International Journal of Pharmaceutics 363 (2008) 1–25

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Review

The gastrointestinal microbiota as a site for the biotransformation of drugs

Tiago Sousa [a], Ronnie Paterson [b], Vanessa Moore [b], Anders Carlsson [c], Bertil Abrahamsson [d], Abdul W. Basit [a,*]

[a] Department of Pharmaceutics, The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX, UK
[b] Early Development, AstraZeneca R&D Charnwood, Loughborough, Leics LE11 5RH, UK
[c] Early Development, AstraZeneca R&D, S-431 83 Mölndal, Sweden
[d] Product Development, AstraZeneca R&D, S-431 83 Mölndal, Sweden

ARTICLE INFO

Article history:
Received 13 March 2008
Received in revised form 7 July 2008
Accepted 8 July 2008
Available online 16 July 2008

Keywords:
Large intestine
Bacteria
Microbiota
Microflora
Xenobiotics
Stability
Fermentation
Metabolism
Bioavailability
Colonic delivery

ABSTRACT

There are 100 trillion microbes in the human gastrointestinal tract with numbers increasing distally. These microbiota secrete a diverse array of enzymes (primarily for carbohydrate and protein fermentation) giving them substantial metabolic potential which can have major implications for drug stability. At least thirty drugs which are, or have been, available commercially, were subsequently shown to be substrates for these bacterial enzymes, and with increasing numbers of new and existing drugs having the potential for contact with the distal gut (through modified release systems or poor solubility/permeability), many more are expected to be discovered. The major concern with bacterial drug degradation is the behaviour of the metabolite; is it more or less active than the parent compound, or has toxicity resulted? For example, there were eighteen deaths in 1993 due to a drug interaction in which a toxic drug metabolite was produced by bacterial fermentation. Thus, the objective of this review is the provision of a comprehensive overview of this area; the gastrointestinal microbiota, their drug substrates and metabolic mechanisms, and approaches to studying this further are discussed.

Contents

* Corresponding author. Tel.: +44 20 7753 5865; fax: +44 20 7753 5865.
E-mail address: abdul.basit@pharmacy.ac.uk (A.W. Basit).

0378-5173/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2008.07.009

1. Introduction

The oral route is the preferred route for drug administration with 84% of the fifty bestselling pharmaceutical products in the US and European markets given by mouth (Lennernäs and Abrahamsson, 2005). However, oral administration is arguably the most complex route of drug delivery. For an orally administered drug to be effective it must (a) dissolve in the fluids of the gastrointestinal (GI) tract (b) remain intact in the lumen (c) cross the epithelial membrane and (d) undergo minimal first-pass metabolism. Oral bioavailability is therefore a multi-factorial process dependent on the solubility, stability, permeability and metabolism of the drug molecule.

While much has been written on the role of dissolution, permeability and first-pass metabolism on oral drug bioavailability (Lindahl et al., 1997; Dressman et al., 1998; Amidon et al., 1995; Lennernäs and Abrahamsson, 2005; McConnell et al., 2008a), less attention has been paid to the stability of the drug in the intestinal lumen. Instability is often associated with pH and/or enzyme mediated degradation in the upper gut. These can be overcome by formulation approaches such as enteric coating. A major stability factor that is often overlooked is the effect of the microbiota in the gastrointestinal tract.

The gastrointestinal tract is populated with large numbers of bacteria that contribute to normal digestive function. Most of these bacteria reside in the large intestine and their primary function is to ferment carbohydrate and protein that escape digestion in the upper gut into absorbable energy. In addition, the metabolic reactions performed by these bacteria and their respective enzymes have the ability to metabolise drugs and other xenobiotics far more extensively than any other part of the body (Scheline, 1973; Abu Shamat, 1993; Mikov, 1994). Scheline has even suggested that the gastrointestinal microbiota has the ability to act as an organ with a metabolic potential at least equal to the liver (Scheline, 1973). There are, however, important differences between hepatic and bacterial metabolism. The liver is primarily responsible for metabolism via *oxidation* and *conjugation* producing polar high molecular weight metabolites, while the gastrointestinal microbiota is involved in *reductive* and *hydrolytic* reactions generating non-polar low molecular weight byproducts. Furthermore, all drugs that are delivered to, or absorbed into, the blood stream, are subject to hepatic metabolism. However, rate and extent of bacterial metabolism will be influenced by the amount of drug that reaches the distal gut.

The majority of drugs are rapidly and completely absorbed in the upper gut and have minimal contact with intestinal bacteria. This goes someway towards explaining why over the last forty years only thirty or so marketed drugs have been identified as substrates for intestinal bacteria. However, the biopharmaceutical complexity of new drug candidates is providing an increasing number of compounds that suffer from low solubility, low permeability or both (Davis, 2005). Drugs that display these properties will reach the lower confines of the gastrointestinal tract, presenting themselves to the host microbiota. Furthermore, drugs that are delivered via the intravenous route or that are fully absorbed from the upper parts of the gastrointestinal tract may still reach the lower gut by secretion or diffusion from the systemic circulation into the intestinal lumen, or may be excreted in the bile, possibly as conjugates following a recycling process known as enterohepatic recirculation. There is an increasing trend to develop modified release preparations

(colon specific or extended release systems) to improve therapy via the oral route (Basit, 2005; Rubinstein, 2005; Ibekwe et al., 2006; Tiwari and Rajabi-Siahboomi, 2008; Ibekwe et al., 2008). In such cases, most if not all the entire drug load will be deposited in the large intestine, providing further opportunity for exposure to the microbiota. Drugs can also come in direct contact with bacteria via rectal administration in the form of suppositories or enemas. Given all this it is expected that opportunities for microbiota-mediated metabolism will increase and drug stability assessment in the presence of intestinal bacteria becomes of increasing importance.

The stability of a drug to the microbiota is clinically relevant: metabolism can render a drug pharmacologically active, inactive or toxic. An important example of the significance of this was seen in Japan in 1993 when sorivudine, a promising antiviral drug was introduced into the Japanese market. This was later discovered to be transformed by gut microbiota into (E)-5-(2-bromovinyl)uracil which can inhibit the metabolism of the anti-cancer drug 5-fluorouracil leading to toxic levels of this drug. Within forty days of reaching the Japanese market this bacterially-metabolized interaction was responsible for the death of eighteen patients who were co-administered sorivudine with oral 5-fluouracil prodrugs (Okuda et al., 1998). Sorivudine was withdrawn from the market a few weeks after these deaths. This highlights the importance of studying metabolism by the microbiota, and this has come to the attention of the pharmceutical industry. For example, AstraZeneca has now started to examine the stability of drugs in early development when relevant using an *in vitro* colonic model (based on an inoculum of human faecal bacteria). New molecules can be screened to assess whether there will be development issues with an extended release formulation option later in the programme. Significantly, of the fifty-one molecules examined since 2004, nineteen underwent degradation in the colonic *in vitro* model.

In light of the significant issue of bacterial drug metabolism, the purpose of this review is to describe the *in vitro* and *in vivo* methods used to assess drug metabolism in the presence of gastrointestinal bacteria and their relative merits. Detailed information on those drug molecules known to be susceptible to bacterial metabolism are also presented with a summary of the metabolic reactions involved. Knowledge of the individual drugs, and drug classes, should be useful for initial identification of chemical groups that are potential substrates: these data could be extrapolated to new drug molecules for further investigation.

2. The human gastrointestinal microbiota and its function

The term microflora has been used to describe microorganisms residing on body surfaces including the gastrointestinal tract. This is because these microorganisms were originally thought to be plants and were incorrectly classified as “flora”. Since this denomination is scientifically inaccurate and misleading, the term microbiota is preferred. We often consider the members of the human intestinal microbiota as key players in maintaining human health and well-being: they are implicated in developmental, immunological and nutrition host functions (Egert et al., 2006). However, in numerical terms, of the total human and bacterial cells making up the body, only 10% are eukaryotic. This has tempted some authors to suggest that man’s role as a host for gut bacteria is simply to function as an advanced fermenter, carefully designed to maximize the productivity of the remaining 90% of prokaryotic microbiotal cells (Nicholson and Wilson, 2005).

