration together with the report that some strains of *A. aerogenes* could aromatize quinic acid (99) suggested that the intestinal bacteria might be responsible for the reaction (78). This hypothesis was supported by the finding that inhibition of the intestinal microorganisms with neomycin prevented the conversion of quinic acid to hippuric acid in man (78). This finding was later confirmed with rats (11). In the latter study an even greater increase in hippuric acid output occurred when shikimic acid (3,4,5-trihydroxy-1-cyclohexene-1-carboxylic acid, LXLVII) was given; this conversion was prevented also by neomycin treatment.

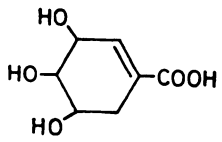
Hippuric acid formation is not the sole pathway in aromatization of quinic acid and shikimic acid as their oral administration to rats increases the excretion of urinary catechol (43). In addition, vanillic acid was also excreted. The extent of conversion of quinic acid to catechol in rats recently has been found to amount to about 1% of an oral dose of 100 mg/animal (213). It therefore seems unlikely that an extensive utilization of this pathway leading to catechol can explain the low values for conversion to benzoic and hippuric acids observed in some species. However, further pathways seem to be involved as 20 to 50% of the ^{14}C-quinic acid given orally was found to be converted to CO_2 (3).

Several studies of the site of catechol formation have shown that this metabolite is produced also from quinic and shikimic acids by intestinal microorganisms (45, 347). The microorganisms responsible have not been identified but quinic acid is known to be converted to 3,4-dihydroxybenzoic acid (protocatechuic acid) by strains of *Pseudomonas*, including some isolated from chicken droppings (392). The latter finding may be relevant in view of the fact that protocatechuic acid is detected sometimes when shikimic acid is incubated with rat cecal microorganisms (347). The further decarboxylation of protocatechuic acid to catechol has been well documented (see section III H). It has been suggested that these polyhydroxy compounds can be converted by intestinal bacteria to protocatechuic acid which can be partly absorbed and methylated to vanillic acid and partly decarboxylated in the intestine to catechol (343).

Studies on the aromatization of steroids by intestinal bacteria have shown that strains of *Clostridium paraputrificum* and *E. coli* can carry out this dehydrogenation reaction (7, 160, 160a).

V. Nitrosamine Formation

Nitrosamines are a group of *N*-nitroso compounds having powerful biological properties. Considerable interest has been shown in such compounds because of their actions, of which carcinogenic, mutagenic, and teratogenic effects are the most striking manifestations. A recent review of the toxicity of nitrosamines dealt with the biological actions of nitroso compounds, their possible occurrence in food and their formation in the stomach (254). The latter point has generated considerable interest and it is now well documented that secondary amines and



LXLVII

nitrites react to form nitrosamines under acid conditions such as those found in the mammalian stomach.

A development of great interest in regard to the present review was the report that several species of nitrate-reducing enterobacteria formed nitrosamines when incubated with nitrate and secondary amines (335). Aryl amines were much better substrates for the reaction than were aliphatic amines. However, nitrosamines formed from secondary aliphatic amines are known to be potent carcinogens and the bacterial formation of dimethylnitrosamine therefore has been investigated (229). This compound was formed when dimethylamine and sodium nitrite were incubated anaerobically at neutral pH with rat cecal microorganisms. It should be noted that nitrite may be made available in the gastrointestinal tract from nitrate, a reduction which may be carried out by bacteria in the human stomach (336), by rat cecal microorganisms (229), and by isolated strains of human intestinal bacteria (193). Dimethylamine is known to be a constituent of some foods, especially fish products and therefore may be ingested along with these. Moreover, its formation was dependent upon the bacterial metabolism of dietary choline in the intestine, although the major portion of urinary dimethylamine arises from endogenous sources (12). The metabolic pathway proposed for choline initially involves formation of trimethylamine by the intestinal microflora followed by demethylation in the tissues to give dimethylamine. This scheme adequately explains the failure of choline and nitrite to form dimethylnitrosamine under incubation conditions with rat cecal bacteria which result in the formation of this metabolite from dimethylamine and nitrite (229).

Another recent investigation has dealt with the formation of nitrosamines by strains of bacteria isolated from the human intestinal tract (193). Many strains of *E. coli*, enterococci, clostridia, bacteroides, and bifidobacteria were tested for their ability to nitrosate diphenylamine when incubated in the presence of nitrate or nitrite. Diphenylnitrosamine formation was detected with some (10–40%) of the strains of all the genera tested. In more extensive studies with *E. coli* many of these strains also nitrosated aliphatic and heterocyclic secondary amines.

W. Acetylation

Histamine was converted partly to *N*-acetylhistamine when incubated with human fecal suspensions (365). Other observations from patients who received chemotherapeutic agents have suggested that histamine acetylation is carried out largely by the intestinal microorganisms rather than by the liver (300). However, the possible significance of the acetylation reaction in the intestinal metabolism of xenobiotic compounds is not known. In a study of the metabolism of sulfanilic acid and *p*-phenylenediamine sulfonic acid in rats, increased amounts of these compounds were found in fecal samples after hydrolysis (353). The bound forms were assumed to be acetylated derivatives and it was suggested that these were formed in the gastrointestinal tract. However, later experiments in which sulfanilic acid was incubated anaerobically with rat cecal contents were unable to demonstrate any acetylation (352). It has been stated that acetylation of aromatic amines by the intestinal microflora has not been detected (260).

X. Esterification

The information given in section III D indicates that many esters may be hydrolyzed by intestinal bacteria. Thus, the formation of such compounds seems unlikely to be a reaction which would be expected to be observed readily. The sterol esters found in human feces were identified as the linoleate, palmitate, and stearate esters of coprostanol (322) and subsequent reports showed that the

esterification could be carried out by fecal bacteria (323, 324). Anaerobic incubation of propyl gallate with mixed cultures of rat cecal bacteria resulted in the formation of methyl gallate, presumably after initial hydrolysis to gallic acid (415).

Y. Other Reactions

The fission of the heterocyclic ring of tartrazine (LXII) by intestinal bacteria has been discussed above (section III L). These results also have shown that a hydrazine derivative (LXVI) can be cleaved to the corresponding amino compound (LXV) by intestinal microorganisms (333).

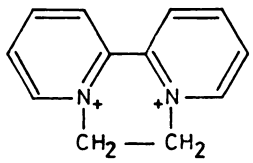
Isomerization by intestinal bacteria has been reported with α-linoleic acid (cis-cis-cis-octadeca-9,12,15-trienoic acid) which was converted to the cis-trans-cis isomer by rumen microorganisms (227).

Various aspects of the metabolism of some pyrrolizidine alkaloids by intestinal bacteria have been mentioned in sections III D, M, and S. In addition, some of these compounds undergo reductive cleavage of the ester linkage when incubated with rumen microorganisms. This was shown with heliotrine (LXVIII) and lasiocarpine (XII) which were reduced to 7α-hydroxy-1-methylene-8α-pyrrolizidine (LXIX) and 7α-angeloxy-1-methylene-8α-pyrrolizidine (XIII), respectively (109, 238). This reductive fission of heliotrine has also been demonstrated in anaerobic incubates with a small Gram-negative coccus isolated from sheep rumen contents (329).

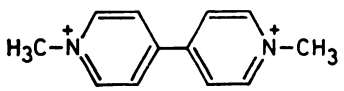
Examples of the oxidative metabolism of xenobiotic compounds by the intestinal microflora are not numerous. The metabolism of chloramphenicol (VIII) has been discussed in the sections covering amide hydrolysis (III C) and nitro reduction (III N). In addition, this antibiotic compound undergoes extensive degradation when incubated with several species of bacteria including *E. coli* and *Pr. vulgaris* (373). The further metabolic reactions found include oxidation of a side chain hydroxyl group to a ketone group as well as fission of a carbon-carbon bond. However, these incubations were carried out under aerobic conditions and are likely to be fairly unrepresentative of the situation existing in the intestinal lumen. The bacterial metabolism of the food color indigo carmine (LXI) has been discussed in section III L and the metabolites formed, isatin-5-sulfonic acid (LXIII) and 5-sulfoanthranilic acid (LXIV), are oxidative products (249). A recent study of the metabolism of a series of benzaldehyde derivatives by rat cecal microorganisms showed that, in addition to the major reductive pathway (section III P), these compounds were also oxidized to the corresponding benzoic acids (351).

Diquat (LXLVIII) and paraquat (LXLIX) are herbicides of the bipyridylium type. Their metabolism in rats has been studied and it was found that they were poorly absorbed from the intestine (89). However, about 70% of the diquat and 30% of the paraquat given orally were found in the feces as metabolic products. These products appear to be capable of absorption from the intestine as small amounts of metabolites not identical with the original compounds were detected in the urine. As incubation of diquat and paraquat with a rat fecal homogenate resulted in extensive degradation of the compounds it was concluded that the intestinal metabolites were the result of microbiological degradation.

The metabolism of tyrosine by decarboxylation and deamination has been mentioned in sections III H and K, respectively. It was also pointed out that this amino acid is further degraded by intestinal microorganisms. Incubation with rat cecal microorganisms resulted in the formation of *p*-hy-



LXLVIII



LXLIX

droxyphenylacetic acid, *p*-hydroxyphenylpropionic acid, *p*-cresol, and phenol (15). The conversion of tyrosine to simple phenols also has been demonstrated in rats fed a diet containing 10% tyrosine (14). The major urinary volatile phenol in these experiments was *p*-cresol and the daily outputs of this metabolite were increased more than 100-fold over the values from rats receiving a similar diet lacking tyrosine.

Studies with germ-free rats have shown that the degradation of choline to trimethylamine is carried out solely by the intestinal microorganisms (308). This conversion was depressed in rats when coprophagy was prevented or when several antibiotics which affect intestinal bacteria were administered (236). The urinary excretion of dimethylamine after choline or lecithin ingestion results partly from the bacterial degradation of these compounds in the intestine (12). This is presumably dependent upon an initial conversion to trimethylamine.

^{14}C-Hydroxyethyl starch is degraded partly to CO_2 when given orally but not intraperitoneally to rats (330). As biliary excretion of radioactivity is minimal it was concluded that the reaction was mediated by the intestinal microorganisms. However, it was not determined whether complete degradation to CO_2 occurred in the intestine or whether the bacteria formed an intermediate which was then absorbed and oxidized in the tissues.

The hydration of oleic acid to hydroxystearic acid is carried out by aerobic and anaerobic intestinal bacteria (408). The reaction was observed with clostridia and *Str. faecalis* and was increased greatly when the bacteria were preincubated with oleic acid.

IV. Factors Affecting the Gastrointestinal Metabolism of Foreign Compounds

A. Species Variations

The list of examples of compounds which undergo qualitative or quantitative differences in metabolism by the gastrointestinal microorganisms in different animal species has been growing quite rapidly. Most of these studies merely illustrate these differences without attempting to correlate them with variations in the microbial populations among the various animal species. However, future work will have to place increased emphasis on this latter aspect as has been done recently in the case of glycoside hydrolysis (192) and cyclamate metabolism (120, 121). In the former investigation numerous strains of six groups of bacteria (*E. coli*, enterococci, lactobacilli, clostridia, bacteroides, and bifidobacteria) were assayed for their production of various glycosidases including β-glucuronidase. A major finding was that levels of the latter enzyme in the small intestine were much lower in man, rabbits, and guinea pigs than in rats or mice. These results are described in greater detail in sections III A 1 and A 2. A microbiological study of the bacteria responsible for hydrolyzing cyclamate to cyclohexylamine showed that enterococci were responsible in man whereas clostridia and enterobacteria were implicated in rats and rabbits, respectively (120, 121).

The 8-methyl ether of xanthurenic acid (XXII) is known to be metabolized by the gastrointestinal microorganisms of rabbits by both *O*-demethylation and dehydroxylation (252). The former reaction was found to account for about 20% of the dose whereas values from about 2 to 9% were found for the dehydroxylation reaction. These reactions were also observed in mice when the compound was given at the same dose; however they accounted for less than 1% of the amount given as over 99% was excreted unchanged (251). Studies on the metabolism of homoprotocatechuic acid in rats and rabbits have shown that dehydroxylation by intestinal microorganisms occurs with part of the orally administered dose (82). The percentage dehydroxylated in rabbits was about twice that observed in rats when the same dose level was employed.

The aromatization of quinic acid (LXLVI) is carried out by intestinal microorganisms and, as mentioned briefly in

section III U, appreciable differences in this ability have been observed among different animal species and also among various groups of a single species. The most comprehensive study of these species variations showed that extensive aromatization of quinic acid occurred in man and Old World monkeys whereas New World monkeys and many species of lower animals showed little or no conversion (3). Lack of conversion was noted in guinea pigs. However, a previous investigation with this species reported that 30% of an ingested dose was excreted in the urine as hippuric acid (78). Variations in the values of conversion to hippuric acid ranging from about 2 to 12% have been obtained from different groups of rats (3, 27, 213). Thus, variations in the ability of the gastrointestinal microorganisms to aromatize quinic acid appear to follow more subtle influences than those which can be related solely to the species of the animal in question. A further example of this situation is seen with coumarin (XLVII) which is metabolized in rats partly to melilotic acid (XLIX) as a result of ring scission (38, 346). This reaction appeared to result from the metabolic activities of the gastrointestinal microflora (346), an assumption which was confirmed subsequently with a coumarin derivative given to germ-free rats (212). However, earlier studies with another population of rats found no scission of coumarin to melilotic acid (217, 262).

The metabolism of flavonoid compounds by the gastrointestinal microflora has been discussed in section III where it was shown that numerous reactions including glycoside hydrolysis and heterocyclic ring fission are carried out. While most of the studies have dealt with the reactions carried out by the microflora of laboratory animals, a few studies in man have shown that the same metabolic products can be formed. This has been shown with the flavonol quercetin (41) and with the flavanol (+)-catechin (LVIII) (93). However, other reports have demonstrated a marked difference in the metabolism of some flavonoids in man compared with that observed in rats and rabbits. The flavanones hesperetin and its glycoside hesperidin are degraded to phenylpropionic acid derivatives in these laboratory animals whereas, in man, a novel metabolite identified as 3-hydroxy-4-methoxyphenylhydracrylic acid (LVII) was formed (36). The metabolism of a related flavanol, naringin, follows the expected route leading to the formation of a C_6—C_3 phenolic acid in rats (37). However, in man, this reaction was not observed and the only urinary metabolites detected were the aglycone naringenin and its glucuronide conjugate. Species differences in the degradation of (+)-catechin when using rat and rabbit intestinal microorganisms have been reported (349).

While the explanation for the species differences in metabolism in most or all of the examples noted above appears most likely to be associated with variations in the gastrointestinal microorganisms of the various animal species, it must also be kept in mind that other species-related variables may also influence the microbial metabolism of compounds. This is especially important in regard to the variations in biliary excretion that exist among different animal species. An excellent example of this is seen with chloramphenicol (VIII). Metabolism studies in man and rats have indicated that appreciable quantities of aromatic amines are formed in the latter species (159). Most of this reduction was found to take place in the intestine. It was shown subsequently that the reaction is dependent upon the biliary excretion of chloramphenicol glucuronide which undergoes bacterial metabolism to amino compounds upon reaching the lower intestine (157). These metabolites are then absorbed and excreted in the urine. In man, however, biliary excretion of chloramphenicol metabolites is very low with the result that only small amounts of amines are detected in the feces.

B. Diet

Several studies have indicated that, in man at least, a dominant feature of the

microflora of the lower intestinal tract is its stability towards nutritional factors (185, 388). However, this stability need not apply to the microbial metabolism taking place and more drastic changes in this regard may be observed, as evidenced by the large variations in urinary indican excretion found in individuals maintained on different diets (185).

An example of the influence of diet on the gastrointestinal metabolism of a compound is seen with the flavonoid glycoside hesperidin (XXXI). It is metabolized extensively by the intestinal bacteria which carry out hydrolysis of the glycosidic linkage, fission of the heterocyclic ring system, demethylation, and dehydroxylation (see appropriate subdivisions in section III). It was found that a rabbit, maintained on a commercial pellet diet, excreted the degradation products of hesperetin (the aglycone of hesperidin) but not those of the glycoside when these compounds were administered orally (36). This indicates that the intestinal flora was not able to carry out the initial hydrolysis of the glycoside. However, when the animal was adjusted to a purified diet based on starch, dextrose, casein, oil, salt, and vitamins, both glycoside hydrolysis and subsequent degradation to numerous phenolic acids were observed.

Another example showing the effect of diet on the gastrointestinal metabolism of a compound has recently been reported with L-dopa. Minor urinary metabolites of this compound have been shown to be *m*-hydroxyphenyl acids which are formed by the intestinal bacteria (see section III K). However, rats given a milk diet failed to show this conversion and this difference was explained on the basis of the different diets used (50). It seems likely that the nature of the diet was responsible for the finding that homoprotocatechuic acid was not dehydroxylated when fed to rats maintained on a purified diet (54). As discussed in section III G 1, this compound is converted in several animals species including the rat partly to *m*-hydroxyphenylacetic acid which is a prominent urinary metabolite. However, this metabolite was not detected when homoprotocatechuic acid was administered after a 5-day feeding period on a synthetic test diet.

The carcinogen 4-dimethylaminoazobenzene is split by a bacterial azo reductase which is present in large amounts in the rat cecum (441). The activity of this reductase was depressed when rats were fed a diet low in riboflavin. Activity was restored when small amounts of the vitamin were added to the cecal incubates.

