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The influence of intestinal bacteria on the absorption and metabolism of foreign compounds

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The food of man and other animals contains many compounds that are not utilized either for the production of energy or for the elaboration of tissue components. In addition to naturally occurring alkaloids, terpenes, and aromatic acids, foods may contain artificial colourings, preservatives, and contaminants such as pesticides. Many of these 'foreign compounds' are potentially toxic and their metabolism may be considered as part of the body's defence against the chemical environment.

The methods by which foreign compounds that enter the body are altered by tissue enzymes have been extensively studied (Williams, 1959; Parke, 1968) but it is only recently that the part played by bacteria on compounds entering the gastrointestinal canal has been appreciated (Scheline, 1968; Drasar, Hill, and Williams, 1970). Studies of the relative importance of the tissue and bacterial metabolic systems have been facilitated by recent developments in methods for the cultivation of the oxygen-sensitive bacteria of the gut (eg, Moore, 1966; Drasar, 1967; Arank, Syed, Kenney, and Freter, 1969) and by the consequential improved understanding of the distribution of the bacteria in the gut (Haenel, 1961; Drasar, Shiner, and McLeod, 1969). There is still, however, a serious lack of precise information about the metabolic activities of many of the gut bacteria, especially the nonsporing anaerobes which are by far the dominant form in most animals. Much of the evidence for the involvement of bacteria in the metabolism of foreign compounds has been derived from studies on animals treated with antibiotics or on germ-free animals. It is, however, often difficult to extrapolate from such experiments to man because of the differing nature of the intestinal bacterial flora in different animals. It is, therefore, convenient to compare first the present knowledge of the bacterial flora of man with that of the small animals commonly used for metabolic studies.

The Bacterial Flora of the Intestine

The bacterial flora of the intestine in man and in some laboratory animals are summarized in Tables I and II and in Figure 1.

The hydrochloric acid of the stomach is bactericidal, but it is probably only in man that anything approaching sterility of the stomach contents is achieved; even in man the stomach contents can be sterile only for short periods between meals and at night. When food enters the stomach the acid is neutralized and any bacteria present in the food can survive. In some animals there are additional sources of bacteria. Rats and mice are coprophagic and mice have, in the stomach, a nonsecretory area of epithelium which is permanently colonized with bacteria.

Since some of the stomach contents enter the

Fig. 1. Approximate total numbers of bacteria recoverable from the contents of the small intestine. For the counts from animals, the horizontal scale has been adjusted to correspond to the human small intestine.

		<i>Enterobacteria</i>	<i>Enterococci</i>	<i>Lactobacilli</i>	<i>Veillonella</i>	<i>Yeasts</i>	<i>Clostridia</i>	<i>Bacteroides</i>	<i>Bifidobacteria</i>
Rabbit	(3) ^a	N ^a	N	N	N	N	N	5·7	5·3
Guinea pig	(9)	N	N	3·9	N	N	3·3	N	5·9
Rat	(8)	4·1	3·9	6·6	5·7	4·1	3·3	7·0	7·6
Mouse	(3)	3·1	2·9	8·3	7·8	6·3	3·6	8·1	8·3
Man	(30)	N	N	N	N	N	N	N	N

Table I *Bacterial flora of the stomach in man and laboratory animals*¹¹Mean log₁₀ viable count/g wet weight sample.^aNumber of specimens examined.^aN = <10 organisms/g sample wet weight.

		<i>Enterobacteria</i>	<i>Enterococci</i>	<i>Lactobacilli</i>	<i>Veillonella</i>	<i>Yeasts</i>	<i>Clostridia</i>	<i>Bacteroides</i>	<i>Bifidobacteria</i>
Rabbit	(3)	2·3	2·3	4·0	N	N	3·8	7·8	7·7
Guinea pig	(9)	2·9	3·1	3·9	4·0	4·0	3·5	7·8	8·4
Rat	(8)	5·9	6·6	7·8	5·6	5·8	3·0	8·2	8·6
Mouse	(3)	5·0	4·8	8·1	6·6	6·6	3·3	8·4	7·8
Man	(30)	6·0	3·5	4·0	3·0	1·0	3·0	10·5	10·5

Table II *Bacterial flora of faeces in man and laboratory animals*

duodenum before the acidity has returned to bactericidal levels, some bacteria enter the small intestine. In animals that feed more continuously than man, there must be an even greater degree of contamination of the small intestine with bacteria from the mouth and from food.