The human gut is sterile at birth, shortly after which a number of microbial strains/species, find their way on to body surfaces and into the alimentary canal. Most of this inoculum is derived largely from the mother’s vaginal and faecal microbiota (conventional birth) or from the outside environment (caesarean delivery). Initially, facultatively anaerobic bacterial species, such as *Escherichia coli* and streptococci populate the gut. These nutritionally undemanding bacteria create an adequate environment for the development of the anaerobic species that later dominate the gut (Cummings et al., 2004; Macfarlane and Macfarlane, 1997).

The mouth, pharynx, oesophagus, stomach, small intestine and large intestine correspond to the primary anatomical regions found in the human gastrointestinal tract. The caecum, colon, rectum and anal canal are collectively referred to as the large intestine. Moving along these different sections of the gut, pH and redox potential undergo extreme variations (McConnell et al., 2008a; Varum et al., 2008) as seen in Table 1. These variations will impact on, and reflect the microbiotal colonisation of the digestive tract. There is a progressive increase in both numbers and species towards the ileo-caecal junction and bacteria find an optimal growth environment once they reach the large intestine (Fig. 1). The intra-luminal pH influences bacterial concentration in each section of the gut: a low pH destroys most bacteria. On the other hand, the redox potential is influenced by the bacterial concentration in each gastrointestinal section and higher bacterial concentrations are responsible for lower redox potentials (Table 1). Redox or oxidation–reduction potential is especially important when studying the activity of the microbiota because it acts as an indicator of the physiological and metabolic state of bacteria (Oktyabrsky and Smirnova, 1989; Celesk et al., 1976). Redox potential is defined as a measurement of the ability of a system to oxidise (lose electrons) or reduce (gain electrons) and the major end products of bacterial fermentation (short chain fatty acids) are electronically charged at the gastrointestinal pH. A lower redox potential is therefore expected in the gastrointestinal regions where there is active bacterial growth and metabolism of substrates into short chain fatty acids.

Bacterial activity is further influenced by other physiological parameters. Gastrointestinal transit time, for example, has been associated with changes in bacterial metabolism. Cummings et al. (1992) recorded transit times for 185 healthy adults (using radio-opaque pellets) and found a mean whole-gut transit time of 70 h

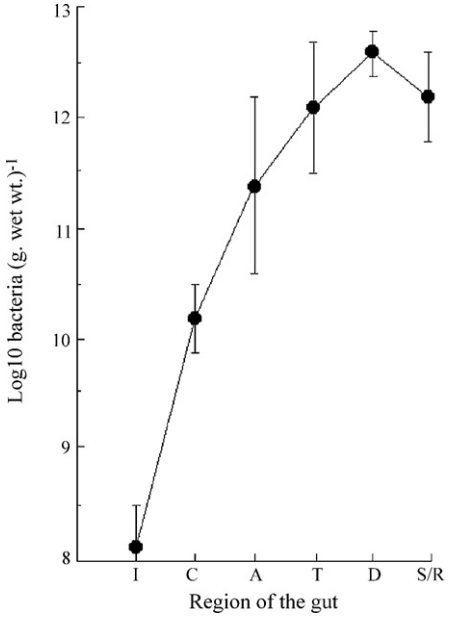

Fig. 1. Total bacterial numbers in distal gastrointestinal tract obtained from human sudden death victims ($n = 10$). I, ileum; C, caecum; A, ascending colon; T, transverse colon; D, descending colon; and S/R, sigmoid/rectum (reproduced with permission from Macfarlane and Macfarlane, 2004).

Table 1
Gastrointestinal intra-luminal pH, redox potential and bacterial concentration

	Mean pH values	Mean redox potential (mV)	Bacterial concentration (CFU/g of contents)
Man			
Stomach	1.0–2.5	+200	10^3
Small intestine			
Proximal	6.6	−66	10^4
Distal	7.5	−197	10^6–10^8
Large intestine			
Proximal	6.4	−415	10^{11}–10^{13}
Distal	7.0	−380	
References	Evans et al. (1988)	Stirrup et al. (1990)	Simon and Gorbach (1984) and Macfarlane and Macfarlane (2004)

with values ranging from 23 to 168 h. Although inter-subject transit times are highly variable, what is common to all subjects is that the greater part of this time (almost 80%) will be spent in the colon rather than in the small bowel (Tuleu et al., 2002; Wilding, 2001; Varum et al., 2008). In general, slow colonic transit times result in reduced activities of saccharolytic bacteria and short chain fatty acid production rates due to substrate deprivation in the distal gut. However, Cummings et al. (1979) did notice that subjects with longer transit times have an increased production of bacterial metabolites (phenols). Slow transit times are also associated with high prevalence of colonic disorders (Burkitt et al., 1972).

As aforementioned, the stomach is not heavily colonised due to a prohibitive pH but ingested bacteria are likely to escape the lowest pH levels in the early post-meal period when pH is around 4–5 and substantial volumes of liquid are moving into the duodenum (Cummings et al., 1989). Flow rate is at its greatest at the top of the small intestine and microorganisms are removed quickly by peristalsis, which, together with bile and pancreatic fluid secretions, does not allow increased microbial multiplication (Drasar and Barrow, 1985). The bacterial concentration in the proximal small bowel is, therefore, modest when compared to bacterial concentrations further along in the gastrointestinal tract (Table 1) (Simon and Gorbach, 1984). As a consequence, the enzymatic activity of bacteria in the small intestine will be less, however these bacteria may be in contact with many more molecules than those bacteria situated in the lower gut. Potentially, small intestinal bacterial enzymes will exert their action on *all* substances taken orally but large intestinal bacterial enzymes will only exert their activity on molecules that are able to reach the lower confines of the gut (Priebe et al., 2002). It is likely that the substantial concentrations of bacteria present in the small intestine may interfere with the metabolism of external substances and conditions such as small intestinal bacterial overgrowth could increase the bacterial concentration in to levels close to colonic bacterial concentrations (Khoshini et al., 2007). This causes concern over a possible rise in the overall bacterial metabolic activity in these diseased situations.

Bacteria exist adherent to the mucosal epithelium or in microhabitats (trapped in the mucous gel layer or in the intestinal lumen probably associated with either food particles or with each other) (Fanaro et al., 2003; Tannock, 1999). In the ascending colon, microorganisms, having plentiful supply of dietary nutrients, tend to grow rapidly, while in the transverse and descending colon substrate availability is lower and bacteria growth slows. Substrates available for microbial fermentation in the human colon include carbohydrates and proteins: these are mostly from dietary origin but can also be host derived, e.g. from mucus (Egert et al., 2006). These materials are degraded by bacterial enzymes which can be located extracellularly, cell-bound or released into the environment by cell lysis. Studies into the location of enzymatic activity have been contradictory and are enzyme specific, for some enzymes like glycosidases their activity is higher in cell associated suspensions (McBain and Macfarlane, 1998), for pectinolytic enzymes for example, their activity is higher extracellularly (Sirotek et al., 2004). The end products of enzymatic action will be oligomers and their component sugars and amino acids, which are then fermented to short chain fatty acids (acetate, propionate, butyrate), branched chain fatty acids (such as isobutyrate, isovalerate and 2-methylbutyrate), H_2, CO_2 and other neutral, acidic and basic end products (Cummings et al., 2004). Short chain fatty acids are, from a nutritional point of view, the major products of fermentation; butyrate is of particular importance because it is the major energy source for colonocytes (the epithelial cells that line the colon). Short chain fatty acids can affect colonic epithelial cell transport, colonocyte metabolism, growth and differentiation, hepatic control of lipids and carbohydrates and provide energy to muscle, kidney, heart and brain (Cummings and Macfarlane, 1997). Bacteria in the large intestine also fulfil important vitamin requirements for the body: they synthesize B complex vitamins including thiamine, riboflavin and vitamin B_{12}, and vitamin K (Hill, 1997). The average diet does not contain enough vitamin K (important for blood clotting) and its synthesis by bacteria is essential (Hill, 1997). A further benefit of the gastrointestinal microbiota is to act as an effective barrier against opportunistic and pathogenic microorganisms (Gibson and Wang, 1994).