The glycoside amygdalin (II) gives rise to cyanide toxicity when administered orally to mice (381). This arises from its intestinal hydrolysis by bacterial enzymes and it was also reported that starvation of the animals for 48 hr to suppress the intestinal bacteria conferred some protection against the toxic effects. Starvation has been shown to affect the intestinal metabolism of a sulfate ester of a laxative of the diphenol type (143). When the compound was placed in colon segments from fed rats it was hydrolyzed to the free diphenol which is responsible for the laxative effect. However, this reaction did not occur when segments lacking fecal material from fasted rats were employed. In agreement with the findings given in section II D 2 which show that prevention of coprophagy often has little influence on the composition of the microflora in the lower intestine, it was shown recently that the metabolism of L-tyrosine to simple phenols which is known to occur in the large intestine was not significantly different in coprophagy-prevented and control rats (1).

C. Age

While the information presented in section II E clearly shows that large changes in the gastrointestinal microflora take place in young animals, very little is known about the effects these may have on the intestinal metabolism of foreign compounds. However, changes in the developing microflora would be expected to give rise to alterations in metabolic patterns. Evidence of this has been presented very recently in the case of

diethylstilbestrol which undergoes a pronounced enterohepatic circulation in rats (139a, 140). An essential part of this process is the deconjugation by bacterial β-glucuronidase of the conjugate excreted in the bile. It was found that very little hydrolysis of the conjugate occurred in the intestinal contents of rats on the day of birth or at the age of 5 days. This activity subsequently developed until it reached an adult level by about the 25th day. It was suggested that a similar metabolic pattern may be seen with many other compounds which are excreted in the bile as conjugates and thereafter undergo enterohepatic circulation. It was pointed out that this delay in the development of intestinal deconjugating enzymes may spare the liver from repeated exposure of reabsorbed unconjugated material during the period in which the conjugating mechanisms of the liver are being developed.

D. Metabolic Adaptation

The effect of foreign compounds on the composition of the gastrointestinal microflora has been discussed in section II G where it was shown that these circumstances may initiate changes in the bacterial population which range from subtle to the most profound. In these cases it is not surprising that the alterations produced may be reflected in changes in the metabolism of other foreign compounds which would otherwise undergo metabolic change by the affected bacteria. The present section is concerned with a more restricted aspect of this general topic and deals with the situation in which a compound influences the gastrointestinal microflora with the result that its own metabolism is affected. This alteration may take place by quantitative shifts among the bacterial groups so that a group becomes more or less abundant or by adaptive changes in a bacterial group which augment its metabolic capabilities. In any case, this metabolic adaptation to a compound may have considerable metabolic and/or toxicological significance as not only may an existing metabolic pathway be enhanced but entirely new pathways may be developed. This latter point is important and it illustrates a fundamental difference between the metabolic adaptation shown by the gastrointestinal microflora compared with that associated with the endoplasmic reticulum. The latter phenomenon is now well documented (see ref. 76 for discussion and further references) but it is one which generally involves quantitative rather than qualitative changes in metabolism.

The metabolic adaptation of the intestinal microflora to a foreign compound given repeatedly appears to have been described in only a few instances. The best known and most thoroughly investigated of these is the conversion of cyclamate to cyclohexylamine. However, other reports have shown that this phenomenon also occurs with widely differing compounds including the flavonoids kaempferol and robinin and the pyrimidine precursor orotic acid (uracil-4-carboxylic acid). The flavonoids are metabolized by rat intestinal bacteria to *p*-hydroxyphenylacetic acid which is excreted in the urine (178). The formation of the metabolite was increased in animals previously maintained on a diet containing the flavonoids. A substantial portion of a dose of ingested orotic acid is excreted unchanged in the feces of conventional and germ-free rats (446). While this fecal excretion continues in the latter group, the intestinal microorganisms in the conventional animals rapidly adapt to catabolize all the non-absorbed orotic acid, which disappears from the feces within 5 days after feeding is begun.

Discussion of the conversion of cyclamate (XVII) to cyclohexylamine (XVIII) by the intestinal bacteria is found in section III E and the present section therefore is devoted to the question of the ability of the bacteria to acquire this metabolic capacity. The availability of several recent articles on this topic (5, 121, 318, 350, 381) makes detailed coverage of the subject unnecessary. The acquisition of cyclamate converting abilities by the intestinal microflora was

demonstrated first in rats (317). Very little cyclohexylamine was excreted after the initial dose but values corresponding to a conversion of about 1 to 35% of the dose were obtained when the rats were given drinking water containing 0.5% calcium cyclamate for 3 months. Upon removal of the animals from the cyclamate-containing diet, production of the metabolite was reduced to a low level in 5 days. The pronounced capacity of rats to acquire this ability after chronic cyclamate administration has been confirmed (420). This ability is acquired also in other species including man although the extent to which this occurs may vary considerably with species and also with individuals (381).

In spite of the small number of reports in this area, the implications of this phenomenon of metabolic adaptation are readily apparent. It seems reasonable to assume that investigations designed to assess this factor will uncover further examples and that these may be of considerable interest in chronic feeding studies.

E. Enzyme Inhibition

Little use has been made of compounds which can inhibit particular intestinal reactions, although this approach should be able to furnish useful information in some cases. A possible advantage of this method is that a metabolic pathway can be blocked without altering the composition of the gastrointestinal microflora. Examples of this method have been demonstrated with the intestinal hydrolysis of compounds containing glycosidic linkages (381). Orally administered amygdalin (II) is toxic because of its hydrolysis by a β-glycosidase present in the intestinal contents. However, pretreatment of animals with lactose protected them from a lethal dose of amygdalin. This effect probably resulted from the competition of lactose, a β-glycoside, for intestinal β-glycosidases as maltose, an α-glycoside, did not confer protection under similar conditions. β-Glucuronidase is inhibited by saccharo-1,4-lactone and oral administration of this compound to rats was shown to reduce markedly the rate and amount of stilbestrol absorbed from the intestine after the intraduodenal infusion of stilbestrol glucuronide.

V. Alterations in Physico-chemical Properties and Membrane Permeability Characteristics as a Result of Gastrointestinal Metabolism

With the exception of those compounds which exert only local effects, biologically active substances must penetrate membranes before reaching their site of action. As is well known, these membranes are lipoid in nature. Furthermore, the concentration gradient of the compound across the membrane is the main driving force behind transmembranal movement. We therefore find that passive diffusion is the most important feature in the movement of foreign compounds into the various compartments of the body and that the properties of lipid solubility, degree of ionization, and molecular size of these compounds are of major importance (72). To a large extent, the physicochemical properties of very many biologically active foreign compounds are such that they readily penetrate biological membranes at physiological pH-values. This fact would have significant effects on the duration and perhaps the depth of action of many compounds if mechanisms for alterations in their polarity did not exist. It is now well known that such processes are carried out by enzyme systems in many tissues of the body and that, in general, the metabolic reactions result in more water-soluble derivatives due to the introduction of more polar functional groups. Thus the metabolites will penetrate less readily into cells and will be excreted more rapidly from the body.

With the above remarks as a background, it is of interest to consider the nature of the metabolic conversion of foreign compounds effected by gastrointestinal microorganisms. Much of what has been said in section III should make it clear that quite different changes in polarity often occur as a result

of bacterial metabolism. Indeed, the alteration may sometimes be the opposite of that which takes place in the tissues as, for example, in the case of glucuronides. The gastrointestinal reactions very often involve the hydrolysis or reduction of compounds or other reactions which remove various groups. They therefore often have a degradative character although it is wise not to use this term in characterizing all microbial metabolism of foreign compounds as some of the reactions do not fit this pattern. However, a general degradative pattern can be expected to enhance the lipoid permeability properties of the metabolite compared with those of the original compound. This may be a result of a reduction in molecular size but will more likely come about from reactions which remove highly polar or highly ionized groups. In the case of compounds containing a glycosidic linkage, the change in permeability characteristics will be profound and this particular subject is one of special importance (see section VI). With many of the gastrointestinal reactions, however, the polarity changes are more subtle and the consequences may not be readily apparent without further study. Increases in lipophilic properties may be expected with reactions involving sulfamate hydrolysis, dehydroxylation, decarboxylation, or aromatization whereas the opposite change may take place in the case of ester hydrolysis or dealkylation. Negligible polarity changes may occur with some of the other reactions. On the whole, however, the trend differs considerably from that which takes place as a result of metabolism in the tissues and it therefore may be of profound significance in determining the pharmacological and toxicological properties of some compounds (see section VII).

VI. Role of the Intestinal Microorganisms in the Enterohepatic Circulation of Compounds

While the excretion of xenobiotic substances by the biliary route has long been recognized, most of the studies and especially the systematic investigations are of recent date. Reviews have appeared on the general aspects of this subject (304, 379, 382, 395) as well as on specific topics including species differences in biliary excretion (380) and the biliary excretion of azo dyes (414). The possible consequences of biliary excretion with regard to the ultimate fate of the excreted compound are greater than is the case with urinary excretion which largely means that the compound is removed from the body. While this is likewise a possibility with biliary metabolites, there is also the chance of the compound being reabsorbed unchanged from the intestinal lumen with the result that an enterohepatic circulation is established. However, a third possibility also exists in that the biliary metabolite may undergo metabolism by the intestinal microorganisms. In fact, it is now realized that the bacterial metabolism of these metabolites is usually a key step in the complex chain of events required for the enterohepatic circulation of compounds. This is best illustrated with glucuronide conjugates which are readily hydrolyzed by bacterial enzymes in the intestine. A further possibility exists for the compound to undergo alternate metabolic pathways or for the initial product of intestinal metabolism to be further metabolized. The factors involved in enterohepatic circulation have been discussed in the general reviews cited above. Several articles have dealt recently with the significance of the intestinal reactions in enterohepatic circulation, with special emphasis on the formation of metabolites possessing enhanced biological activity (350, 381, 444). In view of this extensive coverage of the subject, it is felt that further detailed discussion is unwarranted in the present review. However, numerous examples of the metabolism of biliary metabolites by intestinal microorganisms are summarized in table 2.

The examples in table 2 serve to illustrate several different possibilities including regeneration of the original compound in the intestine and therefore its prolonged retention in the body, liberation of various

metabolites of the administered compound, and, finally, situations where the compound released by intestinal hydrolysis undergoes further intestinal metabolism. Examples of the latter situation are seen with the intestinal *O*-demethylation of 3-*O*-methyldopa (65) and of ferulic acid (345) which are excreted in the bile of rats as conjugates. It has been found recently that vanillin and its metabolites, vanillyl alcohol and vanillic acid, are excreted in bile as glucuronide conjugates which undergo further metabolism by the intestinal microorganisms to decarboxylated compounds and to toluene derivatives (396). However, these minor metabolites are not formed when biliary excretion is prevented, even after the oral administration of vanillin. An example of the first situation, prolonged retention of a compound, which is not included in table 2 is seen with digitoxin. This characteristic has been proposed to be due to its excretion in the bile as conjugates which are hydrolyzed by enzymes present in the intestine (224, 225). These conjugates have been identified tentatively as a glucuronide and a sulfate. A recent pharmacokinetic study of digitoxin in rats has emphasized the major role of enterohepatic circulation with this compound and its metabolites (2).

Another type of compound which can undergo enterohepatic circulation but which is not covered in table 2 is that of the amino acid conjugates of bile acids. However, references to the intestinal hydrolysis of these amides are given in section III C.

While it is readily apparent from table 2 that the intestinal metabolism of biliary metabolites is to a very great extent concerned with glucuronide conjugates, it should be added that a similar situation may exist with other types of glycosides after their parenteral administration. This was shown to be the case with 3′,4′,7-tri-*O*-(β-hydroxyethyl) rutoside which is excreted extensively in the bile of rats (24). After hydrolysis of the glycoside in the intestine the aglycone was excreted in the feces. It seems likely that a similar situation may exist with the glycosides naringin and phloridzin which, after subcutaneous administration, were excreted in the urine as degradation products known to be produced only by the intestinal bacteria (37, see section III L).

The findings briefly summarized in table 2 indicate that the majority of the results now available were obtained in experiments with rats. While this may attest to the widespread use of this experimental animal, it must not be overlooked that biliary excretion in this species, along with the dog, is more extensive with many compounds than is the case with numerous other animal species including the rabbit, guinea pig, rhesus monkey, and man (see 382). Thus, experimental data from rats may overly emphasize the metabolism of biliary metabolites by the intestinal microorganisms insofar as extrapolation to other species is concerned.

VII. Implications of Drug Metabolism by Gastrointestinal Microorganisms

In view of the information presented in sections V and VI, it is to be expected that the metabolism of drugs and other foreign compounds by the gastrointestinal microorganisms may produce alterations in the biological activities of certain compounds. Indeed, the tendency for many of these reactions to reduce the polarity of compounds suggests that increases in pharmacological or toxicological effects may be a common feature. In addition, due consideration must be given to the possibility of the metabolic adaptation of the gastrointestinal microorganisms to some compounds after their repeated administration. While the implications of these situations have only recently received attention, four publications are now available which deal with the toxicological significance of drug metabolism by gastrointestinal microorganisms (119, 350, 381, 443a). In view of this, detailed coverage of this area in the present article is unwarranted. However, the toxicological findings presently available are summarized and, in addition, a number of reports showing

TABLE 2

Metabolism of biliary metabolites by intestinal microorganisms

Compound	Species	Results	References
Glucuronides			
Stilbestrol glucuronide	Rat	Hydrolyzed compound reabsorbed from intestine and undergoes pronounced enterohepatic circulation. Excreted mainly in feces, largely as stilbestrol and unidentified metabolites	69, 139, 381
Etorphine-3-glucuronide	Rat	Largely hydrolyzed in lower intestine where it is reabsorbed. Reappears in bile as conjugate. Fecal rather than urinary excretion predominates	111
18 β-Glycyrrhetic acid glucuronide	Rat	Hydrolysis in intestine and free acid excreted largely in feces	214
4,4′-Dihydroxydiphenyl-(2-pyridyl)-methane glucuronide	Rat	Following intestinal hydrolysis the free diphenol excreted mainly in feces	134, 412
Naphthaleneacetic acid glucuronide	Rat	Conjugate hydrolyzed in intestine and acid mainly reabsorbed and excreted in urine, largely as glycine and glucuronide conjugates	248
3,5-Diiodo-4-(4′-hydroxy-3′-iodophenoxy) benzoic acid glucuronide	Rat	Hydrolyzed in intestine and excreted mainly in feces	279
3,5-Di-*t*-butyl-4-hydroxybenzoic acid ester glucuronide	Rat	Intestinal hydrolysis followed by partial reabsorption of the free acid	90, 203
Glucuronides of pentaerythritol mono-, di-, and tri-nitrate	Rat	Hydrolysis in intestine followed by extensive absorption	79
Glucuronides of myalex and its metabolites	Rat	Metabolites liberated by intestinal hydrolysis and excreted in feces and urine	144
Glucuronides of metabolites of ethynylestradiol 3-cyclopenyl ether	Rat	Biliary metabolites eliminated in feces and/or reabsorbed from large intestine	390
Glucuronides of hydroxylated metabolites of glutethimide	Rat	Conjugates hydrolyzed and partially absorbed from intestine	226
Glucuronides of phenolic metabolites of imipramine	Rat	Conjugates mainly hydrolyzed in lower intestine. Both urine and feces contain considerable amounts of phenolic metabolites. Large individual variations in enterohepatic circulation noted	32
4-Demethylgriseofulvin glucuronide	Rat	4-Demethylgriseofulvin absorbed from intestine and then excreted mainly in urine	398
Glucuronide of metabolites of mepivacaine	Rat	Reabsorption from intestine leads to excretion mainly in urine	190
Glucuronides of hydroxylated derivatives of biphenylyl 4-sulfate, cyclohexylphenyl 4-sulfate and cyclohexylphenyl 2-sulfate	Rat	Hydrolyzed in intestine where the aglycones are absorbed and excreted in urine	194
Glucuronides of hydroxylated derivatives of orphenadrine	Rat	Extensive hydrolysis in and absorption from the intestine. Extensive urinary and fecal excretion	196
p-Hydroxydiphenylhydantoin glucuronide	Rat	Partial hydrolysis in and absorption from the intestine. Subsequently excreted as conjugate in urine	151
Chloramphenicol-3-glucuronide	Rat	Intestinal hydrolysis followed by further intestinal metabolism of liberated chloramphenicol to aryl amines which are partly absorbed and excreted in urine	157

TABLE 2—*Continued*

Compounds	Species	Results	References
Ferulic acid glucuronide	Rat	Hydrolyzed in intestine and further metabolized by intestinal bacteria to *m*-hydroxyphenylpropionic acid which is absorbed and excreted in urine	345
N-Hydroxy-*N*-2-fluorenylacetamide glucuronide	Rat	Hydrolysis in lower intestine followed by further metabolism, both by intestinal bacteria and in tissues after absorption	170, 433, 440
(+)-Catechin glucuronide	Rat	Intestinal hydrolysis followed by further metabolism to lactones and phenolic acids which are absorbed and excreted in urine	97
Morphine glucuronide	Dog	Conjugate hydrolyzed in intestine and partially absorbed. This source responsible for most of the later urinary morphine and most fecal morphine	449
Indomethacin glucuronide	Dog	Enterohepatic circulation leads to excretion mainly in feces. However, in other animal species urinary excretion predominates	208, 451
7-Hydroxyfluphenazine glucuronide	Dog	Conjugate hydrolyzed and phenolic compound excreted in feces	123
Sulfates			
3,5-Diiodo-4-(4′-hydroxy-3′-iodophenoxy)benzoic acid sulfate	Rat	Hydrolyzed in intestine and mainly excreted in feces	279
4,4′-Dihydroxydiphenyl-(2-pyridyl)-methane sulfate	Rat	Ester hydrolyzed in intestine, free phenol partly absorbed and partly retained in intestine where it exerts a laxative effect	143

changes in pharmacological activity as a result of gastrointestinal metabolism are discussed.