Whether for this reason, or for some other such as actual multiplication in the intestine, bacteria are much more numerous in the small intestine in laboratory animals than in man. In man the proximal small intestine contains few bacteria. Lactobacilli and bifidobacteria are the types found most frequently, but all intestinal organisms, including enterobacteria, are isolated occasionally and usually in small numbers. Only in the terminal ileum is there a large bacterial population qualitatively similar to that of faeces.

There is relatively little direct information about the bacterial flora of the large intestine in man but it is reasonable to assume that, as in animals, the distribution of bacteria in faeces reflects that in the large intestine very closely (Smith, 1965).

The bacteria isolated from human faeces are predominantly the nonsporing anaerobes, bacteroides and bifidobacteria. The same two groups of bacteria are dominant in the faeces of all the animals studied, although there are strikingly more lactobacilli and streptococci in the faeces of rats and mice than in man and other animals, and there are notably fewer enterobacteria in rabbits and guinea pigs.

The Influence of Bacteria on Absorption

Bacteria can influence the absorption of foreign compounds from the intestine in at least three ways.

First, they may alter the physical conditions within the intestinal lumen with a resultant alteration in the state of the compound, which might affect its solubility in lipid membranes. For example, pH changes produced by bacterial metabolism could alter the degree of ionization of molecules. Secondly, they might act through their effect on the development of intestinal mucosal cells, as is shown by studies on germ-free animals. And thirdly, bacteria may have some metabolic effect on the compounds in the gut.

The Effect of Bacteria on Mucosal Structure and Functions

Although the lack of immunological development has attracted most attention in studies of germ-free animals, such animals differ from conventional animals in a number of ways that could be relevant to absorption of foreign compounds. For example, the total mucosal surface area in germ-free rats is significantly less than in conventional rats (Gordon and Bruchner-Kardoss, 1961); the migration of the mucosal cells up the villus takes four days in germ-free and only two days in normal animals. The mucosa in germ-free pigs and guinea pigs is more regular than in those harbouring a normal bacterial load. In some species, such as the chick, the germ-free state is associated with a reduction in the level of intestinal enzymes. Moreover, germ-free mice show a decreased gut motility (Luckey, 1963; Kenworthy and Allen, 1966; Abrams, 1967) and an accumulation of mucus, which is apparently normally degraded by bacteria.

The accumulation of mucus in the intestine of

germ-free animals decreases the rate of absorption of water; absorption of foodstuffs is also affected but the changes appear to be species linked (Cole, 1967; Geever, Kan, and Levenson, 1968; Gordon, 1969; Tennant, Reina-Guerra, Harrold, and Gold-man, 1969; Wiech, 1969).

Products of bacterial metabolism are also absorbed from the intestine. Phenol, cresol, indole, skatole, and pyrocatechin is not found in the urine of germ-free guinea pigs. Nor is indican found in the urine of germ-free mice or rats (Wagner, 1958). Although the levels of microsomal drug-metabolising enzymes in the liver of germ-free and normal rats are the same (Short, 1969), it may be that intestinal bacteria are important in the stimulation of inducible enzyme systems involved in metabolism.

The Bacterial Metabolism of Compounds in the Intestine

There are some bacteria able to metabolize virtually any organic molecule and the bacteria present in the intestine have been demonstrated to metabolize a wide variety of compounds, including glucuronides, glycosides, amides, esters, azo dyes, antibiotics, phenols, alcohols, and many others. Moreover prolonged oral administration of any compound will exert a selective pressure on the bacterial flora and may favour the growth of organisms able to metabolize the compound.

Whether a particular organic compound is metabolized by the intestinal flora or not is dependent not only on the nature of the bacteria but also on the site of absorption. Compounds absorbed from the mouth or from the stomach are exposed to bacteria for less time than those absorbed from either the small intestine or the colon. The colonization of the stomach and small intestine normally present in experimental animals means that compounds administered by mouth are more likely to be metabolized by intestinal bacteria in these animals than in man.