The detection and identification of intestinal microbiota has occupied scientists for over a century (Savage, 2001). The first observations of faecal microbes described them as some "apparently randomly appearing bacteria" (Escherich, 1885), however, the current estimate for the total number of prokaryotes that inhabit the human gut goes up to 100 trillion (10^{14}) microbes (Ley et al., 2006). This number makes the human gastrointestinal tract one of the most populated microhabitats on earth (Whitman et al., 1998).

It is not known exactly how many bacterial cells exist in the human gut and which species they all belong to, in fact, attempting to identify and classify microbes is probably one of the most challenging cornerstones of modern microbiology. Most conventional methods dilute the sample and incubate it with specific growth media, the final number of bacteria is determined by multiplying the number of colonies that develop (viable culturable microorganisms) by the degree of dilution (Finegold et al., 1983). However, not all bacteria are able to be cultured in growth media, which can lead to an underestimation of their numbers and there is a risk that the growth media can never be truly specific which allows the possibility of bacteria being counted more than once on different "specific" growth media. Culturing methods are also labourious and time-consuming especially with anaerobic habitats (Vaughan et al., 2000). Based on culturing studies Finegold et al. (1983) proposed an estimated figure of 400 bacterial species inhabiting the human gastrointestinal tract. Current advances have made it possible to study bacterial populations by culture-independent approaches using molecular genetic methodology. Ribosomal RNA gene sequences are ideal for the classification of organisms since they are uni-

versally distributed among all cellular life forms and they possess very slow genetic evolution which allows the comparison between genetic sequences (domains) that have remained the same and sequences that have evolved. Usually a new species is found when less than 98% of domains are similar (Rajilic-Stojanovic et al., 2007). It is still unknown if this is a faultless species distinction. In a recent large-scale comparative analysis of bacteria ribosomal RNA gene sequences to better characterise the adherent colonic mucosal and faecal microbial communities, mucosal tissue and faecal samples were obtained from three healthy adult subjects. This represented the first application of high throughput sequencing on samples of the human gastrointestinal tract and a total of 395 bacterial phylotypes corresponding to different species of bacteria were identified (Eckburg et al., 2005). However, only three individuals provided samples for the aforementioned study which underestimates the true diversity of the human population. Rajilic-Stojanovic et al. (2007), recently compiled different ribosomal RNA studies together with culture-dependent studies, they reported a total of 1183 distinct bacterial species in the human gut (898 species using ribosomal RNA studies alone) and based on the variability seen so far between individuals they predicted an estimated diversity of the human gastrointestinal microbiota in excess of 3000 species.

The species identification from ribosomal RNA gene sequencing techniques occurs by alignment of the sequences with sequences stored in databanks. Using this technique most species identified from healthy subjects using mucosal and faecal samples belong to eight dominant phylogenetic phyla: *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Fusobacteria*, *Verrucomicrobia*, *Cyanobacteria*, *Spirochaeates* and *Actinobacteria* (Eckburg et al., 2005; Wang et al., 2005). *Firmicutes* are by far the most abundant and diverse group that include the *Clostridia* and *Bacilli* class; *Bacteroidetes* are also present in high numbers (Eckburg et al., 2005; Wang et al., 2005).

3. Models to study intestinal bacteria-related drug metabolism

Since the majority of gut bacteria are found in the large intestine this is the main site for biotransformation for endogenous and exogenous molecules. However, the inaccessibility of the human colon prevents the direct examination of the metabolic and ecological activity of the microbiota. Human and animal experiments to study this region are costly and have ethical drawbacks. Toxicity issues also render the use of human in vivo methodologies unviable in early drug development. The use of *in vitro* methodologies is over simplistic due to the complex continuous influx of endogenous compounds throughout the digestive tract such as intestinal secretions, the absorption of fermentation products and the interactions between the host and the bacterial population. Acknowledging these difficulties, research has focused on studying specific features of the colonic environment rather than an attempt to fully simulate the human colon. Studies that specifically focused on bacterial drug metabolism are presented hereafter as well as other methods that, although initially used to study other features of the colon (like the production of short chain fatty acids), could potentially be used for drug metabolic studies. *In vivo* studies are particularly relevant as they can provide unique insight into the *in vivo* relevance of colonic metabolism of a particular compound. There is no one ideal method but rather a combination of methods should be used to elucidate a role for gut bacteria in drug metabolism.

The use of animals or their intestinal contents is essential for some of the models described and Table 2 shows bacterial numbers throughout the gastrointestinal tract of commonly used laboratory animals. In all animals, as in man, bacterial numbers increase progressively from the stomach to the distal small intestine and subsequently to the large intestine and faeces. However, gastrointestinal physiology and diet will influence bacterial colonisation. The stomach and small intestine of rats and mice for example (Table 2) is heavily colonised when compared to man (Table 1); this can be explained by both a sufficiently high stomach pH (which allows bacterial multiplication) and a higher eating frequency (Smith, 1965; McConnell et al., 2008c). Most animals eat more often than humans which permits an almost continuous influx of bacteria carried with the food into the stomach. On the other hand, man is considered to be in a fasting state between meals and very few organisms colonize its stomach. Furthermore, diet composition is important and Smith (1965) found that *Lactobacillus* spp. are found in large numbers in animals fed mainly on cereals compared to those fed mainly on meat. Behavioural differences between different animals should also be considered, rodents for example are known to practise coprophagy and in doing so are continuously re-inoculating themselves with faecal microbes which may cause differences in their intestinal microbiota when compared to non-coprophagic animals.

Rowland et al. (1986) compared the numbers and activity of certain microbial enzymes of five species of laboratory animals with that of man. The sources were caecal contents of rats, mice, hamsters and guinea-pigs and freshly voided faeces from three marmosets and three human volunteers. The rat, guinea-pig and marmoset differed significantly from the human in three of the four enzymes studied and the mouse and hamster in two out of four enzymes (Table 3).

Further evidence of different bacterial activities using intestinal contents removed from animal species (rat and mouse) versus bacterial activities using human faeces was provided by Manning et al. (1988). The study focused on the nitroreduction of 6-nitrochrysene, a carcinogen which is commonly found in diesel engine exhaust and in certain photocopier fluids. The rates of nitroreduction were consistently different among human, rat and mouse intestinal microbiota (Fig. 2) with the human intestinal microbiota metabolizing 6-nitrochrysene to the greatest extent.

These examples demonstrate that there is some degree of variability when using animal bacterial activities for human predictions of bacterial drug metabolism. However, if used qualitatively, animal contents and models might be useful and in certain situations as described hereafter, advantageous.