A. Pharmacological Significance

It has been shown recently that azo reduction by intestinal microorganisms can result in drug activation. Prontosil (LXXXII) and neoprontosil (LXXIV) are converted to the active antibacterial sulfanilamide partly by intestinal bacteria in rats (154). In the case of neoprontosil, most of the reduction took place in the intestine and it was suggested that this also may have occurred in man since the drug, after its injection, would be expected to undergo biliary excretion. Another example of the intestinal reduction of an azo drug is seen with salicylazosulfapyridine (LXXXVII). Although the mode of action of this drug, which is used in the treatment of ulcerative colitis, is not known entirely, it was shown recently that reduction to sulfapyridine is carried out by the intestinal bacteria in rats (297) and, presumably, in man (358).

An investigation of the extrahepatic metabolism of several psychotherapeutic agents including imipramine indicated that the gastrointestinal contents of rats and the lower intestinal contents of man have the ability to demethylate this drug to the active metabolite, desmethylimipramine (271). The data suggested that about an equal extent of metabolism occurred in the liver and in the gastrointestinal tract and it was suggested that this and the probable variation in metabolism which takes place at the latter site may be of importance in imipramine therapy.

In view of the well known differences in the oral and parenteral doses of quaternary anticholinergic drugs needed to produce an identical effect, the fate of methylatropine in animals and man was studied (4). Oral administration was found to result in the formation of two urinary metabolites not

TABLE 3

Examples of reactions by the gastrointestinal microflora which have toxicological implications

Reaction	Compounds	Remarks	References
Hydrolysis of glucuronides	*N*-Hydroxy-*N*-2-fluorenyl-acetamide glucuronide	The glucuronide excreted in bile of rats, hydrolyzed to the carcinogenic *N*-hydroxy compound which can be absorbed or further metabolized by intestinal bacteria	Section III G 2, 170, 433, 440
	Chloramphenicol glucuronide	The glucuronide excreted in bile of rats, hydrolyzed and further metabolized by intestinal bacteria to thyrotoxic arylamines	Sections III A 1 and III N
Hydrolysis of of glycosides	Cycasin	The glycoside, found in cycad plants, hydrolyzed to methylazoxymethanol which is hepatotoxic and carcinogenic	Section III A 2
	Amygdalin	The glycoside hydrolyzed to mandelonitrile which then forms cyanide	Section III A 2
Hydrolysis of amides	Methotrexate	Hydrolyzed in the mouse to 4-amino-4-deoxy-N^{10}-methylpteroylglutamic acid which is less toxic than methotrexate	Section III C
Hydrolysis of sulfamates	Cyclamate	After continued exposure to cyclamate, intestinal bacteria adapt metabolically to produce cyclohexylamine which has sympathomimetic and perhaps carcinogenic effects	Sections III E and IV D
N-Dehydroxylation	*N*-Hydroxy-*N*-2-fluorenylacetamide	The hydroxy compound excreted in the bile of rats following conjugation with glucuronic acid. After intestinal hydrolysis of glucuronide, the toxic *N*-hydroxy compound partly dehydroxylated to *N*-2-fluorenylacetamide which is both absorbed and excreted in feces	Section III G 2
Decarboxylation	Several phenolic carboxylic acids	Produces more toxic simple phenols	Section III H, 18
O-Demethylation	Biochanin A and formononetin	Metabolized to genistein and daidzein, respectively, which are more potent estrogens	Section III I 1
Reduction of nitro groups	Chloramphenicol	Reduction in rats to aryl amines which have thyrotoxic properties	Sections III A 1 and III N
	Parathion	Low toxicity in cattle correlated with rapid reduction to amino compound by rumen microorganisms	Section III N
Reduction of azo groups	Brown FK	Amines derived from the dye absorbed from the intestine and cause muscle toxicity in rats. Toxicity is not seen in mice and this may be due to differences in intestinal microflora	418
Reduction of aldehydes and alcohols	Several phenolic compounds	Compounds containing a *p*-hydroxyl group reduced to more toxic toluene derivatives	Sections III P and III R

TABLE 3—*Continued*

Reaction	Compound	Remarks	References
Aromatization	Steroid compounds	Postulated that particular intestinal floras, as a result of dietary variations, may convert steroids to aromatic hydrocarbon carcinogens	Section III V, 59, 199
Nitrosamine formation	Nitrite and secondary amines	Carcinogenic nitrosamines produced	Section III V
Reductive ester group cleavage	Pyrrolizidine alkaloids	Hepatotoxic alkaloids converted to non-toxic derivatives	Section III Y

seen after parenteral dosage. These were probably hydrolysis products of methylatropine and it was believed that substantial hydrolysis of the drug occurred in the gastrointestinal tract. It is thus possible that the differences in effects after different routes of administration, in addition to the important influence of poor gastrointestinal absorption, may arise also from metabolism of the drug by intestinal microorganisms.

Interest has been expressed recently in the possible relationship between the timing of the clinical response after L-dopa therapy in parkinsonism and the minor metabolic pathways of this drug (337). In particular, the production of *m*-tyrosine, which has the ability to protect monoamine stores from depletion, by *p*-dehydroxylation of L-dopa by intestinal bacteria has been suggested as a factor of possible importance in the response observed with L-dopa.

In addition, some well known examples of drug activation by intestinal bacteria are seen with certain poorly absorbed sulfonamides and some cathartics. The antibacterial effect of succinylsulfathiazole (III) and phthalylsulfathiazole appears to be due to their hydrolysis by the intestinal microflora to the bacteriostatically active sulfathiazole (IV) (431). The anthraquinone cathartics including the glycosides of cascara sagrada and senna act by virtue of their hydrolysis to active components by bacterial glycosidases in the lower intestine (137). A similar metabolic pathway has been demonstrated recently with the sulfate ester analogue of bisacodyl which was shown to be active as a result of its hydrolysis to the free phenol by intestinal bacteria (143) (see section III B). Thus, the metabolic activities of the intestinal microflora can be used to release an active drug in the intestine.

B. Toxicological Significance

Examples of intestinal reactions which have toxicological implications are listed in table 3. Literature references, which are given in only a few cases in the table, are found mainly in the appropriate parts of other sections of this review as indicated. A fuller discussion of most of these examples is available in references 350, 381 and 443a. In addition, the possible role of bacterial enzymes in the production of cancer of the colon, breast, and stomach has been discussed recently (117a, 432). An interesting hypothesis has been put forward linking the metabolic activities of the intestinal flora with the blood dyscrasias that occasionally result from the use of chloramphenicol (204). It was pointed out that this toxic effect has not been seen after the parenteral administration of the antibiotic. It was suggested that a particular type of intestinal flora, consisting of large numbers of enterobacteria, which seems to occur in a low proportion of the patients studied may be able to degrade chloramphenicol to toxic compounds. Enterobacteria have been shown previously to metabolize chloramphenicol to a large number of compounds under suitable conditions (373) although the toxicity of these to the bone marrow has not been assessed. The intestinal decarboxylation of amino acids

was discussed briefly in section III H. This subject may have toxicological interest and it has been suggested that the amines thus formed suppress animal growth (263).

VIII. Conclusions

The findings summarized in this review clearly show that gastrointestinal microorganisms can carry out a large number of metabolic reactions with foreign organic compounds. These reactions are of diverse types although hydrolytic, reductive, and degradative pathways are encountered most commonly. Fairly simple alterations occur in many of these whereas others involve extensive and complex metabolic changes. Reactions of the latter type illustrate the high degree of metabolic ingenuity which is sometimes shown by the gastrointestinal microorganisms. The numerous reactions which are now known furnish us with a reasonably good picture of the general nature of this microbial metabolism. Nonetheless, new routes of metabolism can be expected to be encountered in the future, especially among reactions of a pronounced degradative character.

Most of the articles cited in this review are of quite recent date, a fact which attests to the growing interest in this aspect of foreign compound metabolism. However, this development is understandable in view of the increasing awareness of the important role which microbial metabolism is capable of playing in numerous areas including the enterohepatic circulation of compounds, the metabolism of compounds to more active or toxic metabolites, alterations in metabolism resulting from chronic administration of compounds and species differences in metabolism. We can also expect to see many new and interesting findings in these areas in the future since we now know that the gastrointestinal microflora has the ability to act as an organ with a metabolic potential equal to or sometimes greater than that of the liver. It is expected that these developments will result in the study of the gastrointestinal reactions becoming a natural and integrated part of the larger subject of foreign compound metabolism.

Further studies on the ability of the gastrointestinal microorganisms to adapt to compounds given chronically are greatly needed. The surface of this subject has only been scratched and it is obviously important to know whether the phenomenon is of fairly widespread occurrence or whether such a development, which can lead to the formation of new metabolites of increased activity or toxicity, is to be expected only rarely. Another area which requires considerable further study is that of species variations in metabolism, both in regard to the variations in gastrointestinal metabolism occurring among the various animal species and in how these are related to the types, numbers, and distribution of the microorganisms which are capable of carrying out the particular metabolic reactions. Although numerous investigations have shown clearly that foreign compound metabolism by gastrointestinal microorganisms occurs not only in certain laboratory animals but in all species studied including man, much of our present information is derived from studies in rats, a species which appears to show more than average abilities in this respect. We shall thus need to look more closely at these species variations in order to be able to better assess the role of the microbial metabolism of foreign compounds in other species including man.

There exist, in addition, areas which have hitherto been largely neglected but which should provide fruitful avenues for future investigation. Foremost here are studies of the mechanisms involved in the reactions carried out by the gastrointestinal microorganisms. The present review has used conventional terminology in designating the types of reactions although it is realized that a better understanding of the pathways involved may show that the events which occur are sometimes unlike those which we are accustomed to from tissue metabolism. This may, perhaps, be the situation with the so-called hydrolytic reactions where bond cleavage may involve either

hydrolysis or reductive fission. In fact, the latter pathway has been shown to be used by rumen bacteria in the cleavage of the ester group of pyrrolizidine alkaloids (section III Y). Further information of this nature may reveal some common characteristics of some of the many seemingly diverse types of metabolic reactions carried out by gastrointestinal microorganisms and thereby may be of predictive value in the assessment of the role played by the microflora in the metabolism of foreign organic compounds.

Acknowledgments. The author is grateful to Mrs. Solfrid Dybvik, Mrs. Astrid Hetle, Mrs. Eli Tepstad and Mr. Erik Larsen for typographical assistance.

REFERENCES

1. Aarbakke, J. and Bakke, O. M.: Localisation of microbial L-tyrosine degradation in the intestine of coprophagy-prevented rats. Scand. J. Gastroenterol. 7: 417–421, 1972.
2. Abshagen, U., v. Bergmann, K. and Reitbrock, N.: Evaluation of the enterohepatic circulation after ^{3}H-digoxin administration in the rat. Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol. 275: 1–10, 1972.
3. Adamson, R. H., Bridges, J. W., Evans, M. E. and Williams, R. T.: Species differences in the aromatisation of quinic acid *in vivo* and the role of gut bacteria. Biochem. J. 116: 437–443, 1970.
4. Albanus, L., Sundwall, A. and Vangbo, B.: On the metabolic disposition of methylatropine in animals and man. Acta Pharmacol. Toxicol. 27: 97–111, 1969.
5. Anonymous: Cyclamate still to the fore. Food Cosmet. Toxicol. 10: 237–243, 1972.
6. Aranki, A., Syed, S. A., Kenney, E. B. and Freter, R.: Isolation of anaerobic bacteria from human gingiva and mouse cecum by means of a simplified glove box procedure. Appl. Microbiol. 17: 568–576, 1969.
7. Aries, V. C. and Hill, M. J.: The formation of unsaturated bile acids by intestinal bacteria. Biochem. J. 119: 37P–38P, 1970.
8. Aries, V. and Hill, M. J.: Degradation of steroids by intestinal bacteria. I. Deconjugation of bile salts. Biochim. Biophys. Acta 202: 526–534, 1970.
9. Aries, V. and Hill, M. J.: Degradation of steroids by intestinal bacteria. II. Enzymes catalysing 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, 1970.
10. Asahina, M., Yamaha, T., Watanabe, K. and Sarrazin, G.: Excretion of cyclohexylamine, a metabolite of cyclamate, in human urine. Chem. Pharm. Bull. (Japan) 19: 628–632, 1971.
11. Asatoor, A. M.: Aromatisation of quinic acid and shikimic acid by bacteria and the production of urinary hippurate. Biochim. Biophys. Acta 100: 290–292, 1965.
12. Asatoor, A. M. and Simenhoff, M. L.: The origin of urinary dimethylamine. Biochim. Biophys. Acta 111: 384–392, 1965.
13. Astill, B. D., Terhaar, C. J. and Fassett, D. W.: The toxicology and fate of 2,2,4-trimethyl-1,3-pentanediol diisobutyrate. Toxicol. Appl. Pharmacol. 22: 387–399, 1972.
14. Bakke, O. M.: Urinary simple phenols in rats fed diets containing different amounts of casein and 10% tyrosine. J. Nutr. 98: 217–221, 1969.
15. Bakke, O. M.: Studies on the degradation of tyrosine by rat caecal contents. Scand. J. Gastroenterol. 4: 603–608, 1969.
16. Bakke, O. M.: Minor urinary metabolites of L-dopa in the rat. Acta Pharmacol. Toxicol. 29: 106–107, 1971.
17. Bakke, O. M.: Degradation of dopa by intestinal microorganisms *in vitro*. Acta Pharmacol. Toxicol. 30: 115–121, 1971.
18. Bakke, O. M. and Midtvedt, T.: Influence of germ-free status on the excretion of simple phenols of possible significance in tumour promotion. Experientia (Basel) 26: 519, 1970.
19. Barber, M., Brooksbank, B. W. L. and Haslewood, G. A. D.: Destruction of urinary glucuronide by bacteria. Nature (London) 162: 701–702, 1948.
20. Barker, P. S. and Morrison, F. O.: Breakdown of DDT to DDD in mouse tissue. Can. J. Zool. 42: 324–325, 1964.
21. Barker, P. S. and Morrison, F. O.: The metabolism of TDE by *Proteus vulgaris*. Can. J. Zool. 43: 652–654, 1965.
22. Barker, P. S., Morrison, F. O. and Whitaker, R. S.: Conversion of DDT to DDD by *Proteus vulgaris*, a bacterium isolated from the intestinal flora of a mouse. Nature (London) 205: 621–622, 1965.
23. Barrett, J. F., Pitt, P. A., Ryan, A. J. and Wright, S. E.: The metabolism of 2-phenylazo-1-naphthol-4- and -5-sulphonic acids in the rat. Biochem. Pharmacol. 14: 873–879, 1965.
24. Barrow, A. and Griffiths, L. A.: The biliary excretion of hydroxyethylrutosides and other flavonoids in the rat. Biochem. J. 125: 24P–25P, 1971.
24a. Barrow, A. and Griffiths, L. A.: Metabolism of the hydroxyethylrutosides. Biliary and urinary excretion of 3′,4′,5-(hydroxyethyl-^{14}C), 7-tetra-*O*-(β-hydroxyethyl)-rutoside in rats and monkeys after parenteral administration. Xenobiotica 2: 575–586, 1972.
25. Batterham, T. J., Hart, N. K., Lamberton, J. A. and Braden, A. W. H.: Metabolism of oestrogenic isoflavones in sheep. Nature (London) 206: 509, 1965.
26. Beckett, A. H., Gorrod, J. W. and Jenner, P.: Absorption of (−)-nicotine-1′-*N*-oxide in man and its reduction in the gastrointestinal tract. J. Pharm. Pharmacol. 22: 722–723, 1970.
27. Beer, C. T., Dickens, F. and Pearson, J.: The aromatization of hydrogenated derivatives of benzoic acid in animal tissues. Biochem. J. 48: 222–237, 1951.
28. Behal, F. J. and Folds, J. D.: Arylamidase of *Neisseria catarrhalis*. Arch. Biochem. Biophys. 121: 364–371, 1967.
29. Bergmark, J., Carlsson, A., Granerus, A.-K., Jagenburg, R., Magnusson, T. and Svanborg, A.: Decarboxylation of orally administered L-dopa in the human digestive tract. Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol. 272: 437–440, 1972.
30. Bernhard, K., Vuilleumier, J. P. and Brubacher, G.: Zur Frage der Entstehung der Benzoesäure im Tierkörper. Helv. Chim. Acta 38: 1438–1444, 1955.
31. Bertino, J. R., O'Brien, P. and McCullough, J. L.: Inhibition of growth of leukemia cells by enzymic folate depletion. Science 172: 161–162, 1971.
32. Bickel, M. H. and Weber, H. J.: The total fate of a drug: Kinetics of distribution, excretion, and formation of 14 metabolites in rats treated with imipramine. Arch. Int. Pharmacodyn. Thér. 173: 433–463, 1968.
33. Blaschko, H. and Longlands, M. G.: Some observations on the bacterial decarboxylation of metatyrosine. Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol. 273: 430–433, 1972.
34. Blumberg, A. G. and Heaton, A. M.: The occurrence of cyclohexylamine in urines studied for drug use by thin-layer chromatography. J. Chromatogr. 48: 565–566, 1970.
35. Booth, A. N., Emerson, O. H., Jones, F. T. and DeEds,

F.: Urinary metabolites of caffeic and chlorogenic acids. J. Biol. Chem. 229: 51–59, 1957.