THE ENTEROHEPATIC CIRCULATION

As part of the process of detoxification many compounds are conjugated by the liver to form, for example, glucuronides, and are then excreted in the bile into the intestine. These conjugates are highly polar compounds and so are poorly absorbed from the small intestine. If they remain unhydrolysed they are rapidly eliminated from the body in the faeces or urine. If they are hydrolysed in the small intestine, however, the relatively nonpolar aglycones are often absorbed from the gut and returned to the liver where they are re-conjugated and re-excreted in the bile. This 'enterohepatic circulation' results in the

retention of the compound in the body for a much longer period of time and allows other bacteria time to produce other, possibly more toxic, metabolites. The enterohepatic circulation of drugs has been reviewed by Smith (1966).

For example, stilboestrol is conjugated by the liver to form its glucuronide and, to a smaller extent, the diglucuronide. In the rat, with many bacteria in the small intestine producing glucuronidase, there is an extensive enterohepatic circulation, so that 30% of the stilboestrol administered is still present in the animal after seven days. In the rabbit, on the other hand, most of the stilboestrol is excreted in the faeces and urine within 24 hours, presumably because the relatively small numbers of bacteria in the intestine do not break down so much of the glucuronide. When the rats are treated with antibiotics, stilboestrol is excreted much more rapidly confirming the suggestion that the gut bacteria were responsible for the glucuronide hydrolysis that leads to the enterohepatic circulation.

Although the β -glucuronidase activity of *Escherichia coli* is on average more than four times that of any other bacterial group, the lactobacilli, bacteroides, and bifidobacteria outnumber the *E. coli* to such an extent that *E. coli* contributes only 2% of the total bacterial β -glucuronidase in the proximal small intestine of the rat and less than 0.2% in the distal small intestine (Hawksworth, Drasar, and Hill, 1971).

METABOLISM OF GLYCOSIDES

Just as the glucuronides are poorly absorbed, so the dietary glycosides are also much less readily absorbed from the gut than their aglycones. The aglycones released by bacterial action and absorbed often have distinctive pharmacological effects. An example is the anthraquinone cathartic group. These compounds, which include cascara sagrada and senna, are glycosides related to emodin (1, 3, 8 trihydroxy-6-methyl anthraquinone); the aglycones, which are the active agents, are released by bacterial action in the large intestine. The cardiac glycosides are also hydrolysed by gut bacteria to release an active aglycone. The lanatosides are a group of compounds containing an aglycone attached to three digitoxose and a terminal glucose. Incubation of lanatoside C with intestinal bacteria results in the removal of the terminal glucose to give acetyl digoxin and, on occasion, removal of the acetyl group to release digoxin (Hawksworth *et al.*, 1971).

Cycasin, which is methyl azoxymethanol, β D-glucoside, has no hepatotoxic or carcinogenic effect on germ-free rats and is excreted unmetabolized in their faeces (Spatz, McDaniel, Laqueur, 1966). In conventional rats, however, only 18-35% of the

dose is recovered in the faeces and the rats die with liver necrosis or hepatic carcinoma within two weeks; the gut bacteria produce the toxic and carcinogenic aglycone methylazoxymethanol.

Amygdalin, which is a diglucoside, is hydrolysed by gut bacteria to release the aglycone, which is absorbed and metabolized by the liver to release cyanide (Williams, 1970) which is, of course, toxic.

In all these examples highly polar, and therefore poorly absorbed, glucosides are hydrolysed by gut bacteria to give readily absorbable aglycones with distinctive pharmacological effects. Studies of the intestinal bacteria revealed that enterococci produce, on average, three times more β -glucosidase than do strains of other groups of bacteria. However, as with the β -glucuronidase of intestinal bacteria, the bacteroides, bifidobacteria, and lactobacilli are present in such relatively large numbers that they produce 99% of the bacterial glucosidase and the enterococci contribute a very small proportion of the total.