3.1. Animal in vivo studies

3.1.1. Elucidation of bacterial metabolism by comparing bile metabolites with faecal metabolites

The simplest *in vivo* studies are based on the identification of the organs responsible for the production of specific metabolites. They are usually performed in rats with cannulated bile ducts kept

Table 2
Bacterial numbers in different sections of the gastrointestinal tract in commonly used laboratory animals (adapted with permission from Drasar et al., 1970)

	Stomach	Proximal small intestine	Distal small intestine	Large intestine	Rectum and faeces
Rabbit	$0–10^6$	$0–10^5$	$10^6–10^7$	$10^8–10^9$	$10^9–10^{10}$
Guinea-pig	$10^5–10^6$	$10^5–10^6$	$10^6–10^7$	$10^8–10^9$	$10^9–10^{10}$
Rat	$10^7–10^9$	$10^6–10^8$	$10^7–10^8$	$10^8–10^9$	$10^9–10^{10}$
Mouse	$10^7–10^9$	$10^7–10^9$	$10^7–10^8$	$10^8–10^9$	$10^9–10^{10}$

Table 3
Bacterial numbers and enzyme activities of caecal contents or faeces from different animal species (reproduced with permission from Rowland et al., 1986)

Species	Weight of caecal contents or faeces (g)	Bacterial numbers (log/g)	Enzymes activities (μmol/h per g caecal contents or faeces)				
			β-Glucosidase	β-Glucoronidase	Azo reductase	Nitro reductase	Nitrate reductase
Rat	2.9	10.8 ± 0.8	34.3 ± 5.5	156.3 ± 28.8	2.0 ± 0.6	4.0 ± 0.7	3.9 ± 1.0
Mouse	0.3	10.2 ± 0.2	55.6 ± 22.0	42.9 ± 4.6	2.3 ± 0.7	6.5 ± 0.7	1.8 ± 0.4
Hamster	1.5	10.4 ± 0.1	30.1 ± 2.3	60.8 ± 14.8	2.9 ± 0.3	3.9 ± 0.6	1.7 ± 0.2
Guinea-pig	46.2	10.3 ± 0.1	8.4 ± 3.0	11.3 ± 1.5	1.4 ± 0.3	0.4 ± 0.1	5.6 ± 1.8
Marmoset	1.7	10.8 ± 0.3	35.1 ± 8.0	11.7 ± 5.5	2.1 ± 0.7	0.6 ± 0.2	1.9 ± 1.0
Human	110	11.3 ± 0.2	49.5 ± 8.1	35.5 ± 19.9	Not available	1.0 ± 0.2	8.0 ± 2.3

in stainless steel metabolic cages for urine and faecal collection. The quantification of metabolites in faeces relative to the metabolites found in bile after oral administration of a compound can give an idea of the location where metabolism occurs. With the drug risperidone, for example, a group of metabolites is exclusively found in faeces and not in bile of rats providing strong evidence that the reaction is a result of bacterial action (Meuldermans et al., 1994). These types of *in vivo* studies normally require simultaneous *in vitro* evaluation to detect which metabolites can result from bacterial action to strengthen evidence for colonic bacterial metabolism.

3.1.2. Elucidation of bacterial metabolism by comparing lower gut metabolites with upper gut metabolites

Another methodology for locating the production of specific metabolites includes the removal of the stomach, small intestines, caecum and colon of rats at 1 h and 6 h after an oral dose of the compound being analysed (Yoshisue et al., 2000). The contents of these organs are taken out for quantification of metabolites of the administered compound. If after 6 h there is an increased amount of certain metabolites in caecal and colonic contents as compared to gastric and small intestinal contents then there is indication of colonic metabolism. The authors complemented this work with further *in vitro* and *in vivo* work emphasising the role of colonic bacteria in the metabolism of this particular compound (potassium oxonate).

3.1.3. Elucidation of bacterial metabolism by comparing gnotobiotic or antibiotic-treated animals with conventional animals

The previous methods are not stand alone techniques to prove bacterial metabolism, most require further *in vivo* or *in vitro* methodologies to strengthen evidence of colonic bacterial metabolism of a particular compound. This is due to the fact that they are unable to distinguish between metabolites produced in the colonic lumen by colonic bacteria and metabolites that are the result of intracellular metabolism by the colonic mucosa. To overcome this, a methodology was designed to specifically eliminate from an animal its bacterial metabolism. Such animals can be gnotobiotic animals or antibiotic-treated animals. Drug absorption and metabolism using these animals is then compared against using conventional animals.

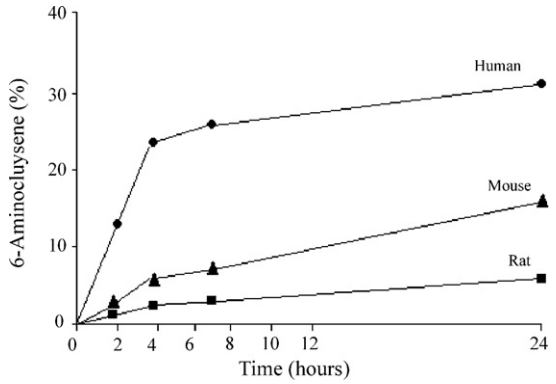

Fig. 2. Nitroreduction of 6-nitrochrysene to 6-aminochrysene by anaerobic bacterial suspensions from human faeces (●) and from mouse (▲) and rat (■) intestinal contents (reproduced with permission from Manning et al., 1988).

A gnotobiotic animal is defined as one in which all the life forms are known. It may be germ-free if it is free from any detectable microbes, or it may be associated with any number of strains of microorganisms the identity of which are known. The gnotobiotic animal is a useful tool in which to investigate the influence of indigenous microbiota on the host (Coates et al., 1988). Thus, the microbiota is implicated in a reaction sequence when a drug metabolite is found in the conventional animal (harbouring the indigenous microbiota), but not in the germ-free animal (Goldman, 1984).

Germ-free animals are, however, physiologically different from conventional controls and thus may show differences in their *in vivo* handling of xenobiotics (Ilett et al., 1990). Morphologically, the germ-free rat caecum is greatly enlarged and, being filled with liquid contents, fails to develop its normal musculature. The overall mass of the small intestine is decreased, its surface area is smaller, the crypts are shorter and the villi of the small intestine are more uniform and slender than in their respective conventional control animals (Heneghan, 1984). Germ-free rats also show a slower intestinal transit and while active transport seems unaffected by the presence or absence of microbiota, passive absorption tends to be increased in the germ-free state (Heneghan, 1984).

Animals lacking a gut microbiota can be obtained more conveniently by pretreatment with antibiotics and the differential sensitivity of microbiota to various antibiotics offers an experimental means of selectively modifying bacterial populations in the gut (Ilett et al., 1990). By comparison to gnotobiotic animals only relatively short-term experiments are possible, which are usually adequate for metabolic studies but not for long-term toxicity or carcinogenicity assays (Coates et al., 1988).

It has been suggested that a good model to study human colonic fermentation is the germ-free rat colonised with bacteria from human faecal samples. It has been demonstrated that rats, born germ-free, maintained in isolators and associated with human microbiota by orally dosing with fresh human faecal suspension retain bacteriological and metabolic characteristics of the human microbiota (Edwards and Parrett, 1999). The labour intensiveness of this technique and the impracticality of keeping these animals with a stable microbiota for long periods of time have prevented their use for drug metabolite studies.

3.2. Human in vivo studies

The use of animal models has some economical and logistical advantages over the use of human models: easier experimental set up and less stringent ethic regulations apply. However, for the

study of bacteria-related metabolism, as previously mentioned, there is evidence of variability between animal species and man. This inter-species variability has led scientists to use human volunteers instead of animals to determine how bacteria degrade drug substrates in the human gut.

3.2.1. Elucidating bacterial metabolism by comparing extended release and immediate release formulations

This technique compares the kinetic profiles of parent drug and metabolites using oral formulations that have different release profiles. A targeted drug release can deliver the drug to known locations where certain metabolites can be produced. One example of such a study demonstrated the bacterial metabolism of digoxin. The kinetics of a poorly absorbed digoxin tablet was compared with the kinetics of a well absorbed formulation (Lindenbaum et al., 1981). Poorly absorbed formulations release the drug distally where there is a higher concentration of bacteria. If the administration of poorly absorbed formulations causes a sharp increase in the concentration of metabolites then the most probable explanation for this is that metabolite formation occurs as a result of metabolism by colonic bacteria. Similar conclusions can be drawn from the comparison of immediate release formulations versus sustained release formulations used to study metronidazole (Koch and Goldman, 1979). One of the drawbacks of these studies using oral formulations with different release profiles is related to the fact that they are dependent on the formulation to deliver the drug successfully.