36. Booth, A. N., Jones, F. T. and DeEds, F.: Metabolic fate of hesperidin, eriodictyol, homoeriodictyol and diosmin. J. Biol. Chem. 230: 661–668, 1958.

37. Booth, A. N., Jones, F. T. and DeEds, F.: Metabolic and glucosuria studies on naringin and phloridsin. J. Biol. Chem. 233: 280–282, 1958.

38. Booth, A. N., Masri, M. S., Robbins, D. J., Emerson, O. H., Jones, F. T. and DeEds, F.: Urinary metabolites of coumarin and *o*-coumaric acid. J. Biol. Chem. 234: 946–948, 1959.

39. Booth, A. N., Masri, M. S., Robbins, D. J., Emerson, O. H., Jones, F. T. and DeEds, F.: The metabolic fate of gallic acid and related compounds. J. Biol. Chem. 234: 3014–3016, 1959.

40. Booth, A. N., Murray, C. W., DeEds, F. and Jones, F. T.: Metabolic fate of rutin and quercetin. Fed. Proc. 14: 321, 1955.

41. Booth, A. N., Murray, C. W., Jones, F. T. and DeEds, F.: The metabolic fate of rutin and quercetin in the animal body. J. Biol. Chem. 223: 251–257, 1956.

42. Booth, A. N., Robbins, D. J., Jones, F. T., Emerson, O. H. and Masri, M. S.: Xanthurenic acid dehydroxylation by fecal microflora. Proc. Soc. Exp. Biol. Med. 120: 546–548, 1965.

43. Booth, A. N., Robbins, D. J., Masri, M. S. and DeEds, F.: Excretion of catechol after ingestion of quinic and shikimic acids. Nature (London) 187: 691, 1960.

44. Booth, A. N. and Williams, R. T.: Dehydroxylation of caffeic acid by rat and rabbit caecal contents and sheep rumen liquor. Nature (London) 198: 684–685, 1963.

45. Booth, A. N. and Williams, R. T.: Dehydroxylation of catechol acids by intestinal contents. Biochem. J. 88: 66P–67P, 1963.

46. Booth, V. H.: Vitamin A activity of orange peel pigment. Biokhimiya 12: 21–25, 1947.

47. Booth, V. H.: The splitting of carotenoid esters in the alimentary tract of the rat. Biochim. Biophys. Acta 84: 188–191, 1964.

48. Bornside, G. H. and Cohn, I.: The normal microbial flora: Comparative bacterial flora of animals and man. Amer. J. Dig. Dis. 10: 844–852, 1965.

49. Bornside G. H. Welsh, J. S. and Cohn, I.: Bacterial flora of the human small intestine. J. Amer. Med. Ass. 196: 1125–1127, 1966.

50. Borud, O., Midtvedt, T. and Gjessing, L. R.: Urinary phenolic compounds in gnotobiotic and conventional rats on a free diet, and before and after L-DOPA loading on a milk diet. Acta Pharmacol. Toxicol. 30: 185–192, 1971.

51. Boulton, A. A. and Quan, L.: Formation of *p*-tyramine from DOPA and DOPamine in rat brain. Can. J. Biochem. 48: 1287–1291, 1970.

52. Bray, H. G., Hyes, Z., James, S. P. and Thorpe, W. V.: The metabolism of 2,3,5,6- and 2,3,4,5-tetrachloronitrobenzenes in the rabbit and the reduction of aromatic nitro compounds in the intestine. Biochem. J. 53: 266–273, 1953.

53. Bray, H. G., Thorpe, W. V. and Wood, P. B.: The fate of certain organic acids and amides in the rabbit. 6. Nitrobensoic acids and amides. Biochem. J. 44: 39–45, 1949.

54. Braymer, H. D.: The Metabolic Fate of Some Phenolic Compounds in the Rat, Ph.D. Thesis, University of Oklahoma, 1960.

55. Bridges, J. W., Davies, D. S. and Williams, R. T.: Dealkylation of diethyltin by the rat. Biochem. J. 98: 14P–15P, 1965.

56. Bridges, J. W., Davies, D. S. and Williams, R. T.: The fate of ethyltin and diethyltin derivatives in the rat. Biochem. J. 105: 1261–1267, 1967.

56a. Broitman, S. A. and Giannella, R. A.: Gut microbial ecology and its relationship to gastrointestinal disease. *In* Topics in Medicinal Chemistry, vol. 4, Absorption Phenomena, ed. by J. L. Rabinowitz and R. A. Myerson, pp. 265–321, Wiley-Interscience, New York, London, Sydney and Toronto, 1971.

57. Bryant, M. P.: Normal flora-rumen bacteria. Amer. J. Clin. Nutr. 23: 1440–1450, 1970.

58. Buehler, H. J., Katzman, P. A. and Doisy, E. A.: Studies on β-glucuronidase from *E. coli*. Proc. Soc. Exp. Biol. Med. 76: 672–676, 1951.

59. Burkitt, D. P.: Epidemiology of cancer of the colon and rectum. Cancer 28: 3–13, 1971.

60. Caldwell, J. and Hawksworth, G. M.: The demethylation of methamphetamine by intestinal microflora. J. Pharm. Pharmacol. 25: 422–424, 1973.

61. Calne, D. B., Karoum, F., Ruthven, C. R. J. and Sandler, M.: The metabolism of orally administered L-dopa in parkinsonism. Brit. J. Pharmacol. 37: 57–68, 1969.

62. Cayen, M. N., Carter, A. L. and Common, R. H.: The conversion of genistein to equol in the fowl. Biochim. Biophys. Acta 86: 56–64, 1964.

63. Cayen, M. N., Tang, G. and Common, R. H.: Urinary conversion products of biochanin A and formononetin in the fowl. Biochim. Biophys. Acta 111: 349–357, 1965.

64. Chalmers, J. P., Davies, D. S., Draffan, G. H., Reid, J. L. and Thorgeirsson, S. S.: Demethylation of 3-O-methyldopa. Brit. J. Pharmacol. 43: 455P–456P, 1971.

65. Chalmers, J. P., Draffan, G. H., Reid, J. L., Thorgeirsson, S. S. and Davies, D. S.: Demethylation of 3-O-methyldopa in the rat. Life Sci. 10: 1243–1251, 1971.

66. Chasseaud, L. F., Fry, B. J., Hawkins, D. R., Lewis, J. D., Sword, I. P., Taylor, T. and Hathway, D. E.: The metabolism of asiatic acid, madecassic acid and asiaticoside in the rat. Arzneimittel-Forschung 21: 1379–1384, 1971.

67. Cheng, K.-J., Jones, G. A., Simpson, F. J. and Bryant M. P.: Isolation and identification of rumen bacteria capable of anaerobic rutin degradation. Can. J. Microbiol. 15: 1365–1371, 1969.

68. Childs, J. J., Nakajima, C. and Clayson, D. B.: The metabolism of 1-phenylaso-2-naphthol in the rat with reference to the action of the intestinal flora. Biochem. Pharmacol. 16: 1555–1561, 1967.

69. Clark, A. G., Fischer, L. J., Millburn, P., Smith, R. L. and Williams, R. T.: The role of gut flora in the enterohepatic circulation of stilboestrol in the rat. Biochem. J. 112: 17P, 1969.

70. Clark, W. G. and MacKay, E. M.: The absorption and excretion of rutin and related flavonoid substances. J. Amer. Med. Ass. 143: 1411–1415, 1950.

71. Closon, J., Salvatore, G., Michel R. and Roche, J.: Dégradation de l'ester sulfurique de la 3:5:3'-triiodo-L-thyronine par les bactéries intestinales du rat. C.R. Séances Soc. Biol. 153: 1120–1125, 1959.

72. Cohn, V. H.: Transmembrane movement of drug molecules. *In* Fundamentals of drug metabolism and drug disposition, ed. by B. N. LaDu, H. G. Mandel and E. L. Way, pp. 3–21, Williams & Wilkins, Baltimore, 1971.

73. Cole, M.: Hydrolysis of penicillins and related compounds by the cell-bound penicillin acylase of *Escherichia coli*. Biochem. J. 115: 733–739, 1969.

74. Cole, M.: Deacylation of acylamino compounds other than penicillins by the cell-bound penicillin acylase of *Escherichia coli*. Biochem. J. 115: 741–745, 1969.

75. Conn, H. O. and Floch, M. H.: Effects of lactulose and *Lactobacillus acidophilus* on the fecal flora. Amer. J. Clin. Nutr. 23: 1588–1594, 1970.

76. Conney, A. H.: Environmental factors influencing drug metabolism. *In* Fundamentals of drug metabolism and drug disposition, ed. by B. N. LaDu, H. G. Mandel and E. L. Way, pp. 253–278, Williams & Wilkins, Baltimore, 1971.

77. Cook, J. W.: In vitro destruction of some organophosphate pesticides by bovine rumen fluid. J. Agr. Food Chem. 5: 859–863, 1957.

78. Cotran, R., Kendrick, M. I. and Kass, E. H.: Role of intestinal bacteria in aromatisation of quinic acid in man

and guinea pig. Proc. Soc. Exp. Biol Med. 104: 424–426, 1960.

79. Crew, M. C., Gala, R. L., Haynes, L. J. and DiCarlo, F. J.: Biliary excretion and biotransformation of pentaerythritol trinitrate in rats. Biochem. Pharmacol. 20: 3077–3089, 1971.
80. Curzon, G.: Identification of an Ehrlich-reacting substance in human urine. Biochem. J. 66: 27P–28P, 1957.
81. Curzon, G. and Pratt, R. T. C.: Origin of urinary resorcinol sulphate. Nature (London) 204: 383–384, 1964.
82. Dacre, J. C., Schelins, R. R. and Williams, R. T.: The role of the tissues and gut flora in the metabolism of (^{14}C)-homoprotocatechuic acid in the rat and rabbit. J. Pharm. Pharmacol. 20: 619–625, 1968.
83. Dacre, J. C. and Williams, R. T.: Dehydroxylation of (^{14}C)protocatechuic acid in the rat. Biochem. J. 84: 81P, 1962.
84. Dacre J. C. and Williams, R. T.: The role of the tissues and gut micro-organisms in the metabolism of (^{14}C)protocatechuic acid in the rat. Aromatic dehydroxylation. J. Pharm. Pharmacol. 20: 610–618, 1968.
85. Dahlqvist, A., Bull, B. and Gustafsson, B. E.: Rat intestinal 6-bromo-2-naphthyl glycosidase and disaccharidase activities. 1. Enzymic properties and distribution in the digestive tract of conventional and germ-free animals. Arch. Biochem. Biophys. 109: 150–158, 1965.
86. Dajani, R. M., Gorrod, J. W. and Beckett, A. H.: Hepatic and extrahepatic reduction of nicotine 1′-*N*-oxide in rats. Biochem. J. 130: 88P, 1972.
87. Dalderup, L. M., Keller, G. H. M. and Schouten, F.: Cyclamate and cyclohexylamine. Lancet 845, 1970.
88. Daniel J. W.: The excretion and metabolism of edible food colors. Toxicol. Appl. Pharmacol. 4: 572–594, 1962.
89. Daniel J. W. and Gage, J. C.: Absorption and excretion of diquat and paraquat in rats. Brit. J. Ind. Med. 23: 133–136, 1966.
90. Daniel, J. W., Gage, J. C. and Jones, D. I.: The metabolism of 3,5-di-*tert.*-butyl-4-hydroxytoluene in the rat and in man. Biochem. J. 106: 783–790, 1968.
91. Darrell, J. H.: Three more penicillins. Prescribers' J. 9: 43–47, 1969.
92. Das, N. P.: Studies on flavonoid metabolism. Degradation of (+)-catechin by rat intestinal contents. Biochim. Biophys. Acta 177: 668–670, 1969.
93. Das, N. P.: Studies on flavonoid metabolism. Absorption and metabolism of (+)-catechin in man. Biochem. Pharmacol. 20: 3435–3445, 1971.
94. Das, N. P. and Griffiths, L. A.: Metabolism of flavone in the guinea pig. Biochem. J. 98: 488–492, 1966.
95. Das, N. P. and Griffiths, L. A.: Studies on flavonoid metabolism. Metabolism of (+)-catechin in the guinea pig. Biochem. J. 110: 449–456, 1968.
96. Das, N. P. and Griffiths, L. A.: Studies on flavonoid metabolism. Metabolism of (+)-(^{14}C)catechin in the rat and guinea pig. Biochem. J. 115: 831–836, 1969.
97. Das, N. P. and Sothy, S. P.: Studies on flavonoid metabolism. Biliary and urinary excretion of metabolites of (+)-(U-^{14}C) catechin. Biochem. J. 125: 417–423, 1971.
98. Davies, D. S. and Williams, R. T.: Metabolism of ethyltin and diethyltin and its oxide in rats. Toxicol. Appl. Pharmacol. 8: 337, 1966.
99. Davis, B. D. and Weiss, U.: Aromatic biosynthesis. VIII. The roles of 5-dehydroquinic acid and quinic acid. Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol. 220: 1–15, 1953.
100. Davis, T. R. A., Adler, N. and Opsahl, J. C.: Excretion of cyclohexylamine in subjects ingesting sodium cyclamate. Toxicol. Appl. Pharmacol. 15: 106–116, 1969.
101. Dawson, I. and Peyse-Davies, J.: The distribution of certain enzyme system in the normal human gastrointestinal tract. Gastroenterology 44: 745–760, 1963.
102. Dawson, R. M. C. and Kemp, P.: The effect of defaunation of the phospholipids and on the hydrogenation of unsaturated fatty acids in the rumen. Biochem. J. 115: 351–352, 1969.
103. Dayman, J. and Jepson, J. B.: The metabolism of caffeic acid in humans: the dehydroxylating action of intestinal bacteria. Biochem. J. 113: 11P, 1969.
104. Decuypere, J. and Van der Heyde, H.: Study of the gastrointestinal microflora of suckling piglets and early weaned piglets reared using different feeding systems. Zentrabl. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. Orig. 221: 492–510, 1972.
105. DeEds, F.: Physiological effects and metabolic fate of flavonoids. *In* The Pharmacology of Plant Phenolics, ed. by J. W. Fairbairn, pp. 91–99, Academic Press, London and New York, 1959.
106. DeEds, F.: Flavonoid metabolism. *In* Comprehensive Biochemistry, vol. 20, Metabolism of Cyclic Compounds, ed. by M. Florkin and E. H. Stotz, pp. 127–171, Elsevier, Amsterdam, London and New York, 1968.
107. DeEds, F., Booth, A. N. and Jones, F. T.: Methylation and dehydroxylation of phenolic compounds by rats and rabbits. J. Biol. Chem. 225: 615–621, 1957.
108. Di Carlo, F. J., Coutinho, C. B. and Crew, M. C.: Sites of absorption of pentaerythritol tetranitrate. Arch. Int. Pharmacodyn. Thér. 167: 163–170, 1967.
109. Dick, A. T., Dann, A. T., Bull, L. B. and Culvenor, C. C. J.: Vitamin B_{12} and the detoxification of hepatotoxic pyrrolizidine alkaloids in rumen liquor. Nature (London) 197: 207–208, 1963.
110. Dieckhues, B.: Untersuchungen zur reduktiven Spaltung der Azofarbstoffe durch Bakterien. Zentrabl. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. Orig. 180: 244–249, 1960.
111. Dobbs, H. E. and Hall, J. M.: Metabolism and biliary excretion of etorphine (M 99-Reckitt)—an extremely potent morphine-like drug. *In* Sensitization to Drugs, vol. 10, Proceedings of the European Society for the Study of Drug Toxicity, pp. 77–86, Excerpta Medica Foundation, Amsterdam, 1969.
112. Dodgson, K. S.: Observations on the arylsulphatase of *Proteus vulgaris*. Enzymol. Acta Biocatal. 20: 301–312, 1959.
113. Dohlman, C. H.: The fate of the sulfate group of chondroitin sulfate after administration to rats. Acta Physiol. Scand. 37: 220–234, 1956.
114. Dolly, J. O., Curtis, C. G., Dodgson, K. S. and Rose, F. A.: Metabolism of sodium oestrone (^{35}S)sulphate in the rat. Biochem. J. 123: 261–266, 1971.
115. Donaldson, R. M.: Normal bacterial populations of the intestine and their relation to intestinal function. N. Engl. J. Med. 270: 938–945, 1964.
116. Douglass, C. D. and Hogan, R.: Formation of protocatechuic acid from quercetin by rat kidney *in vitro*. J. Biol. Chem. 230: 625–629, 1958.
117. Drasar, B. S.: Cultivation of anaerobic intestinal bacteria. J. Pathol. Bacteriol. 94: 417–427, 1967.
117a. Drasar, B. S. and Hill, M. J.: Intestinal bacteria and cancer. Amer. J. Clin. Nutr. 25: 1399–1404, 1972.
118. Drasar, B. S., Hill, M. J. and Shiner, M.: The deconjugation of bile salts by human intestinal bacteria. Lancet 1237–1238, 1966.
119. Drasar, B. S., Hill, M. J. and Williams, R. E. O.: The significance of the gut flora in safety testing of food additives. *In* Metabolic Aspects of Food Safety, ed. by F. J. C. Roe, pp. 245–260, Blackwell Scientific Publications, Oxford, 1970.
120. Drasar, B. S., Renwick, A. G. and Williams, R. T.: The conversion of cyclamate into cyclohexylamine by gut bacteria. Biochem. J. 123: 26P–27P, 1971.
121. Drasar, B. S., Renwick, A. G. and Williams, R. T.: The role of the gut flora in the metabolism of cyclamate. Biochem. J. 129: 881–890, 1972.
122. Drasar, B. S., Shiner, M. and McLeod, G. M.: Studies on the intestinal flora. I. The bacterial flora of the gastro-

intestinal tract in healthy and achlorhydric persons. Gastroenterology **56:** 71-79, 1969.