METABOLISM OF FOOD ADDITIVES AND COMPONENTS

With modern techniques of food preserving and canning there has been a growth in the use of synthetic colourings to restore the food to its former beauty, or even to improve on it. Almost all of these colours are sulphonated diazo compounds which would not be absorbed from the intestine. However, they may be metabolized by the gut bacteria with reduction of the azo linkage to produce sulphonated amines which are absorbed from the gut and excreted in the urine. We examined 20 water-soluble dyes used as food additives and found all of them to be metabolized by intestinal bacteria (Linnecar and Drasar, in preparation). The enzymology of this reaction has been studied more extensively by Walker and Gingell (1971).

The artificial sweetening agent calcium cyclamate is poorly absorbed and is excreted in the faeces. If fed to rats or humans in large doses daily for a month a proportion of the dose is metabolized to cyclohexylamine which is readily absorbed from the gut and excreted in the urine. Examination of the intestinal bacteria showed that strains of *Clostridium* spp. and *Streptococcus faecalis* were able to perform this hydrolytic reaction (A. G. Renwick, B. S. Drasar, and R. E. O. Williams, in preparation).

It has been shown that bacteria are able to produce nitrosamines from secondary amines and nitrate (Sander, 1968). Secondary amines, principally dimethylamine, piperidine, and pyrrolidine, are excreted in large amounts in the urine and in much smaller amounts in faeces and are derived by bacterial action on lecithin and a number of amino acids, such as proline which is decarboxylated to

pyrrolidine (Hawksworth and Hill, 1971). Nitrate is used extensively in the food industry as a preservative and is also present in a number of vegetables in quite high concentration and in drinking water in some areas. Thus nitrate is being ingested with food and secondary amines are produced by bacterial action as well as being present in some foodstuffs (eg, fish). There are two places where both could be present together with bacteria—in the gut and in infected urine. Nitrate is absorbed from the small intestine and so the amount reaching the areas of the intestine with considerable bacterial colonization areas may be very small. The significance of the bacterial production of nitrosamines has still to be determined but is potentially great in view of the increasing proportion of people drinking water with a high nitrate content.

In people with lactase deficiency the β -galactosidase activity of the intestinal mucosa is lost and lactose, which would normally be hydrolysed and absorbed, passes unabsorbed to the terminal ileum and colon where it is metabolized by the bacterial β -galactosidase to produce very acid conditions which may aggravate the osmotic induced diarrhoea.

BACTERIAL METABOLISM OF DRUGS AND ANTIBIOTICS

Many antibiotics are given orally and modify the intestinal flora. In addition the antibiotic may be modified by the gut flora. Antibiotics modified in this way include prontosil, chloramphenicol, and succinyl-sulphathiazole. Prontosil, the first antibacterial agent to be used successfully, is not in itself antibacterial, but is hydrolysed to release sulphanilamide—the active antibiotic. Succinylsulphathiazole is not antibacterial *in vitro*. When administered orally it is poorly absorbed and so gives a high concentration of the compound in the gut where it is hydrolysed to release sulphathiazole—the active antibiotic (Goodman and Gillman, 1965). The main effect of this is to reduce the numbers of enterobacteria in the intestinal flora.

Chloramphenicol has been extensively studied (Glazko, Carnes, Kazenko, and Wolf, 1957) and has been shown to be rapidly absorbed from the gut and conjugated by the liver to give the glucuronide, which is excreted in bile and then hydrolysed by the gut bacteria and so enterohepatically circulated. The glucuronide has no antibacterial action. During enterohepatic circulation other microbial metabolites are formed; the major product is the deacetylated form (which also has no antibacterial action) but there is also reduction of the nitro group to give an arylamine. These compounds are toxic and may be responsible for the disorders associated with the prolonged administration of this antibiotic.

Conclusion

It is clear that the bacteria of the intestine may play a very important role in the metabolism of ingested compounds and intestinal secretions. Investigation of this role is being facilitated by the availability of germ-free animals, and by the development of more effective methods for the isolation and identification of the bacteria. However, these methods still leave much to be desired in convenience and precision, and animal experimentation, though often illustrative of possible effects, suffers from the difficulty that the intestinal flora of man differs in many ways from that of animals. There is a clear need for the further development of methods for studying the effect of the bacterial flora in man.

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