3.2.2. Elucidating bacterial metabolism by comparing intravenous drug delivery with oral drug delivery

Bacterial metabolites can only be present if the drug has contact with bacteria. By administering the drug intravenously, contact of the drug molecule with bacteria is minimised whilst the oral administration will allow this contact in the distal gastrointestinal tract. Any increased or new metabolites found after oral administration that are quantitatively less or absent from the intravenous formulation may be derived from bacterial metabolism (Yoshisue et al., 2000). One of the weaknesses of this approach is that molecules administered intravenously might be secreted into the gastrointestinal lumen via the bile duct from the liver, or via efflux mechanisms in the gastrointestinal tract and can therefore come in contact with the microbiota.

3.2.3. Comparing metabolism in ileostomy patients with healthy volunteers

Most of these previous *in vivo* methods attempt to prevent or favour drug contact with the section of the gut where bacterial metabolism is likely to occur by modifying the way the drug is delivered. A different approach is the use of patients that lack that section of the gut, i.e. ileostomy patients where the small intestine is brought to the outside, rather than carrying on into the colon. Although the presence of microbes has been detected to some extent in ileostomy effluent (Gorbach et al., 1967), it is believed that in this situation the drug will only present itself to a limited microbiota and probably for a very short period of time when compared to healthy individuals. Ileostomy patients have been used to assess the metabolism of the drugs olsalazine, sulfinpyrazone and sulindac (Wadworth and Fitton, 1991; Strong et al., 1987). Parent drug and metabolite concentration–time curves can be compared after oral administration to healthy volunteers versus ileostomy patients and if increased metabolites are found in healthy volunteers then they are likely the result of bacterial metabolism. The availability of ileostomy patients is, however, very limited and not possible in early drug development.

3.3. In vitro studies

In vivo determination of drug metabolism in the human colon is, in most cases, invasive, time-consuming, and requires medical supervision and ethical approval. In early drug development *in vitro* methods are needed for routine screenings of molecules or to further study a specific class of compounds. The biggest challenge with *in vitro* simulation of the colonic environment is the creation of a fermentation system containing bacterial numbers and diversity similar to the human colon. Considering, however, that the inaccessibility of the human colon does not permit the use of human colonic contents for *in vitro* experiments, it is believed that such an environment is more closely simulated with the use of human faecal material (that possesses microbiota derived directly from the microbiota present in the human colon) than with the use of intestinal contents from other animals. The methods of modelling the human colon *in vitro* are summarised.

3.3.1. Static batch cultures

The simplest *in vitro* fermenters are static batch cultures. These cultures can use specific strains of bacteria, caecal or intestinal contents of animals (normally rat) or faeces (animal or human), which then are placed into a suitable medium (buffer or nutritious media) (Coates et al., 1988). The drug is added in solution at time zero and regular samples are withdrawn and quantified for the amount of drug and its metabolites. Important variables include the maintenance of an anaerobic environment, a pH that reflects the colonic environment and the employment of continuous mixing of the contents to allow the drug and bacteria to be evenly distributed.

One of the disadvantages of this methodology is its suitability only for very short incubation periods. This is due to the continuously changing conditions in the cultures: marked and progressive changes in pH, redox potential, and bacterial population (Rumney and Rowland, 1992). It has been suggested that these fermenters can be used for periods of 24–48 h with the limitation that the culture follows a typical bacterial growth curve, thus short time course experiments provide more accurate results (Gibson and Fuller, 2000). Fortunately, incubation periods of up to 2 h are sufficient for most drug metabolic studies.

These static batch cultures have the advantage that a range of compounds can be screened in a relatively short period of time, and that only small amounts of drug are required which is essential in early drug development studies (Williams et al., 2005). Static batch cultures can make use of an anaerobic cabinet or use continuous sparging of nitrogen to keep the environment anaerobic. Different drugs or other variables can be tested simultaneously in different vials; the size of the vials is also changeable according to the amount of drug/culture source available. The simplicity, ease of use and affordability in terms of materials used makes this model a practical and useful *in vitro* method for short-term drug metabolite studies simulating the colonic environment. Such models have also been used to assess drug release from bacteria-sensitive film coatings for colonic delivery (Wilson and Basit, 2005; Basit et al., 2004; McConnell et al., 2007, 2008b; Siew et al., 2004; Yang, 2008).

3.3.2. Semi-continuous culture systems

Semi-continuous culture systems have never been used to study drug metabolism but employing them would offer the advantage of longer periods of incubation compared to static batch cultures due to the addition of fermentable substrates (nutrient for bacteria) into the system. A detailed description of semi-continuous systems is given by Rumney and Rowland (1992) and is summarised as follows. The system contains a fermenter vessel held within a 37 °C water bath and is equipped with a number of portholes for

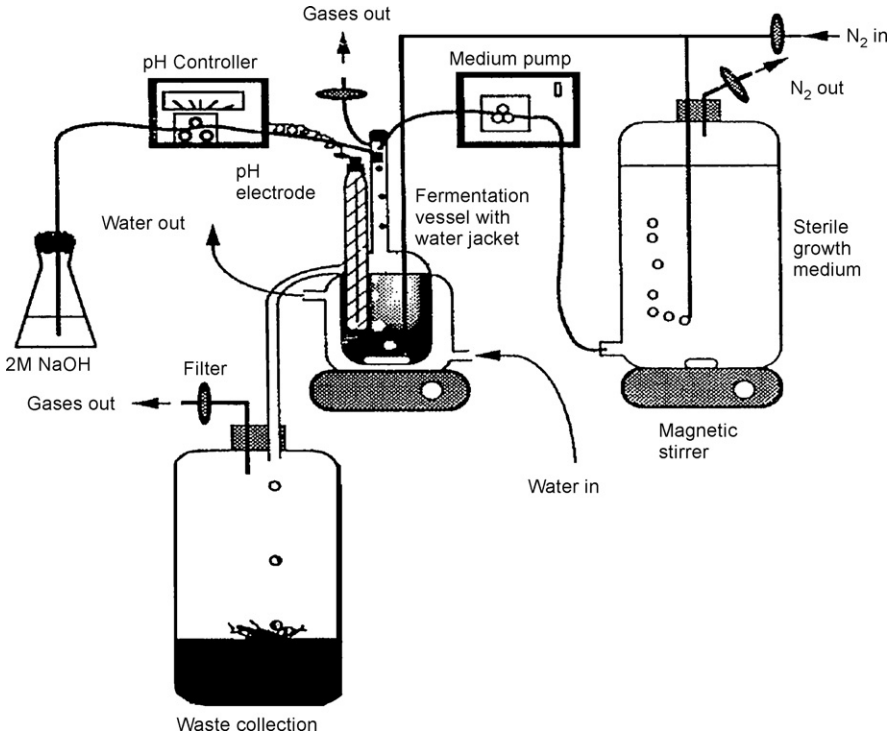

Fig. 3. Single-stage continuous culture system (reproduced with permission from Macfarlane et al., 1989).

sampling, addition of nutrient medium, and removal of culture contents. A pH meter and pH control facilities (acid and alkali infusion pumps) may be incorporated into the system, as can an electrometer for the measurement of redox potential. The mixed culture is maintained at a pH of 6.5–6.8 in an autoregulatory manner, thus making external pH control unnecessary.

3.3.3. Continuous culture systems

In the semi-continuous system described, part of the culture media is removed and replenished at intervals, this does not reproduce the continuous flow system that exists in the human colon. To more closely model the dynamic equilibrium naturally present within the gut, a system was designed to continuously add fresh growth medium and simultaneously remove spent culture (Coates et al., 1988). These types of systems were named continuous culture systems and are able to closely control the bacterial microbiota and their numbers, the pH, temperature, redox potential and nutrient status (Marsh, 1995; Ilett et al., 1990).