123. Dreyfuss, J., Ross, J. J. and Schreiber, E. C.: Biological disposition and metabolic fate of fluphenazine-^{14}C in the dog and rhesus monkey. J. Pharm. Sci. **60:** 821-825, 1971.
124. Dubos, R. J., Savage, D. C. and Schaedler, R. W.: The indigenous flora of the gastrointestinal tract. Dis. Colon Rectum **10:** 23-34, 1967.
125. Dubos, R. J. and Schaedler, R. W.: The effect of diet on the fecal bacterial flora of mice and on their resistance to infection. J. Exp. Med. **115:** 1161-1172, 1962.
126. Dubos, R. and Schaedler, R. W.: The digestive tract as an ecosystem. Amer. J. Med. Sci. **248:** 49-54, 1964.
127. Dubos, R., Schaedler, R. W. and Costello, R.: Composition, alteration, and effects of intestinal flora. Fed. Proc. **22:** 1322-1329, 1963.
128. Dubos, R., Schaedler, R. W., Costello, R. and Hoet, P.: Indigenous, normal and autochthonous flora of the gastrointestinal tract. J. Exp. Med. **122:** 67-76, 1965.
129. Dubos, R., Schaedler, R. W. and Stephens, M.: The effect of antibacterial drugs on the fecal flora of mice. J. Exp. Med. **117:** 231-243, 1963.
130. Emmerson, J. L. and Anderson, R. C.: Metabolism of trifluralin in the rat and dog. Toxicol. Appl. Pharmacol. **9:** 84-97, 1966.
131. Engler, R., Holtz, P. and Raudonat, H. W.: Über die Spaltung herzwirksamer Glykoside im Tierkörper. Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol. **233:** 393-408, 1958.
132. Eriksson, H. and Gustafsson, J.-Å.: Steroids in germfree and conventional rats. Sulpho- and glucuronohydrolase activities of caecal contents from conventional rats. Eur. J. Biochem. **13:** 198-202, 1970.
133. Euler, U. S. von and Lishajko, F.: Excretion of catechol in human urine. Nature (London) **183:** 1123, 1959.
134. Ferlemann, G. and Vogt, W.: Entacetylierung und Resorption von phenolischen Laxantien. Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol. **250:** 479-487, 1965.
135. Ferrini, R. and Glässer, A.: In-vitro decarboxylation of new phenylamine derivatives. Biochem. Pharmacol. **13:** 798-801, 1964.
136. Finegold, S. M.: Interaction of antimicrobial therapy and intestinal flora. Amer. J. Clin. Nutr. **23:** 1466-1471, 1970.
137. Fingl, E.: Cathartics and laxatives. *In* The Pharmacological Basis of Therapeutics, ed. by L. S. Goodman and A. Gilman, pp. 1020-1031, Macmillan, London, 1970.
138. Finkle, B. J., Lewis, J. C., Corse, J. W. and Lundin, R. E.: Enzyme reactions with phenolic compounds: Formation of hydroxystyrenes through the decarboxylation of 4-hydroxycinnamic acids by *Aerobacter*. J. Biol. Chem. **237:** 2926-2931, 1962.
139. Fischer, L. J., Millburn, P., Smith, R. L. and Williams, R. T.: The fate of (^{14}C)stilboestrol in the rat. Biochem. J. **100:** 69P, 1966.
139a. Fischer, L. J., Kent, T. H. and Weissinger, J. L.: Absorption of diethylstilbestrol and its glucuronide conjugate from the intestines of five- and twenty-five-day-old rats. J. Pharmacol. Exp. Ther. **185:** 163-170, 1973.
140. Fischer, L. J. and Weissinger, J. L.: Development in the newborn rat of the conjugation and de-conjugation processes involved in the enterohepatic circulation of diethylstilboestrol. Xenobiotica **2:** 399-412, 1972.
141. Fitzgerald, R. J., Gustafsson, B. E. and McDaniel, E. G.: Effects of coprophagy prevention on intestinal mictoflora in rats. J. Nutr. **84:** 155-160, 1964.
142. Fore, H., Walker, R. and Golberg, L.: Studies on brown FK. II. Degradative changes undergone *in vitro* and *in vivo*. Food Cosmet. Toxicol. **5:** 459-473, 1967.
143. Forth, W., Nell, G., Rummel, W. and Andres, H.: The hydragogue and laxative effect of the sulfuric acid ester and the free diphenol of 4,4'-dihydroxydiphenyl-(pyridyl-2)-methane. Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol. **274:** 46-53, 1972.
144. Foulkes, D. M.: The metabolism of C^{14}-ICI 54,450 (Myalex) in various species—an *in vivo* NIH shift. J. Pharmacol. Exp. Ther. **172:** 115-121, 1970.
145. Fouts, J. R. and Brodie, B. B.: The enzymatic reduction of chloramphenicol, *p*-nitrobenzoic acid and other aromatic nitro compounds in mammals. J. Pharmacol. Exp. Ther. **119:** 197-207, 1957.
146. Fowler, L. R. and Rammler, D. H.: Sulfur metabolism of *Aerobacter aerogenes*. II. The purification and some properties of a sulfatase. Biochemistry **3:** 230-237, 1964.
147. Fries, G. R., Marrow, G. S. and Gordon, C. H.: Metabolism of o,p'- and p,p'-DDT by rumen microorganisms. J. Agr. Food Chem. **17:** 860-862, 1969.
148. Gábor, M.: The anti-inflammatory action of flavonoids. Akadémiai Kiadó, Budapest, 1972.
149. Gall, L. S.: Normal fecal flora of man. Amer. J. Clin. Nutr. **23:** 1457-1465, 1970.
150. Garton, G. A. and Williams, R. T.: Studies in detoxication. **26.** The fates of phenol, phenylsulphuric acid and phenylglucuronide in the rabbit, in relation to the metabolism of benzene. Biochem. J. **45:** 158-163, 1949.
151. Gerber, N., Weller, W. L., Lynn, R., Rangno, R. E., Sweetman, B. J. and Bush, M. T.: Study of dose-dependent metabolism of 5,5-diphenylhydantoin in the rat using new methodology for isolation and quantitation of metabolites *in vivo* and *in vitro*. J. Pharmacol. Exp. Ther. **178:** 567-579, 1971.
151a. Gingell, R.: Substrate non-specificity of *Streptococcus faecalis* azoreductase. Xenobiotica **3:** 165-169, 1973.
152. Gingell, R. and Bridges, J. W.: Intestinal azo reduction and glucuronide conjugation of prontosil. Biochem. J. **125:** 24P, 1971.
153. Gingell, R., Bridges, J. W. and Williams, R. T.: Gut flora and the metabolism of prontosils in the rat. Biochem. J. **114:** 5P-6P, 1969.
154. Gingell, R., Bridges, J. W. and Williams, R. T.: The role of the gut flora in the metabolism of prontosil and neoprontosil in the rat. Xenobiotica **1:** 143-156, 1971.
155. Gingell, R. and Walker, R.: Mechanisms of azo reduction by *Streptococcus faecalis*. II. The role of soluble flavins. Xenobiotica **1:** 231-239, 1971.
156. Glazko, A. J., Carnes, H. E., Kazenko, A., Wolf, L. M. and Reutner, T. F.: Succinic acid esters of chloramphenicol. *In* Antibiotics Annual, 1957-1958, ed. by H. Welch and F. Marti-Ibanez, pp. 792-802, Medical Encyclopedia Inc., New York, 1958.
157. Glazko, A. J., Dill, W. A. and Wolf, L. M.: Observations on the metabolic disposition of chloramphenicol (Chloromycetin) in the rat. J. Pharmacol. Exp. Ther. **104:** 452-458, 1952.
158. Glazko, A. J., Dill, W. A., Wolf, L. M. and Kazenko, A.: Studies on the metabolism of reserpine. J. Pharmacol. Exp. Ther. **118:** 377-387, 1956.
159. Glazko, A. J., Wolf, L. M., Dill, W. A. and Bratton, A. C.: Biochemical studies on chloramphenicol (Chloromycetin). II. Tissue distribution and excretion studies. J. Pharmacol. Exp. Ther. **96:** 445-459, 1949.
160. Goddard, P. and Hill, M. J.: Aromatization of androst-4-ene-3,17-dione by human intestinal bacteria. Biochem. J. **124:** 73P, 1971.
160a. Goddard, P. and Hill, M. J.: Degradation of steroids by intestinal bacteria IV. The aromatisation of ring A. Biochim. Biophys. Acta **280:** 336-342, 1972.
161. Golab, T., Herberg, R. J., Day, E. W., Raun, A. P., Holzer, F. J. and Probst, G. W.: Fate of carbon-14 trifluralin in artificial rumen fluid and in ruminant animals. J. Agr. Food Chem. **17:** 576-580, 1969.
162. Golberg, L. Parekh, C., Patti, A. and Soike, K.: Cyclamate degradation in mammals and in vitro. Toxicol. Appl. Pharmacol. **14:** 654, 1969.
162a. Goldin, B. R. and Goldman, P.: The metabolism of dopa: The role of the intestinal microflora. Fed. Proc. **32:** 798, 1973.

163. GORBACH, S. L.: On the intestinal flora. Gastroenterology **57:** 231–232, 1969.

164. GORBACH, S. L.: Intestinal microflora. Gastroenterology **60:** 1110–1129, 1971.

165. GORBACH, S. L., NAHAS, L., LERNER, P. I. AND WEINSTEIN, L.: Studies on intestinal microflora I. Effects of diet, age, and periodic sampling on numbers of fecal microorganisms in man. Gastroenterology **53:** 845–855, 1967.

166. GORBACH, S. L., PLAUT, A. G., NAHAS, L., WEINSTEIN, L., SPANKNEBEL, G. AND LEVITAN, R.: Studies on intestinal microflora II. Microorganisms of the small intestine and their relations to oral and fecal flora. Gastroenterology **53:** 856–867, 1967.

167. GORDON, J. H. AND DUBOS, R.: The anaerobic bacterial flora of the mouse cecum. J. Exp. Med. **132:** 251–260, 1970.

168. GRABER, C. D., O'NEAL, R. M. AND RABIN, E. R.: Effect of high fat diets on intestinal microflora and serum cholesterol in rats. J. Bacteriol. **89:** 47–51, 1965.

169. GRANT, D. J. W. AND PATEL, J. C.: The non-oxidative decarboxylation of *p*-hydroxybenzoic acid, gentisic acid, protocatechuic acid and gallic acid by *Klebsiella aerogenes* (*Aerobacter aerogenes*). Antonie van Leeuwenhoek J. Microbiol. Serol. **35:** 325–343, 1969.

170. GRANTHAM, P. H., HORTON, R. E., WEISBURGER, E. K. AND WEISBURGER, J. H.: Metabolism of the carcinogen N-2-fluorenylacetamide in germ-free and conventional rats. Biochem. Pharmacol. **19:** 163–171, 1970.

171. GRANTHAM, P. H., WEISBURGER, E. K. AND WEISBURGER, J. H.: Dehydroxylation and deacetylation of N-hydroxy-N-2-fluorenylacetamide by rat liver and brain homogenates. Biochim. Biophys. Acta **107:** 414–424, 1965.

172. GREGORY, P. A., OLAVESEN, A. H., CURTIS, C. G. AND POWELL, G. M.: The fate of the sulphate esters of diethylstilboestrol in the rat. Biochem. J. **125:** 77P–78P, 1971.

173. GRIFFITHS, L. A.: *m*-Hydroxyphenylpropionic acid, a major urinary metabolite of (+)-catechin in the rat. Nature (London) **194:** 869–870, 1962.

174. GRIFFITHS, L. A.: Studies on flavonoid metabolism. Identification of the metabolites of (+)-catechin in rat urine. Biochem. J. **92:** 173–179, 1964.

175. GRIFFITHS, L. A.: Metabolism of sinapic acid and related compounds in the rat. Biochem. J. **113:** 603–609, 1969.

176. GRIFFITHS, L. A.: 3,5-Dihydroxyphenylpropionic acid, a further metabolite of sinapic acid. Experientia (Basel) **26:** 723–724, 1970.

177. GRIFFITHS, L. A. AND SMITH, G. E.: *In vitro* degradation of flavonoids by the intestinal microflora of the rat. *In* Abstracts Seventh International Meeting of the Federation of European Biochemical Societies, Bulgaria, p. 317, Academic Press, New York, 1971.

178. GRIFFITHS, L. A. AND SMITH, G. E.: Metabolism of apigenin and related compounds in the rat. Metabolite formation *in vivo* and by the intestinal microflora *in vitro*. Biochem. J. **128:** 901–911, 1972.

179. GRIFFITHS, L. A. AND SMITH, G. E.: Metabolism of myricetin and related compounds in the rat. Metabolite formation *in vivo* and by the intestinal microflora *in vitro*. Biochem. J. **130:** 141–151, 1972.

180. GUSTAFSSON, B. E., BERGSTRÖM, S., LINDSTEDT, S. AND NORMAN, A.: Turnover and nature of fecal bile acids in germfree and infected rats fed cholic acid-24-^{14}C. Bile acids and steroids. 41. Proc. Soc. Exp. Biol. Med. **94:** 467–471, 1957.

181. GUSTAFSSON, B. E. AND FITZGERALD, R. J.: Alteration in intestinal microbial flora of rats with tail cups to prevent coprophagy. Proc. Soc. Exp. Biol. Med. **104:** 319–322, 1960.

182. GUSTAFSSON, B. E., MIDTVEDT, T. AND NORMAN, A.: Isolated fecal microorganisms capable of 7α-dehydroxylating bile acids. J. Exp. Med. **123:** 413–432, 1966.

183. GUSTAFSSON, B. E., MIDTVEDT, T. AND NORMAN, A.: Metabolism of cholic acid in germfree animals after the establishment in the intestinal tract of deconjugating and 7α-dehydroxylating bacteria. Acta Pathol. Microbiol. Scand. **72:** 433–443, 1968.

184. HAENEL, H.: Some rules in the ecology of the intestinal microflora of man. J. Appl. Bacteriol. **24:** 242–251, 1961.

185. HAENEL, H.: Human normal and abnormal gastrointestinal flora. Amer. J. Clin. Nutr. **23:** 1433–1439, 1970.

186. HAGEN, C. A., SHEFNER, A. M. AND EHRLICH, R.: Intestinal microflora of normal hamsters. Lab. Anim. Care **15:** 185–193, 1965.

187. HAMILTON-MILLER, J. M. T.: Penicillinacylase. Bacteriol. Rev. **30:** 761–771, 1966.

188. HANAHAN, D. J. AND EVERETT, N. B.: The metabolism of S^{35}-sodium estrone sulfate in the adult female rat. J. Biol. Chem. **185:** 919–925, 1950.

189. HANSEN, I. L. AND CRAWFORD, M. A.: Bacterial degradation of the aromatic amino acid side chain. Biochem. Pharmacol. **17:** 338–342, 1968.

190. HANSSON, E., HOFFMANN, P. H. AND KRISTERSON, L.: Fate of mepivacaine in the body: II. Excretion and biotransformation. Acta Pharmacol. Toxicol. **22:** 213–223, 1965.

191. HAWKINS, J. B. AND YOUNG, L.: Biochemical studies of toxic agents. 5. Observations on the fate of ^{35}S-labelled arylsulphuric acids following their administration to the rat. Biochem. J. **56:** 166–170, 1954.

192. HAWKSWORTH, G., DRASAR, B. S. AND HILL, M. J.: Intestinal bacteria and the hydrolysis of glycosidic bonds. J. Med. Microbiol. **4:** 451–459, 1971.

193. HAWKSWORTH, G. M. AND HILL, M. J.: Bacteria and the N-nitrosation of secondary amines. Brit. J. Cancer **25:** 520–526, 1971.

194. HEARSE, D. J., POWELL, G. M., OLAVESEN, A. H. AND DODGSON, K. S.: The influence of some physico-chemical factors on the biliary excretion of a series of structurally related aryl sulphate esters. Biochem. Pharmacol. **18:** 181–195, 1969.

195. HENNESSEY, T. D.: Inducible β-lactamase in Enterobacter. J. Gen. Microbiol. **49:** 277–285, 1967.

196. HESPE, W. AND KAFOE, W. F.: Aspects of the biliary excretion of orphenadrine and its N-demethylated derivative, Tofenacin, in the rat. Eur. J. Pharmacol. **13:** 113–122, 1970.

197. HILL, M. J. AND DRASAR, B. S.: Bacterial degradation of bile salts. Biochem. J. **104:** 55P–56P, 1967.

198. HILL, M. J. AND DRASAR, B. S.: Degradation of bile salts by human intestinal bacteria. Gut **9:** 22–27, 1968.

199. HILL, M. J., DRASAR, B. S., ARIES, V., CROWTHER, J. S., HAWKSWORTH, G. AND WILLIAMS, R. E. O.: Bacteria and aetiology of cancer of the large bowel. Lancet **95–100**, 1971.

200. HOFFMANN, K.: Beeinflussungsmöglichkeiten der bakteriellen Darmbesiedlung. Deut. Med. Wochenschr. **92:** 1431–1432, 1967.

201. HOFFMANN, K. AND BIRCHER, J.: Veränderungen der bakteriellen Darmbesiedlung nach Laktulosegaben. Schweiz. Med. Wochenchr. **99:** 608–609, 1969.

202. HOFFMANN, K., MOSSEL, D. A. A., KORUS, W. AND VAN DE KAMER, J. H.: Untersuchungen über die Wirkungsweise der Lactulose (β-Galactosido-Fructose) im Darm. Klin. Wochenschr. **42:** 126–130, 1964.

203. HOLDER, G. M., RYAN, A. J., WATSON, T. R. AND WIEBE, L. I.: The biliary metabolism of butylated hydroxytoluene (3,5-di-t-butyl-4-hydroxytoluene) and its derivatives in the rat. J. Pharm. Pharmacol. **22:** 832–838, 1970.

204. HOLT, R.: The bacterial degradation of chloramphenicol. Lancet 1259–1260, 1967.

205. HORÁKOVÁ, Z., ZIERDT, C. H. AND BEAVEN, M. A.: Identification of *Lactobacillus* as the source of bacterial histidine decarboxylase in rat stomach. Eur. J. Pharmacol. **16:** 67–77, 1971.

206. HORWITT, M. K.: Observations on behavior of the anthocyan pigment from concord grapes in the animal body. Proc. Soc. Exp. Biol. Med. **30:** 949–951, 1933.

207. HUCKER, H. B., GRADY, L. T., MICHNIEWICZ, STAUFFER, S. C. WHITE, S. E., MAHA, G. E. AND MCMAHON, F. G.: Metabolism of a new hypolipidemic agent, 2-acetamidoethyl (*p*-chlorophenyl) (*m*-trifluoromethylphenoxy)-acetate (Halofenate) in the rat, dog, rhesus monkey and man. J. Pharmacol. Exp. Ther. **179:** 359–371, 1971.

208. Hucker, H. B., Zacchei, A. G., Cox, S. U., Brodie, D. A. and Cantwell, N. H. R.: Studies on the absorption, distribution and excretion of indomethacin in various species. J. Pharmacol. Exp. Ther. 153: 237–249, 1966.

209. Hülsmann, W. C. and Statius van Eps, L. W.: The metabolism of *p*-aminohippuric acid and *p*-acetylaminohippuric acid. Clin. Chim. Acta 15: 233–239, 1967.

210. Ikeda, G. J.: Conversion of a substituted 3,4,5-trimethoxycinnamide to a 3,5-dihydroxy compound in the rat and inhibition of the conversion by neomycin. Biochem. Pharmacol. 21: 2150–2153, 1972.

211. Indahl, S. R. and Scheline, R. R.: Decarboxylation of 4-hydroxycinnamic acids by *Bacillus* strains isolated from rat intestine. Appl. Microbiol. 16: 667, 1968.

212. Indahl, S. R. and Schline, R. R.: The metabolism of umbelliferone and herniarin in rats and by rat intestinal microflora. Xenobiotica 1: 13–24, 1971.

213. Indahl, S. R. and Scheline, R. R.: Quinic acid aromatization in the rat. Urinary hippuric acid and catechol excretion following the singular or repeated administration of quinic acid. Xenobiotica 3: 549–556, 1973.

214. Iveson, P., Lindup, W. E., Parke, D. V. and Williams, R. T.: The metabolism of carbenoxolone in the rat. Xenobiotica 1: 79–95, 1971.

215. Jondorf, W. R., Parke, D. V. and Williams, R. T.: Studies in detoxication. 76. The metabolism of halogenobenzenes. 1:2:3:4-, 1:2:3:5- and 1:2:4:5-tetrachlorobenzenes. Biochem. J. 69: 181–189, 1958.

216. Jones, R., Ryan, A. J. and Wright, S. E.: The metabolism and excretion of tartrazine in the rat, rabbit and man. Food Cosmet. Toxicol. 2: 447–452, 1964.

217. Kaighen, M. and Williams, R. T.: The metabolism of (3-^{14}C)coumarin. J. Med. Chem. 3: 25–43, 1961.

218. Kaihara, M. and Price, J. M.: Quinaldylglycyltaurine: A urinary metabolite of quinaldic acid and kynurenic acid in the cat. J. Biol. Chem. 236: 508–511, 1961.

219. Kaihara, M. and Price, J. M.: The metabolism of quinaldic acid, kynurenic acid, and xanthurenic acid in the rabbit. J. Biol. Chem. 237: 1727–1729, 1962.

220. Kaihara, M. and Price, J. M.: The effect of feeding neomycin on the dehydroxylation of xanthurenic acid to 8-hydroxyquinaldic acid by the rabbit. J. Biol. Chem. 238: 4082–4084, 1963.

221. Kallianos, A. G., Petrakis, P. L., Shetlar, M. R. and Wender, S. H.: Preliminary studies on degradation products of quercetin in the rat's gastrointestinal tract. Arch. Biochem. Biophys. 81: 430–433, 1959.

222. Kalser, M. H., Arteaga, I., Yawn, E., Hoffert, W., Frazier, D., Cohen, R. and Mayoral, L.: Microflora of the small intestine. Amer. J. Dig. Dis. 10: 839–843, 1965.

223. Kalser, M. H., Cohen, R., Arteaga, I., Yawn, E., Mayoral, L., Hoffert, W. R. and Frazier, D.: Normal viral and bacterial flora of the human small and large intestine. N. Engl. J. Med. 274: 500–505 and 558–563, 1966.

224. Katzung, B. G. and Meyers, F. H.: Excretion of radioactive digitoxin in the dog. J. Pharmacol. Exp. Ther. 149: 257–262, 1965.

225. Katzung, B. G. and Meyers, F. H.: Biotransformation of digitoxin in the dog. J. Pharmacol. Exp. Ther. 154: 575–580, 1966.

226. Keberle, H., Hoffman, K. and Bernhard, K.: The metabolism of glutethimide (Doriden). Experientia (Basel) 18: 105–111, 1962.

227. Kemp, P. and Dawson, R. M. C.: Isomerization of linolenic acid by rumen micro-organisms. Biochem. J. 109: 477–478, 1968.

228. Kemp, P. and White, R. W.: The biohydrogenation of linolenic and linoleic acids by bacteria isolated from an ovine rumen. Biochem. J. 106: 55P, 1968.

229. Klubes, P. and Jondorf, W. R.: Dimethylnitrosamine formation from sodium nitrite and dimethylamine by bacterial flora of rat intestine. Res. Commun. Chem. Pathol. Pharmacol. 2: 24–34, 1971.

230. Kobayashi, A. and Matsumoto, H.: Studies on methylazoxymethanol, the aglycone of cycasin. Isolation, biological and chemical properties. Arch. Biochem. Biophys. 110: 373–380, 1965.

231. Koechlin, B. A., Rubio, F., Palmer, S., Gabriel, T. and Duschinsky, R.: The metabolism of 5-fluorocytosine-2 ^{14}C and of cytosine ^{14}C in the rat and the disposition of 5-fluorocytosine-2 ^{14}C in man. Biochem. Pharmacol. 15: 435–466, 1966.

232. Kojima, S. and Ichibagase, H.: Studies on synthetic sweetening agents. VIII. Cyclohexylamine, a metabolite of sodium cyclamate. Chem. Pharm. Bull. (Japan) 14: 971–974, 1966.

233. Krishnamurty, H. G., Cheng, K.-J., Jones, G. A., Simpson, F. J. and Watkin, J. E.: Identification of products produced by the anaerobic degradation of rutin and related flavonoids by *Butyrivibrio sp.* C_2. Can. J. Microbiol. 16: 759–767, 1970.

234. Kuchar, E. J., Gebnty, F. O., Griffith, W. P. and Thomas, R. J.: Analytical studies of metabolism of terraclor in beagle dogs, rats and plants. J. Agr. Food Chem. 17: 1237–1240, 1969.

235. Kutches, A. J. and Church, D. C.: DDT-^{14}C metabolism by rumen bacteria and protozoa in vitro. J. Dairy Sci. 54: 540–543, 1971.

236. Kwong, E. and Barnes, R. H.: Effect of coprophagy prevention and antibiotics on choline degradation. J. Nutr. 100: 711–714, 1970.

237. Ladd, J. N. and Walker, D. J.: The fermentation of lactate and acrylate by rumen micro-organism LC. Biochem. J. 71: 364–373, 1959.

238. Lanigan, G. W. and Smith, L. W.: Metabolism of pyrrolizidine alkaloids in the ovine rumen. I. Formation of 7α-hydroxy-1α-methyl-8α-pyrrolisidine from heliotrine and lasiocarpine. Aust. J. Agr. Res. 21: 493–500, 1970.

239. Laquer, G. L.: Carcinogenic effects of cycad meal and cycasin, methylazoxymethanol glycoside, in rats and effects of cycasin in germfree rats. Fed. Proc. 23: 1386–1388, 1964.

240. Lauterbach, F. and Repke, K.: Über die Verteilung herzglykosidspaltender Fermente im tierischen Organismus. Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol. 240: 45–71, 1960.

241. Leahy, J. S., Taylor, T. and Rudd, C. J.: Cyclohexylamine excretors among human volunteers given cyclamate. Food Cosmet. Toxicol. 5: 595–596, 1967.

242. Leahy, J. S., Wakefield, M. and Taylor, T.: Urinary excretion of cyclohexylamine following oral administration of sodium cyclamate to man. Food Cosmet. Toxicol. 5: 447, 1967.

243. Lee, A., Gordon, J. and Dubos, R.: Enumeration of the oxygen sensitive bacteria usually present in the intestine of healthy mice. Nature (London) 220: 1137–1139, 1968.

244. Lee, C.-C., Anderson, R. C., Henderson, F. G., Worth, H. M. and Harris, P. N.: Pharmacology and toxicology of erythromycin propionate. *In* Antibootics Annual 1958–1959., ed. by H. Welch and F. Marti-Ibanes, pp. 354–363, Medical Encyclopedia Inc., New York, 1959.

245. Levy, C. C. and Goldman, P.: The enzymatic hydrolysis of methotrexate and folic acid. J. Biol. Chem. 242: 2933–2938, 1967.

246. Levy, G. and Angelino, N. J.: Hydrolysis of aspirin by rat small intestine. J. Pharm. Sci. 57: 1449–1450, 1968.

247. Levy, G., Weintraub, L., Matsuzawa, T. and Oles, S.: Absorption metabolism and excretion of salicylic phenolic glucuronide in rats. J. Pharm. Sci. 55: 1319–1320, 1966.

248. Lethco, E. J. and Brouwer, E. A.: The metabolism of naphthaleneacetic acid-1-C^{14} in rats. J. Agr. Food Chem. 14: 532–535, 1966.

249. Lethco, E. J. and Webb, J. M.: The fate of FD and C blue no. 2 in rats. J. Pharmacol. Exp. Ther. 154: 384–389 1966.

250. Litchfield, M. H. and Swan, A. A. B.: Cyclohexylamine production and physiological measurements in subjects ingesting sodium cyclamate. Toxic. Appl. Pharmacol. 18: 535–541, 1971.

251. Lower, G. M. and Bryan, G. T.: The metabolism of the 8-methyl ether of xanthurenic acid in the mouse. Cancer Res. 29: 1013–1018, 1969.

252. Lower, G. M. and Bryan, G. T.: The metabolism of the 8-methyl ether of xanthurenic acid in the rabbit. J. Biol. Chem. 244: 2567–2571, 1969.

253. Luckey, T. D.: Antibiotics in nutrition. *In* Antiobiotics, Their Chemistry and Non-medical Uses, ed. by H. S. Goldberg, pp. 174–321, Van Nostrand, Princeton, N. J., Toronto, London and New York, 1959.

254. Magee, P. N.: Toxicity of nitrosamines: Their possible human health hazards. Food Cosmet. Toxicol. 9: 207–218, 1971.

255. Malone, T. C.: *In vitro* conversion of DDT to DDD by the intestinal microflora of the northern anchovy, *Engraulis mordax*. Nature (London) 227: 848–849, 1970.

256. Marsh, C. A., Alexander, F. and Levvy, G. A.: Glucuronide decomposition in the digestive tract. Nature (London) 170: 163–164, 1952.

257. Masri, M. S., Booth, A. N. and DeEds, F.: The metabolism and acid degradation of quercetin. Arch. Biochem. Biophys. 85: 284–286, 1959.

258. Matsumoto, H. and Strong, F. M.: The occurrence of methylazoxymethanol in *Cycas circinalis* L. Arch. Biochem. Biophys. 101: 299–210, 1963.

259. McCullough, W. G., Piligian, J. T. and Daniel, I. J.: Enzymatic decarboxylation of the aminobenzoates. J. Amer. Chem. Soc. 79: 628–630, 1957.

260. McMahon, K. A. and O'Reilly, W. J.: Metabolism and biliary excretion of arylaminosulphonic acids in the rat. Food Cosmet. Toxicol. 7: 497–500, 1969.

261. McNutt W.: The enzymatic deamination and amide cleavage of folic acid. Arch. Biochem. Biophys. 101: 1–6, 1963.

262. Mead, J. A. R., Smith, J. N. and Williams, R. T.: Studies in detoxication 72. The metabolism of coumarin and *o*-coumaric acid. Biochem. J. 68: 67–74, 1958.

263. Melnykowycz, J. and Johansson, K. R.: Formation of amines by intestinal microorganisms and the influence of chlortetracycline. J. Exp. Med. 101: 507–517, 1955.

264. Mendel, J. L. and Walton, M. S.: Conversion of p,p'-DDT to p,p'-DDD by intestinal flora of the rat. Science 151: 1527–1528, 1966.

265. Meyer, T. and Scheline, R. R.: 3,4,5-Trimethoxycinnamic acid and related compounds. I. Metabolism by the rat intestinal microflora. Xenobiotica 2: 383–390, 1972.

266. Meyer, T. and Scheline, R. R.: 3,4,5-Trimethoxycinnamic acid and related compounds. II. Metabolism in the rat. Xenobiotica 2: 391–398, 1972.

267. Midtvedt, T. and Norman, A.: Bile acid transformations by microbial strains belonging to genera found in intestinal contents. Acta Pathol. Microbiol. Scand. 71: 629–638, 1967.

268. Midtvedt, T. and Norman, A.: Parameters in 7α-dehydroxylation of bile acids by anaerobic lactobacilli. Acta Pathol. Microbiol. Scand. 72: 313–329, 1968.

269. Milazzo, F. H. and Fitzgerald, J. W.: A study of arylsulfatase activity in *Proteus rettgeri*. Can. J. Microbiol. 12: 735–744, 1966.

270. Miller, B., Mitchison, R., Tabaqchali, S. and Neale, G.: The effects of excessive bacterial proliferation on protein metabolism in rats with self-filling jejunal sacs. Eur. J. Clin. Invest. 2: 23–31, 1971.

271. Minder, R., Schnetzer, F. and Bickel, M. H.: Hepatic and extrahepatic metabolism of the psychotropic drugs, chlorpromazine, imipramine and imipramine-N-oxide. Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol. 268: 334–347, 1971.

272. Miskus, R. P., Blair, D. P. and Casida, J. E.: Conversion of DDT to DDD by bovine rumen fluid, lake water, and reduced porphyrins. J. Agr. Food Chem. 13: 481–483, 1965.

273. Moody, J. P. and Williams, R. T.: The fate of 4-nitrophenylarsonic acid in hens. Food Cosmet. Toxicol. 2: 695–706, 1964.

274. Moody, J. P. and Williams, R. T.: The metabolism of 4-hydroxy-3-nitrophenylarsonic acid in hens. Food Cosmet. Toxicol. 2: 707–715, 1964.

275. Moore, J. H., Noble, R. C., Steele, W. and Czerkawski, J. W.: Differences in the metabolism of esterified and unesterified linoleic acid by rumen microorganisms. Brit. J. Nutr. 23: 869–878, 1969.

276. Murrells, D. F.: The Reduction of Food Azo Dyes by Rat Caecal Microflora, with Particular Reference to *Streptococcus faecalis*, Ph.D. Thesis, University of Reading, 1969.

277. Nair, P. P., Gordon, M. and Reback, J.: The enzymatic cleavage of the carbon-nitrogen bond in 3α, 7α, 12α-trihydroxy-5β-cholan-24-oylglycine. J. Biol. Chem. 242: 7–11, 1967.

278. Nakagawa, Y., Shetlar, M. R. and Wender, S. H.: Urinary products from quercetin in neomycin-treated rats. Biochim. Biophys. Acta 97: 233–241, 1965.

279. Nakai, Y., Shirakawa, Y., Tanayama, S., Fujita, T., Suzuoki, Z., Kato, S. and Hayashi, N.: Metabolism of a thyroxine analogue, 3,5-diiodo-4-(4'-acetoxy-3'-iodophenoxy)benzoic acid in rats. Xenobiotica 2: 277–291, 1972.

280. Nelson, D. P. and Mata, L. J.: Bacterial flora associated with the human gastrointestinal mucosa. Gastroenterology 58: 56–61, 1970.

281. Nilsson, A.: Demethylation of the plant oestrogen biochanin A in the rat. Nature (London) 192: 358, 1961.

282. Nilsson, A.: On the *in vitro* metabolism of the plant estrogen biochanin A in rumen fluid. Ark. Kemi. 17: 305–310, 1961.

283. Nilsson, A.: Demethylation of the plant estrogen formononetin to daidzein in rumen fluid. Ark. Kemi. 19: 549–550, 1962.

284. Nilsson, A., Hill, J. L. and Davies, H. L.: An *in vitro* study of formononetin and biochanin A metabolism in rumen fluid from sheep. Biochim. Biophys. Acta 148: 92–98, 1967.

285. Norman, A. and Grubb, R.: Hydrolysis of conjugated bile acids by clostridia and enterococci. Acta Pathol. Microbiol. Scand. 36: 537–547, 1955.