These continuous culture systems were traditionally single-stage chemostats (Fig. 3) that soon became outdated given the complexity of large intestinal fermentation, different parts of the colon have different nutrient availability and different physical conditions (Allison et al., 1989). A three-stage compound continuous culture system was developed and validated (Gibson et al., 1988; Macfarlane et al., 1998) that enables the activities of intestinal bacteria to be studied under the low pH, carbohydrate-excess conditions that characterise the proximal colon and ultimately the carbohydrate-depleted, nonacidic environment that is analogous to the distal bowel.

This continuous culture system (Fig. 4) consists of three vessels, V1, V2, and V3, with respective operating volumes of 0.22, 0.32, and 0.32 l. Temperature (37 °C) and pH were automatically controlled, pH 5.5, 6.2 and 6.8 in each vessel, respectively. Each fermenter is magnetically stirred and maintained under an atmosphere of CO_2 and the growth medium is continuously sparged with O_2-free N_2 and fed by peristaltic pump to V1, which sequentially supply V2 and V3 via a series of weirs.

3.3.4. Simulator of the human intestinal microbial ecosystem (SHIME)

A more intricate system aiming to study the ecological system present in the gastrointestinal tract was developed by Molly et al. (1993) consisting of a five-stage reactor named simulated human intestinal microbial ecosystem (SHIME) (Fig. 5). The small intestine is simulated by a two-step "fill and draw" system (300 ml working volume in each reactor), the large intestine by a three-step reactor (1000 ml, 1600 ml and 1200 ml working volume, respectively). Each reactor vessel has eight ports: for input and output of medium; sampling of liquid phase and headspace gases; pH electrode; pH control (acid and base); and for the headspace flushing. Vessels 1 and 2 are inoculated over eight consecutive days with 10 ml of a diet suspension and vessels 3, 4 and 5 are inoculated with 50 ml of 20% faecal suspension. Adding soygerm powder to the vessels aids in achieving the fermentation balance (De Boever et al., 2000). The analysis of a number of microorganism-associated activities resembled those appearing *in vivo*, thus, this reactor can evaluate the dynamics of the microbial ecology of the gastrointestinal tract and could possibly be used for drug metabolism studies (Molly et al., 1994). One of the major disadvantages of the SHIME simulator is that it requires a minimum of two weeks period to stabilise the microbial community before it can be used for *in vitro* investigations (Possemiers et al., 2004).

3.3.5. Computer-controlled system with peristaltic mixing, water absorption and absorption of fermentation products

Minekus et al. (1999) introduced a new type of system that combines the removal of metabolites and water with peristaltic mixing. This system (Fig. 6) consists of four glass units, each with a flexible wall inside, connected to each other. It is kept at 37 °C with a computer-controlled sequential squeezing of the walls causing a

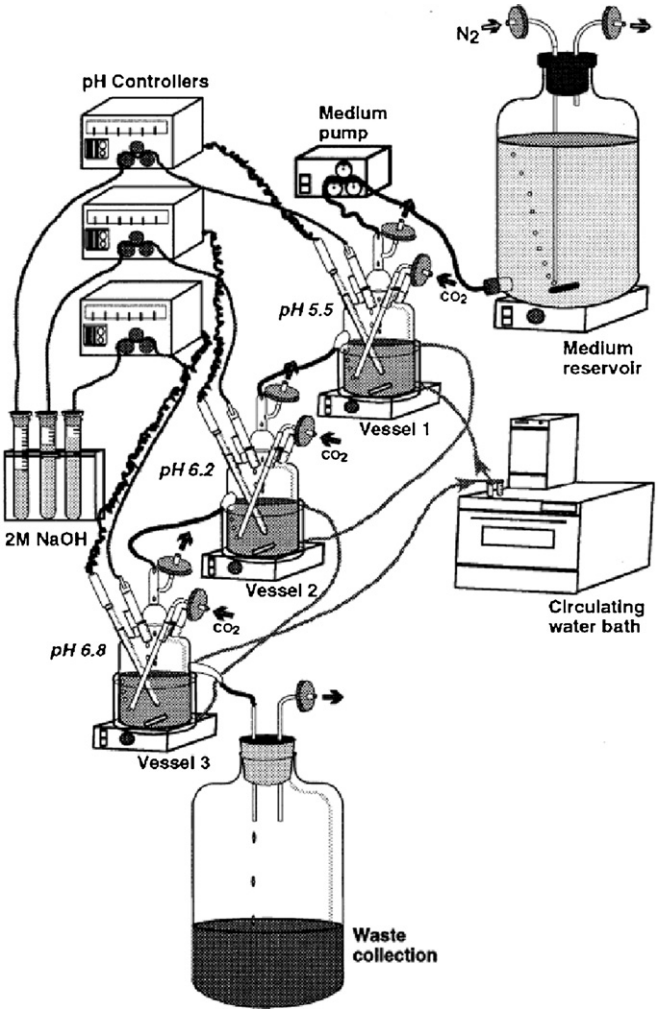

Fig. 4. The three-stage compound continuous culture system (reproduced with permission from Macfarlane et al., 1998).

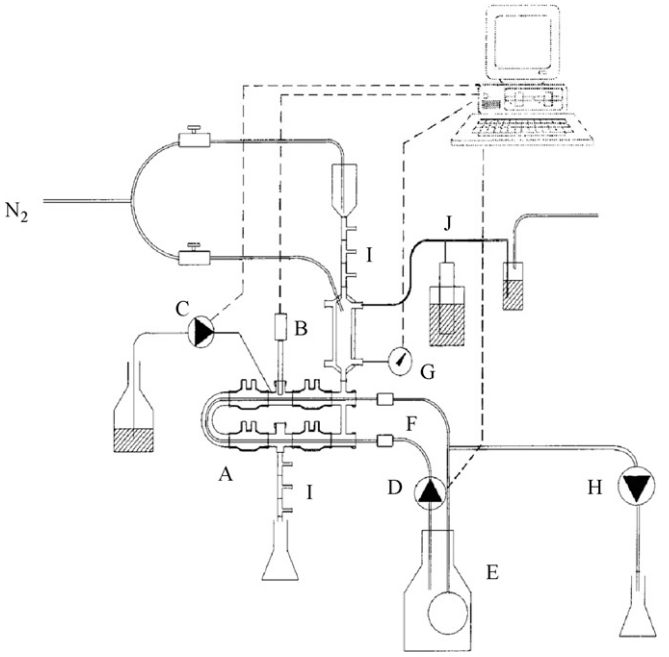

Fig. 6. Schematic presentation of the system to simulate conditions in the large intestine. A, mixing units; B, pH electrode; C, alkali pump; D, dialysis pump; E, dialysis light; F, dialysis circuit with hollow fibres; G, level sensor; H, water absorption pump; I, peristaltic valve pump; J, gas outlet with water lock (reproduced with permission from Minekus et al., 1999).

peristaltic wave, which forces the chyme to circulate through the loop-shaped system. The inoculum can be either fresh faecal samples or microbial cultures taken from previous fermenters, both approaches achieved stable cultures. The pH is controlled by the addition of 5 M NaOH, a dialysis liquid is used to maintain the appropriate electrolyte concentrations and is pumped from a bottle through hollow-fibre membranes positioned in the lumen of the reactor. The amount of chyme in the reactor is monitored with a pressure sensor and kept at a set level by the absorption of water, with a pump in the dialysis circuit. The feeding medium was mixed and kept anaerobic with nitrogen, and was introduced into the reactor with the peristaltic valve system and a peristaltic valve pump was used to remove chyme from the reactor.

This system is a useful tool to study the fate of undigested components and their effect on microbial metabolism and ecology in the lumen of the large intestine (Minekus et al., 1999). Due to the complexity of the system, it might not be such a useful system to use in short-term drug metabolism studies when compared to other *in vitro* models which are easier to set up.