286. Norman, A. and Widstrøm, O. A.: Hydrolysis of conjugated bile acids by extracellular enzymes present in rat intestinal contents. Proc. Soc. Exp. Biol. Med. 117: 442–444, 1964.

287. Norseth, T.: Biotranformation of methyl mercuric salts in germ free rats. Acta Pharmacol. Toxicol. 30: 172–176, 1971.

288. Okabayashi, T. and Yoshimoto, A.: Reduction of 4-nitroquinoline 1-oxide by microorganisms. Chem. Pharm. Bull. (Japan) 10: 1221–1226, 1962.

289. Oser, B. L., Carson, S., Vogin, E. E. and Sonders, R. C.: Conversion of cyclamate to cyclohexylamine in rats. Nature (London) 220: 178–179, 1968.

290. Oshima, Y. and Watanabe, H.: The mechanisms of catechins metabolism. II. Neutral substances in the urine of rabbits administered (+)-catechin. J. Biochem. (Tokyo) 45: 973–977, 1958.

291. Oshima, Y., Watanabe, H. and Isakari, S.: The mechanisms of catechins metabolism. I. Acidic substances in the urine of rabbits administered (+)-catechin. J. Biochem. (Tokyo) 45: 861–865, 1958.

292. Pala, G., Coppi, G. and Crescenzi, E.: On the laxative properties of sulfuric esters of phenols, with particular reference to 4,4'-(2-picolylidene)-bis-phenylsulfuric acid disodium salt (Picosulfol). Arch. Int. Pharmacodyn. Thér. 164: 356–369, 1966.

293. Parke, D. V. and Williams, R. T.: Studies in detoxication. 81. The metabolism of halogenobenzenes: (*a*) Penta- and hexachlorobenzenes. (*b*) Further observations on 1:3:5-trichlorobenzene. Biochem. J. 74: 5–9, 1960.

294. Patel, J. C. and Grant, D. J. W.: The formation of phenol in the degradation of *p*-hydroxybenzoic acid by *Klebsiella aerogenes* (*Aerobacter aerogenes*). Antonie van Leeuwenhoek J. Microbiol. Serol. 35: 53–64, 1969.

295. Patki, V. M. and Shirsat, M. V.: Chemotherapy of intestinal infections. Part IV. Absorption, excretion and

deacylation of N⁴-aliphatic acyl derivatives of sulphadiazine. J. Sci. Ind. Res. (India) **20C**: 181–185, 1961.

296. Peppercorn, M. A. and Goldman, P.: Caffeic acid metabolism by bacteria of the human gastrointestinal tract. J. Bacteriol. **108**: 996–1000, 1971.
297. Peppercorn, M. A. and Goldman, P.: The role of intestinal bacteria in the metabolism of salicylazosulfapyridine. J. Pharmacol. Exp. Ther. **181**: 555–562, 1972.
297a. Peppercorn, M. A. and Goldman, P.: Caffeic acid metabolism by gnotobiotic rats and their intestinal bacteria. Proc. Nat. Acad. Sci. U.S.A. **69**: 1413–1415, 1972.
297b. Peppercorn, M. A. and Goldman, P.: Distribution studies of salicylazosulfapyridine and its metabolites. Gastroenterology **64**: 240–245, 1973.
298. Pérez Silva, M. G. and Rodríguez Sánchez, D.: Deshidroxilación del ácido cafeico por varias especies de pseudomonas. Boln R. Soc. esp. Hist. nat. (Biol.) **65**: 401–405, 1967.
299. Perez-Silva, G., Rodriguez, D. and Perez-Silva, J.: Dehydroxylation of caffeic acid by a bacterium isolated from rat faeces. Nature (London) **212**: 303–304, 1966.
300. Perry, T. L., Hestrin, M., MacDougall, L. and Hansen, S.: Urinary amines of intestinal bacterial origin. Clin. Chim. Acta **14**: 116–123, 1966.
301. Peterson, J. E. and Robison, W. H.: Metabolic products of p,p'-DDT in the rat. Toxicol. Appl. Pharmacol. **6**: 321–327, 1964.
302. Petrakis, P. L., Kallianos, A. G., Wender, S. H. and Shetlar, M. R.: Metabolic studies of quercetin labelled with C^{14}. Arch. Biochem. Biophys. **85**: 264–271, 1959.
303. Pittard, A. J., Gibson, F. and Doy, C. H.: A possible relationship between the formation of *o*-dihydric phenols and tryptophan biosynthesis by *Aerobacter aerogenes*. Biochim. Biophys. Acta **57**: 290–298, 1962.
304. Plaa, G. L.: Biliary and other routes of excretion of drugs. *In* Fundamentals of Drug Metabolism and Drug Disposition, ed. by B. N. LaDu, H. G. Mandel and E. L. Way, pp. 131–145 Williams & Wilkins, Baltimore, 1971.
305. Plaut, A. G., Gorbach, S. L., Nahas, L., Weinstein, L., Spanknebel, G. and Levitan, R.: Studies on intestinal microflora III. The microbial flora of human small intestinal mucosa and fluids. Gastroenterology **53**: 868–873, 1967.
306. Polan, C. E., McNeill, J. J. and Tove, S. B.: Biohydrogenation of unsaturated fatty acids by rumen bacteria. J. Bacteriol. **88**: 1056–1064, 1964.
307. Porter, J. R. and Rettger, L. F.: Influence of diet on distribution of bacteria in stomach, small intestine and cecum of white rat. J. Infect. Dis. **66**: 104–110, 1940.
308. Prentiss, P. G., Rosen, H., Brown, N., Horowitz, R. E., Malm, O. J. and Levenson, S. M.: The metabolism of choline by the germfree rat. Arch. Biochem. Biophys. **94**: 424–429, 1961.
309. Prosky, L. and O'Dell R. G.: *In vivo* conversion of ^{14}C-labeled cyclamate to cyclohexylamine. J. Pharm. Sci. **60**: 1341–1343, 1971.
310. Quick, A. J.: The conjugation of benzoic acid in man. J. Biol. Chem. **92**: 65–85, 1931.
311. Quick, A. J.: The relationship between chemical structure and physiological response. II. The conjugation of hydroxy- and methoxybenzoic acids. J. Biol. Chem. **97**: 403–419, 1932.
312. Radomski, J. L. and Mellinger, T. J.: The absorption, fate and excretion in rats of the water-soluble azo dyes, FD & C red no. 2, FD & C red no. 4, and FD & C yellow no. 6. J. Pharmacol. Exp. Ther. **136**: 259–266, 1962.
313. Raibaud, P., Dickinson, A. B., Sacquet, E., Charlier, H. and Mocquot, G.: La microflore du tube digestif du rat I. Techniques d'étude et milieux de culture proposés. Ann. Inst. Pastuer, Paris **110**: 568–590, 1966.
314. Raibaud, P., Dickinson, A. B., Sacquet, E., Charlier, H. and Mocquot, G.: La microflore du tube digestif du rat II. Dénombrement de différents genres microbiens dans l'estomac et l'intestin de rats conventionnels. Variations quantitatives individuelles et en fonction de l'age. Ann. Inst. Pasteur, Paris **110**: 861–876, 1966.
315. Rammler, D. H., Grado, C. and Fowler, L. R.: Sulfur metabolism of *Aerobacter aerogenes*. I. A repressible sulfatase. Biochemistry **3**: 224–230, 1964.
316. Ranney, R. E. and Eich, S. J.: The metabolism of sodium amylopectin sulfate in the rat. Toxicol. Appl. Pharmacol. **15**: 593–603, 1969.
317. Renwick, A. G. and Williams, R. T.: Gut bacteria and the metabolism of cyclamate in the rat. Biochem. J. **114**: 78P, 1969.
318. Renwick, A. G. and Williams, R. T.: The fate of cyclamate in man and other species. Biochem. J. **129**: 869–879, 1972.
319. Roche, J., Michel, R. and Stützel, M.: Présence de la 3,5,3'-triiodothyronamine dans le contenu intestinal du rat apres administration de 3,5,3'-triiodo-L-thyronine. Biochim. Biophys. Acta **63**: 496–500, 1962.
320. Rosebury T.: Microorganisms Indigenous to Man, McGraw-Hill, New York, Toronto and London, 1962.
321. Rosenberg, I. H., Hardison, W. G. and Bull, D. M.: Abnormal bile-salt patterns and intestinal bacterial overgrowth associated with malabsorption. N. Engl. J. Med. **276**: 1391–1397, 1967.
322. Rosenfeld, R. S.: The isolation of coprostanol from sterol esters of human feces. Arch. Biochem. Biophys. **108**: 384–385, 1964.
323. Rosenfeld, R. S.: Biosynthesis of coprostanyl esters. Arch. Biochem. Biophys. **112**: 621–625, 1965.
324. Rosenfeld, R. S., Paul, I. and Yamauchi, T., Sterol esterification in feces. Arch. Biochem. Biophys. **122**: 653–657. 1967.
325. Roxon, J. J. Ryan, A. J., Welling, P. G. and Wright, S. E.: Origin of urinary sulphanilic acid from tartrazine. Food Cosmet. Toxicol. **5**: 447–449, 1967.
326. Roxon, J. J., Ryan, A. J. and Wright, S. E.: Reduction of tartrazine by a Proteus species isolated from rats. Food Cosmet. Toxicol. **4**: 419–426, 1966.
327. Roxon, J. J., Ryan, A. J. and Wright, S. E.: Reduction of water-soluble azo dyes by intestinal bacteria. Food Cosmet. Toxicol. **5**: 367–369, 1967.
328. Roxon, J. J., Ryan, A. J. and Wright, S. E.: Enzymatic reduction of tartrazine by *Proteus vulgaris* from rats. Food Cosmet. Toxicol. **5**: 645–656, 1967.
329. Russell, G. R. and Smith, R. M.: Reduction of heliotrine by a rumen microorganism. Aust. J. Biol. Sci. **21**: 1277–1290, 1968.
330. Ryan, A. J., Holder, G. M., Mate, C. and Adkins, G. K.: The metabolism and excretion of hydroxyethyl starch in the rat. Xenobiotica **2**: 141–146, 1972.
331. Ryan, A. J., Roxon, J. J. and Sivayavirojana, A.: Bacterial azo reduction: a metabolic reaction in mammals. Nature (London) **219**: 854–855, 1968.
332. Ryan, A. J. and Welling, P. G.: Some observations on the metabolism and excretion of the bisazo dyes sudan III and sudan IV. Food Cosmet. Toxicol. **5**: 755–761, 1967.
333. Ryan, A. J., Welling P. G. and Roxon J. J.: Metabolism of a tartrazine analogue by intestinal bacteria. Food Cosmet. Toxicol. **7**: 297–299, 1969.
334. Ryan, A. J., Welling, P. G. and Wright, S. E.: Further studies on the metabolism of tartrazine and related compounds in the rat. Food Cosmet. Toxicol. **7**: 287–295, 1969.
335. Sander, J.: Nitrosaminsynthese durch Bakterien. Hoppe-Seyler's Z. Physiol. Chem. **349**: 429–432, 1968.
336. Sander, J. and Seif, F.: Bakterielle Reduktion von Nitrat im Magen des Menschen als Ursache einer Nitrosamin-Bildung. Arzneimittel-Forschung. **19**: 1091–1093, 1969.
337. Sandler, M., Goodwin, B. L., Ruthven, C. R. J. and Calne, D. B.: Therapeutic implications in parkinsonism of m-tyramine formation from L-dopa in man. Nature (London) **229**: 414–416, 1971.
338. Sandler, M., Karoum, F., Ruthven, C. R. J. and Calne,

D. B.: *m*-Hydroxyphenylacetic acid formation from L-dopa in man: Suppression by neomycin. Science **166:** 1417–1418, 1969.

330. Savage, D. C.: Associations of indigenous microorganisms with gastrointestinal mucosal epithelia. Amer. J. Clin. Nutr. **23:** 1495–1501, 1970.
340. Saz, A. K. and Marmur, J.: The inhibition of organic nitro-reductase by aureomycin in cell-free extracts. Proc. Soc. Exp. Biol. Med. **82:** 783–784, 1953.
341. Saz, A. K. and Slie, R. B.: The inhibition of organic nitro reductase by aureomycin in cell-free extracts. II. Cofactor requirements for the nitro reductase enzyme complex. Arch. Biochem. Biophys. **51:** 5–16, 1954.
342. Saz, A. K. and Slie, R. B.: Reversal of aureomycin inhibition of bacterial cell-free nitro reductase by manganese. J. Biol. Chem. **210:** 407–412, 1954.
343. Scheline, R. R.: The decarboxylation of some phenolic acids by the rat. Acta Pharmacol. Toxicol. **24:** 275–285, 1966.
344. Scheline, R. R.: 4-Methylcatechol, a metabolite of homoprotocatechuic acid. Experientia (Basel) **23:** 493–494, 1967.
345. Scheline, R. R.: Metabolism of phenolic acids by the rat intestinal microflora. Acta Pharmacol. Toxicol. **26:** 189–205, 1968.
346. Scheline, R. R.: Studies on the role of the intestinal microflora in the metabolism of coumarin in rats. Acta Pharmacol. Toxicol. **26:** 325–331, 1968.
347. Scheline, R. R.: The metabolism of drugs and other organic compounds by the intestinal microflora. Acta Pharmacol. Toxicol. **26:** 332–342, 1968.
348. Scheline, R. R.: Drug metabolism by intestinal microorganisms. J. Pharm. Sci. **57:** 2021–2037, 1968.
349. Scheline, R. R.: The metabolism of (+)-catechin to hydroxyphenylvaleric acids by the intestinal microflora. Biochim. Biophys. Acta **222:** 228–230, 1970.
350. Scheline, R. R.: Toxicological implications of drug metabolism by intestinal bacteria. *In* Toxicological Problems of Drug Combinations, vol. 13, Proceedings of the European Society for the Study of Drug Toxicity, pp. 35–43, Excerpta Medica Foundation, Amsterdam, 1972.
351. Scheline, R. R.: The metabolism of some aromatic aldehydes and alcohols by the rat intestinal microflora. Xenobiotica **2:** 227–236, 1972.
352. Scheline, R. R.: Unpublished observations.
353. Scheline, R. R. and Longberg, B.: The absorption, metabolism and excretion of the sulphonated azo dye, acid yellow, by rats. Acta Pharmacol. Toxicol. **23:** 1–14, 1965.
354. Scheline, R. R. and Midtvedt, T.: Absence of dehydroxylation of caffeic acid in germ-free rats. Experientia (Basel) **26:** 1068–1069, 1970.
355. Scheline, R. R. Nygaard R. T. and Longberg B.: Enzymatic reduction of the azo dye, acid yellow, by extracts of *Srtreptococcus faecalis* isolated from rat intestine. Food Cosmet. Toxicol. **8:** 55–58, 1970.
356. Scheline, R. R. and Strand, L. P.: Reduction of vanillin and vanilly alcohol to toluene derivatives in the rat. Proceedings, Fifth International Congress on Pharmacology, San Francisco, July 23–28, 1972, in press.
357. Scheline, R. R., Williams, R. T. and Wit, J. G.: Biological dehydroxylation. Nature (London) **188:** 849–850, 1960.
358. Schröder, H. and Campbell, D. E. S.: Absorption, metabolism, and excretion of salicylazosulfapyridine in man. Clin. Pharmacol. Ther. **13:** 539–551, 1972.
359. Scott, T. W., Ward, P. F. V. and Dawson, R. M. C.: Formation of aromatic fatty acids from amino acids by rumen micro-organisms. Biochem. J. **87:** 3P–4P, 1963.
360. Scott, T. W. Ward. P. F. V. and Dawson, R. M. C.: The formation and metabolism of phenyl-substituted fatty acids in the ruminant. Biochem. J. **90:** 12–24, 1964.
361. Shaw, K. N. F., Gutenstein, M. and Jepson, J. B.: Intestinal flora and diet in relation to *m*-hydroxyphenyl acids of human urine. Proceedings of the Fifth International Congress on Biochemistry, Moscow 1961, Vol. 5, ed. by N. M. Sissakian, p. 427, Pergamon Press, Oxford, 1963.
362. Shimoyama, M., Tanigawa, Y., Ito, T., Murashima, R., Ueda, I. and Tomoda, T.: Nicotinamide deamidation by microorganisms in rat stomach. J. Bacteriol. **108:** 191–195, 1971.
363. Simpson, F. J., Jones, G. A. and Wolin, E. A.: Anaerobic degradation of some bioflavonoids by microflora of the rumen. Can. J. Microbiol. **15:** 972–974, 1969.
364. Sink, J. D., Varela-Alvarez, H. and Hess, C.: Metabolism of ^{14}C-DDT by ovine rumen fluid *in vitro*. J. Agr. Food Chem. **20:** 7–9, 1972.
365. Sjaastad, O.: Histamine formation and catabolism in the faeces with special reference to dystrophia myotonica. Scand. J. Gastroenterol. **1:** 173–187, 1966.
366. Sjaastad, O.: Fate of histamine and N-Acetylhistamine administered into the human gut. Acta Pharmacol. Toxicol. **24:** 189–202, 1966.
367. Sjaastad, Ø. V. and Kay, R. N. B.: *In vivo* rupture of the imidazole ring of histamine. Experimentia (Basel) **26:** 1197–1198, 1970.
368. Sjaastad, Ø. V. and Sjaastad, O.: Rupture of the imidazole ring of histamine and N-acetylhistamine by human intestinal contents. Acta Pharmacol. Toxicol. **30:** 366–371, 1971.
369. Smith, A. A.: Dehydroxylation of norepinephrine. Biochim. Biophys. Acta **69:** 203–204, 1963.
370. Smith, A. A., Fabrykant, M., Kaplan, M. and Gavitt, J.: Dehydroxylation of some catecholamines and their products. Biochim. Biophys. Acta **86:** 429–439, 1964.
371. Smith, G. E. and Griffiths, L. A.: Metabolism of myricetin, myricitrin and 3,4,5-trihydroxyphenylacetic acid. Biochem. J. **118:** 53P–54P, 1970.
372. Smith, G. N. and Worrel, C. S.: Enzymatic reduction of chloramphenicol (Chloromycetin). Arch. Biochem. **24:** 216–223, 1949.
373. Smith, G. N. and Worrel, C. S.: The decomposition of Chloromycetin (chloramphenicol) by microorganisms. Arch. Biochem. **28:** 232–241, 1950.
374. Smith, G. N., Worrel, C. S. and Lilligren, B. L.: The enzymatic hydrolysis of chloramphenicol (Chloromycetin). Science **110:** 297–298, 1949.
375. Smith, H. W.: Observations on the flora of the alimentary tract of animals and factors affecting its composition. J. Path. Bact. **89:** 95–122, 1965.
376. Smith, H. W.: The development of the flora of the alimentary tract in young animals. J. Pathol. Bacteriol. **90:** 495–513, 1965.
377. Smith, H. W.: Effect of antibiotics on bacterial ecology in animals. Amer. J. Clin. Nutr. **23:** 1472–1479, 1970.
378. Smith, H. W. and Crabb, W. E.: The faecal bacterial flora of animals and man: its development in the young. J. Pathol. Bacteriol. **82:** 53–66, 1961.
379. Smith, R. L.: The biliary excretion and enterohepatic circulation of drugs and other organic compounds. *In* Progress in Drug Research, vol. 9, ed. by E. Jucker, pp. 301–360, Birkhäuser, Basel and Stuttgart, 1966.
380. Smith, R. L.: Species differences in the biliary excretion of drugs. *In* The Problems of Species Differences and Statistics in Toxicology, vol. 11, Proceedings of the European Society for the Study of Drug Toxicity, pp. 19–32, Excerpta Medica Foundation, Amsterdam, 1970.
381. Smith, R. L.: The role of the gut flora in the conversion of inactive compound to active metabolites. *In* A Symposium on Mechanisms of Toxicity, ed. by W. N. Aldridge, pp. 229–247, Macmillan, 1971.
382. Smith, R. L.: Excretion of drugs in bile. *In* Handbook of Experimental Pharmacology, vol. XXVIII/1, Concepts in Biochemical Pharmacology, ed. by B. B. Brodie and J. R. Gillette, pp. 354–389, Springer, Berlin, Heidelberg and New York 1971.
383. Soleim, H. A. and Scheline, R. R.: Metabolism of xeno-