3.3.6. *Immobilisation of faecal microbiota*

The ability to maintain a stable ecological environment *in vitro* is fundamental for the success of a large intestinal simulator. Most systems show a high variability of bacterial numbers and species

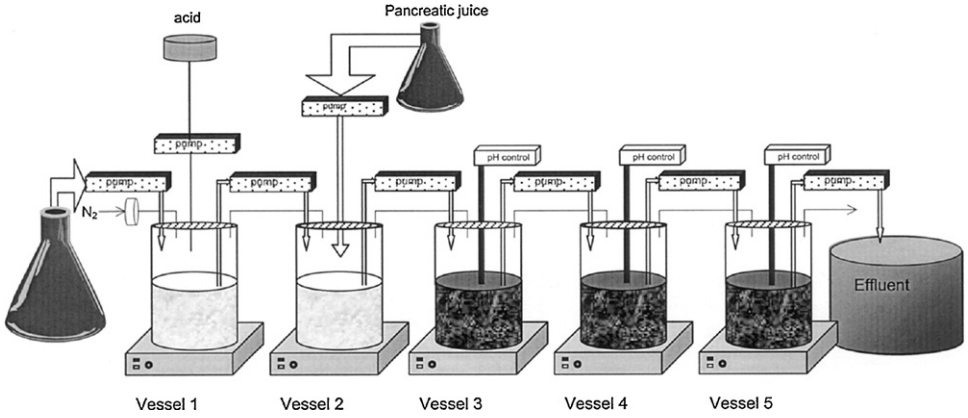

Fig. 5. Schematic representation of the Simulator of the Human Intestinal Microbial Ecosystem (SHIME) (reproduced with permission from De Boever et al., 2000).

over time or require a long stabilisation period before any investigation can occur. Researchers believe that the variability is due to the free movement of bacterial cells in the vessels, by moving freely, cells may be removed in an non-uniform way from the system when spent media is removed. This is because within the fermenter, bacteria can adhere to particles or grow in agglomerates, and dead and new growing bacterial cells coexist together. Ideally only dead cells and spent media would be removed for an optimum growth.

Cinquin et al. (2004) proposed the use of cell immobilisation and continuous fermentation to overcome these difficulties. Immobilising the bacteria in a layer ensures that new bacterial cells are not being removed from the system. The immobilisation was achieved by entrapment of bacteria cells (isolated from infant faeces) within a porous polysaccharide matrix. The bacterial cells are trapped in cell beads than can be grown in any suitable nutritious media. When immobilised cells are transferred in a growth medium a high-cell density layer is formed extending from the bead surface to a radial depth where lack of substrate prevents cell growth (Cinquin et al., 2004). The main advantage of bacterial immobilisation is the stability over long experimental trials, due to the continuous inoculation of the medium by shedding of free cells from highly colonised beads retained in the reactor. This ability allows the system to rapidly restore its previous equilibrium.

The advantage of this model is the ability to test different parameters using the same conditions by using beads precolonised with the same microbiota, this can be useful to compare metabolic rates of different compounds. The lack of diversity can, on the other hand, be a disadvantage since the human colon is a continuously evolving fermentation ecosystem. The use of fresh samples rather than precolonised beads to inoculate *in vitro* systems ensures bacterial and nutrient diversity that is likely to occur *in vivo*.

4. Drug metabolic reactions performed by the gastrointestinal microbiota

The following section highlights over 30 drug substrates for the gastrointestinal microbiota. A detailed understanding of the metabolic reactions involved and the types of studies to confirm a role for gut microbiota in metabolism is also presented (summarised in Table 4). The section is subdivided according to the class of reaction.

4.1. Reduction

4.1.1. Azo reduction: prontosil, neoprontosil, sulfasalazine, balsalazide and olsalazine

The reduction of the azo bond has been the basis of many prodrugs and delivery systems that target the colonic region of the gastrointestinal tract. This reduction reaction occurs with the aid of azoreductase enzymes produced by the large intestinal microbiota. The conversion of prontosil and neoprontosil to sulfanilamide are the first known examples where bacteria have an impact on prodrug activation. Both drugs were used as antibacterial agents in man and in 1937 (Fuller, 1937) it was discovered that the antibacterial action of prontosil was due to the molecule sulfanilamide.

In the rabbit, prontosil was found to be most actively metabolised to sufanilamide in the liver and kidney (Fouts et al., 1957). Antibiotics were later found to suppress the conversion of orally administered prontosil to sulfanilamide in the rat, so prontosil can be split to sulfanilamide partly in the gut before absorption and partly in the tissues after absorption (Gingell et al., 1971). Even when given intraperitoneally prontosil could be split by gut bacteria since it can be transported into the gut by the bile (Gingell et al., 1971). The reaction takes place presumably in two steps, the formation of the hydrazo compound, followed by the reductive splitting of the nitrogen bond (Fig. 7(A)).

Neoprontosil is water-soluble and highly polar, as a result it is not readily absorbed from the intestine and when given intraperitoneally a large proportion is excreted in the bile unchanged. Gingell et al. (1971) suggests that, in the rat, neoprontosil, whether given orally or intraperitoneally is split to sulfanilamide mainly by the gut microbiota (Fig. 7(B)). *In vitro* evidence was provided that neoprontosil is readily reduced by rat caecal and faecal homogenates, the output of sulfanilamide in the urine after administering neoprontosil is furthermore suppressed by antibiotics.

Prontosil and neoprontosil belong to the chemical group of sulfonamide drugs. Sulfonamide drugs were the first antimicrobial drugs and initiated the antibiotic revolution in medicine. The innovative idea of combining a sulfonamide molecule such as sulfapyridine with an anti-inflammatory moiety like salicylic acid for the treatment of inflammatory conditions that were believed to be of bacterial origin was first postulated in 1941 (Bachrach, 1988; Svartz, 1988). Linking salicylic acid (specifically 5-aminosalicylic acid) to sulfapyridine was achieved by a diazo coupling, yielding sulfasalazine. It was later found that sulfasalazine was extremely beneficial for the treatment of ulcerative colitis. Sulfasalazine has limited small intestinal absorption and, in the colon, its azo bond is reduced by bacteria (Fig. 7(C)) releasing 5-aminosalicylic acid (the topical active) and sulfapyridine which is systemically absorbed (Peppercorn and Goldman, 1976, 1973; Hayllar and Bjarnason, 1991).

The cleavage of the azo bond in sulfasalazine by intestinal bacteria was documented in 1972 (Peppercorn and Goldman), these studies showed that the excreta of rats fed sulfasalazine did not contain unchanged sulfasalazine. However, with antibiotic-treated or with germ-free rats unchanged sulfasalazine was recovered in the caecum and faeces. Intestinal bacterial strains were also cultured in the presence of sulfasalazine and in all cases the azo bond cleavage was detected. In addition, in humans, a pharmacokinetic study performed in healthy volunteers (Schröder and Campbell, 1972) demonstrated no faecal and a very small urinary excretion of the intact drug.

Sulfasalazine is more effective in ulcerative colitis than in Crohn's disease, the latter is not always localised in the colonic region of the gastrointestinal tract, which is consistent with the drug being more effective when the pathology is in a region where bacterial breakdown is more likely to occur (Peppercorn and Goldman, 1973).

Sulfasalazine causes side effects in some patients (anorexia, nausea, skin rash, blood dyscrasias). It is believed that circulating sulfapyridine is the main cause of unwanted effects and effort has been made to replace sulfapyridine by carrier molecules thought unlikely to produce side effects. As a consequence, balsalazide was synthesized from 4-aminobenzoyl-β-alanine by diazo coupling with salicylic acid. The results of metabolic studies in man showed that trace amounts of unchanged drug were found in normal faeces and urine (Chan et al., 1983). These studies suggested that reduction to 5-aminosalicylic acid and the carrier molecule was almost complete (Fig. 7(D)).