biotics by strains of intestinal bacteria. Acta Pharmacol. Toxicol. 31: 471–480, 1972.

384. Sonders, R. C., Netwal, J. C. and Wiegand, R. G.: Site of conversion of cyclamate to cyclohexylamine. Pharmacologist 11: 241, 1969.

385. Spatz, M., McDaniel, E. G. and Laqueur, G. L.: Cycasin excretion in conventional and germfree rats. Proc. Soc. Exp. Biol. Med. 121: 417–422, 1966.

386. Spatz, M., Smith, D. W. E., McDaniel, E. G. and Laqueur, G. L.: Role of intestinal microorganisms in determining cycasin toxicity. Proc. Soc. Exp. Biol. Med. 124: 691–697, 1967.

387. Spears, R. W. and Freter, R.: Improved isolation of anaerobic bacteria from the mouse cecum by maintaining continuous strict anaerobiosis. Proc. Soc. Exp. Biol. Med. 124: 903–908, 1967.

388. Speck, R. S., Calloway, D. H. and Hadley, W. K.: Human fecal flora under controlled diet intake. Amer. J. Clin. Nutr. 23: 1488–1494, 1970.

389. Spink, W. W., Hurd, F. W. and Jermsta, J.: *In vitro* conversion of prontosil-soluble to sulfanilamide by various types of microorganisms. Proc. Soc. Exp. Biol. Med. 43: 172–175, 1940.

390. Steinetz, B. G., Meli, A., Giannina, T. and Beach, V. L.: Studies on biliary metabolites of orally administered ethylestradiol (EE) and its 3-cyclopentyl ether (EECPE-Quinestrol). Proc. Soc. Exp. Biol. Med. 124: 1283–1289, 1967.

391. Stenersen, J. H. V.: DDT-metabolism in resistant and susceptible stable-flies and in bacteria. Nature (London) 207: 660–661, 1965.

392. Stewart, D. J.: Formation of protocatechuic from quinic acid by fluorescent pseudomonads. Nature (London) 208: 200, 1965.

393. Stewart, G. T.: Inactivation of β-lactam antibiotics by bacterial enzymes. *In* Antibiotics. Advances in Research, Production and Clinical Use. Proceedings of the Congress on Antibiotics, Prague, June 15–19, 1964, ed. by M. Herold, pp. 25–31, Butterworths, London, 1966.

394. Stimmel, B. F.: Reduction of radioactive estrone to estradiol by human feces. Fed. Proc. 13: 305–306, 1954.

395. Stowe, C. M. and Plaa, G. L.: Extrarenal excretion of drugs and chemicals. Annu. Rev. Pharmacol. 8: 337–351, 1968.

396. Strand, L. P. and Scheline, R. R.: Unpublished observations.

397. Syed, S. A.: Biochemical characteristics of *Fusobacterium* and *Bacteroides* species from mouse cecum. Can. J. Microbiol. 18: 169–174, 1972.

398. Symchowicz, S., Staub, M. S. and Wong, K. K.: A comparative study of griseofulvin-^{14}C metabolism in the rat and rabbit. Biochem. Pharmacol. 16: 2405–2411, 1967.

399. Tabaqchali, S.: The pathophysiological role of small intestinal bacterial flora. Scand. J. Gastroent. Suppl. 6: 139–163, 1970.

400. Tabaqchali, S. and Booth, C. C.: Relationship of the intestinal bacterial flora to absorption. Brit. Med. Bull. 23: 285–290, 1967.

401. Takahashi, H., Kaihara, M. and Price, J. M.: The conversion of kynurenic acid to quinaldic acid by humans and rats. J. Biol. Chem. 223: 705–708, 1956.

402. Takahashi, H. and Price, J. M.: Dehydroxylation of xanthurenic acid to 8-hydroxyquinaldic acid. J. Biol. Chem. 233: 150–153, 1958.

403. Tanigawa, Y., Shimoyama, M., Murashima, R., Ito, T., Yamaguchi, K. and Ueda, I.: The role of microorganisms as a function of nicotinamide deamidation in rat stomach. Biochim. Biophys. Acta 201: 394–397, 1970.

404. Taurog, A., Briggs, F. N. and Chaikoff, I. L.: I^{131}-Labeled L-thyroxine. II. Nature of the excretion product in bile. J. Biol. Chem. 194: 655–668, 1952.

405. Teuchy, H., Quatacker, J., Wolf, G. and Van Sumere, C. F.: Quantitative investigation of the hippuric acid formation in the rat after administration of some possible aromatic and hydroaromatic precursors. Arch. Int., Physiol. Biochim. 79: 573–587, 1971.

406. Teuchy, H. and Van Sumere, C. F.: The metabolism of DL-phenylalanine-1-^{14}C, cinnamic acid-3-^{14}C and ferulic acid-2-^{14}C in the rat. Arch. Int. Physiol. Biochim. 76: 953–954, 1968.

407. Teuchy, H. and Van Sumere, C. F.: The metabolism of (1-^{14}C)phenylalanine, (3-^{14}C)cinnamic acid and (2-^{14}C)-ferulic acid in the rat. Arch. Int. Physiol. Biochim. 79: 589–618, 1971.

408. Thomas, P. J.: In vitro conversion of oleic acid to hydroxy stearic acid by intestinal bacteria. Clin. Res. 18: 609, 1970.

409. Tompsett, S. L.: The determination and excretion of polyhydroxy (catecholic) phenolic acids in urine. J. Pharm. Pharmacol. 10: 157–161, 1958.

410. Uno, T. and Kono, M.: Studies on the metabolism of sulfisoxasole. V. On the deacetylation of N^1-acetylsulfisoxasole by intestinal bacteria. J. Pharm. Soc. Japan 81: 1434–1436, 1961.

411. Valerino, D. M., Johns, D. G., Zaharko, D. S. and Oliverio, V. T.: Studies on the metabolism of methotrexate by intestinal flora. I. Identification and study of biological properties of the metabolite 4-amino-4-deoxy-N^{10}-methylpteroic acid. Biochem. Pharmacol. 21: 821–831, 1972.

412. Vogt, W., Schmidt, G. and Dakhil, T.: Die Bedeutung der Glucuronidbildung und -spaltung für das Schicksal von Dihydroxydiphenyl-pyridylmethan. Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol. 256: 488–495, 1965.

413. Vos, B. J., Radomski, J. L. and Fuyat, H. N.: Cathartic actions of F D & C orange no. I and other coal-tar food dyes. Fed. Proc. 12: 396, 1953.

414. Walker, R.: The metabolism of azo compounds: A review of the literature. Food Cosmet. Toxicol. 8: 659–676, 1970.

415. Walker, R. and Banns, H. E.: Personal communication.

416. Walker, R. and Gingell, R.: An assay for azo-reductase activity in micro-organisms. Biochem. J. 122: 18P, 1971.

417. Walker, R., Gingell, R. and Murrells, D. F.: Mechanisms of azo reduction by *Streptococcus faecalis*. I. Optimization of assay conditions. Xenobiotica 1: 221–229, 1971.

418. Walker, R., Grasso, P. and Gaunt, I. F.: Myotoxicity of amine metabolites from Brown FK. Food Cosmet. Toxicol. 8: 539–542, 1970.

419. Walker, R. and Ryan, A. J.: Some molecular parameters influencing rate of reduction of azo compounds by intestinal microflora. Xenobiotica 1: 483–486, 1971.

420. Wallace, W. C., Lethco, E. T. and Brouwer, E. A.: The metabolism of cyclamates in rats. J. Pharmacol. Exp. Ther. 175: 325–330, 1970.

421. Watanabe, H.: The chemical structure of the intermediate metabolites of catechin. I. Chemical properties of the intermediate metabolites (G and H) and their derivatives. Bull. Agr. Chem. Soc. Japan 23: 257–259, 1959.

422. Watanabe, H.: The chemical structure of the intermediate metabolites of catechin. II. Oxidative decomposition of the intermediate metabolites (G and H). Bull. Agr. Chem. Soc. Japan 23: 260–262, 1959.

423. Watanabe, H.: The chemical structure of the intermediate metabolites of catechin. III. Synthesis of the intermediate metabolites (G and H). Bull. Agr. Chem. Soc. Japan 23: 263–267, 1959.

424. Watanabe, H.: The chemical structure of the intermediate metabolites of catechin. IV. Structure of the intermediate metabolite (F). Bull. Agr. Chem. Soc. Japan 23: 268–271, 1959.

425. Watanabe, H. and Oshima, Y.: Metabolism of gallic acid and tea catechin by rabbit. Agr. Biol. Chem. 29: 90–93, 1965.

426. Watson, W. C.: Intestinal hydrogenation of dietary fatty acids. Clin. Chim. Acta 12: 340–342, 1965.

427. Wedemeyer, G.: Dechlorination of DDT by *Aerobacter aerogenes*. Science 152: 647, 1966.

428. Wedemeyer, G.: Dechlorination of 1,1,1-trichloro-2-2-bis

p(chlorophenyl)ethane by *Aerobacter aerogenes*. I. Metabolic products. Appl. Microbiol. **15**: 569–574, 1967.

429. WEDEMEYER, G.: Biodegradation of dichlorodiphenyltrichloroethane: Intermediates in dichlorodiphenylacetic acid metabolism by *Aerobacter aerogenes*. Appl. Microbiol. **15**: 1494–1495, 1967.

430. WEDEMEYER, G.: Role of intestinal microflora in the degradation of DDT by rainbow trout (*Salmo gairdneri*). Life Sci. **7**: 219–223, 1968.

431. WEINSTEIN, L.: The sulfonamides. *In* The pharmacological basis of therapeutics, ed. by L. S. Goodman and A. Gilman, pp. 1177–1203, Macmillan, London, 1970.

432. WEISBURGER, J. H.: Colon carcinogens: their metabolism and mode of action. Cancer **28**: 60–70, 1971.

433. WEISBURGER, J. H., GRANTHAM, P. H., HORTON, R. E. AND WEISBURGER, E. K.: Metabolism of the carcinogen N-hydroxy-N-2-fluorenylacetamide in germ-free rats. Biochem. Pharmacol. **19**: 151–162, 1970.

434. WESTLAKE, D. W. S., TALBOT, G., BLAKLEY, E. R. AND SIMPSON, F. J.: Microbial decomposition of rutin. Can. J. Microbiol. **5**: 621–629, 1959.

435. WESTÖÖ, G.: On the metabolism of tartrazine in the rat. Acta Chem. Scand. **19**: 1309–1316, 1965.

436. WHITE, R. W., KEMP, P. AND DAWSON, R. M. C.: Isolation of a rumen bacterium that hydrogenates oleic acid as well as linoleic acid and linolenic acid. Biochem. J. **116**: 767–768, 1970.

437. WHITING, G. C. AND CARR, J. G.: Metabolism of cinnamic acid and hydroxy-cinnamic acids by *Lactobacillus pastorianus* var. *quinicus*. Nature (London) **184**: 1427–1428, 1959.

438. WILDE, P. F. AND DAWSON, R. M. C.: The biohydrogenation of α-linoleic acid and oleic acid by rumen microorganisms. Biochem. J. **98**: 469–475, 1966.

439. WILLIAMS, B., DRING, L. G. AND WILLIAMS, R. T.: Benzene as a metabolite of triphenyl-lead acetate in the rat. Biochem. J. **127**: 24P–25P, 1971.

440. WILLIAMS, J. R., GRANTHAM, P. H., MARSH, H. H., WEISBURGER, J. H. AND WEISBURGER, E. K.: Participation of liver fractions and of intestinal bacteria in the metabolism of N-hydroxy-N-2-fluorenylacetamide in the rat. Biochem. Pharmacol. **19**: 173–188, 1970.

441. WILLIAMS, J. R., GRANTHAM, P. H., YAMAMOTO, R. S. AND WEISBURGER, J. H.: Effect of dietary riboflavin on azo dye reductase in liver and in bacteria of cecal contents of rats. Biochem. Pharmacol. **19**: 2523–2525, 1970.

442. WILLIAMS, P. P. AND FEIL, V. J.: Identification of trifluralin metabolites from rumen microbial cultures. Effect of trifluralin on bacteria and protozoa. J. Agr. Food Chem. **19**: 1198–1204, 1971.

443. WILLIAMS, R. T.: Detoxication Mechanisms. The Metabolism and Detoxication of Drugs, Toxic Substances and Other Organic Compounds. Chapman & Hall, London, 1959.

443a. WILLIAMS, R. T.: Toxicological implications of biotransformation by intestinal microflora. Toxicol. Appl. Pharmacol. **23**: 769–781, 1972.

444. WILLIAMS, R. T., MILLBURN, P. AND SMITH, R. L.: The influence of enterohepatic circulation on toxicity of drugs. Ann. N. Y. Acad. Sci. **123**: 110–122, 1965.

445. WILSON, G. S. AND MILES, A. A.: Topley and Wilson's Principles of Bacteriology and Immunity, 5th Ed., vol. 2, pp. 2461–2482, Arnold, London, 1964.

446. WINDMUELLER, H. G., MCDANIEL, E. G. AND SPAETH, A.: Orotic acid-induced fatty liver. Metabolic studies in conventional and germ-free rats. Arch. Biochem. Biophys. **109**: 13–19, 1965.

447. WINKLER, W. O.: Report on hydrocyanic glucosides. J. Ass. Off. Agr. Chem. **34**: 541–548, 1951.

448. WINKLER, W. O.: Report on methods for glucosidal HCN in lima beans. J. Ass. Off. Agr. Chem. **41**: 282–287, 1958.

449. WOODS, L. A.: Distribution and fate of morphine in nontolerant and tolerant dogs and rats. J. Pharmacol. Exp. Ther. **112**: 158–175, 1954.

450. YANG, M. G. AND MICKELSEN, O.: Cycads. *In* Toxic Constituents of Plant Foodstuffs, ed. by I. E. Liener, pp. 159–167, Academic Press, New York and London, 1969.

451. YESAIR, D. W., CALLAHAN, M., REMINGTON, L. AND KENSLER, C. J.: Role of the entero-hepatic cycle of indomethacin on its metabolism, distribution in tissues and its excretion by rats, dogs and monkeys. Biochem. Pharmacol. **19**: 1579–1590, 1970.

452. YOKOYAMA, M. T. AND DAVIS, C. L.: Hydrogenation of unsaturated fatty acids by *Treponema* (*Borrelia*) strain $B_2 5$, a rumen spirochete. J. Bacteriol. **107**: 519–527, 1971.

453. ZAHARKO, D. S., BRUCKNER, H. AND OLIVERIO, V. T.: Antibiotics alter methotrexate metabolism and excretion. Science **166**: 887–888, 1969.

454. ZAHARKO, D. S. AND OLIVERIO, V. T.: Reinvestigation of methotrexate metabolism in rodents. Biochem. Pharmacol. **19**: 2923–2925, 1970.