Olsalazine is a prodrug consisting of two 5-aminosalycilic acid moieties bridged by an azo bond. Little olsalazine is metabolised before entry into the colon where azoreductase-containing bacteria split unabsorbed olsalazine into two 5-aminosalicylic acid molecules (Fig. 7(E)) (Wadworth and Fitton, 1991).

In healthy volunteers, urinary recovery of 5-aminosalicylic acid was found in the first 8 h after administration of a single oral dose of olsalazine. This contrasts with ileostomy patients where no 5-aminosalicylic acid was detected in the urine following olsalazine's

Table 4
Examples of drug substrates which undergo bacterial metabolism and the studies which established this

Substrates (references)	*In vitro* studies—static batch cultures					*In vivo* studies—elucidation of bacterial metabolism				
	Human faeces	Rat intestinal contents	Rat faeces	Other animal's intestinal or faecal contents	Bacterial cultures	Location of metabolite production	Comparing extended and immediate release formulations	Comparing intravenous with oral formulations	Comparing ileostomy with healthy volunteers	Using gnotobiotic or antibiotic-treated animals
Prontosil (Gingell et al., 1971)		√	√							√
Neoprontosil (Gingell et al., 1971)		√	√							√
Sulfasalazine (Peppercorn and Goldman, 1972)					√	√				√
Balsalazide (Chan et al., 1983)						√				
Olsalazine (Wadworth and Fitton, 1991)									√	
L-Dopa (Goldin et al., 1973)		√								√
Chloramphenicol (Holt, 1967)					√					
5-Aminosalicylic acid (Dull et al., 1987; Deloménie et al., 2001)	√	√	√	√	√					
Phenacetin (Smith and Griffiths, 1974)		√								
Digoxin (Lindenbaum et al., 1981)	√						√	√		√
Ranitidine (Basit and Lacey, 2001)	√									
Nizatidine (Basit et al., 2002)	√									
Quercetin-3-glucoside (Schneider et al., 2000)										√
Insulin (Tozaki et al., 1997)			√							
Calcitonin (Tozaki et al., 1997)			√							
Nitrazepam (Takeno and Sakai, 1990; Rafii et al., 1997)					√					√
Clonazepam (Elmer and Remmel, 1984)		√								√
Sulfinpyrazone (Strong et al., 1987)									√	
Sulindac (Strong et al., 1987)									√	
Isosorbide Dinitrate (Abu Shamat, 1993)	√				√					
Glyceryl Trinitrate (Abu Shamat and Beckett, 1983)	√	√								
Omeprazole (Watanabe et al., 1995)		√								√
Levamisole (Shu et al., 1991)	√				√					
Metronidazole (Koch et al., 1979; Abu Shamat, 1993)		√			√		√			√
Misonidazole (Koch et al., 1980)		√			√					√
Risperidone (Meuldermans et al., 1994)		√				√				
Methamphetamine (Caldwell and Hawksworth, 1973)				√						
Zonisamide (Kitamura et al., 1997)				√	√					√
Azetirelin (Sasaki et al., 1997)	√	√	√	√						√
Sorivudine (Okuda et al., 1998)								√		√
Potassium Oxonate (Yoshisue et al., 2000)		√				√		√		
Flucytosine (Vermes et al., 2003; Harris et al., 1986)	√				√					
Hesperidin (Lee et al., 2004)	√		√							
Daidzein (Rafii et al., 2004)				√						√

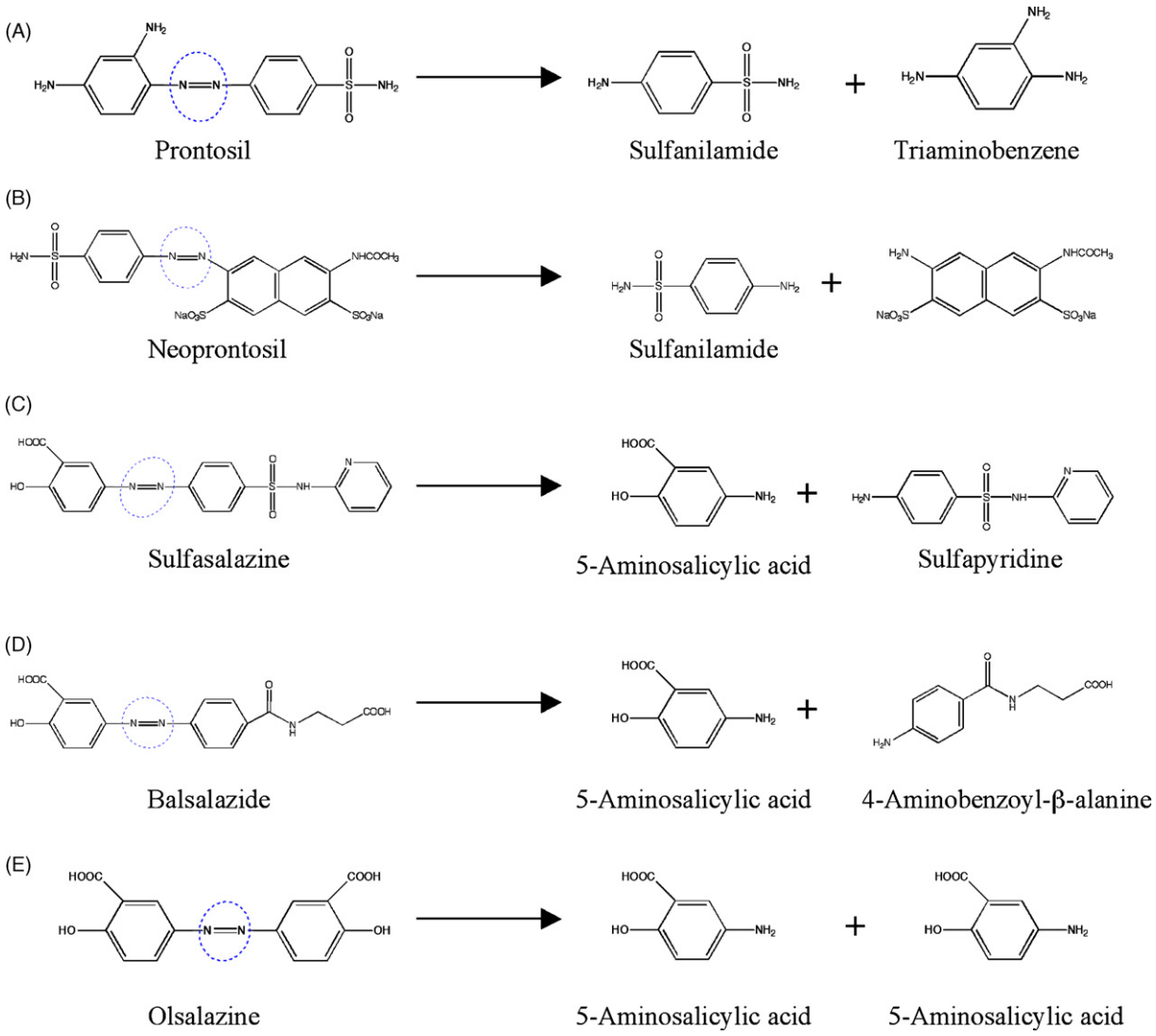

Fig. 7. Proposed reactions for the azo reduction of prontosil (A), neoprontosil (B), sulfasalazine (C), balsalazide (D) and olsalazine (E).

administration. Studies in both healthy volunteers and patients with ulcerative colitis demonstrated that a dose of olsalazine is almost completely metabolised due to the low faecal recovery of unchanged olsalazine (Wadworth and Fitton, 1991).

4.1.2. Nitrazepam

Nitrazepam is responsible for fetal abnormalities and Takeno and Sakai (1990) tried to determine whether nitrazepam is the active teratogenic substance or whether metabolic activation is required for the teratogenic response. They also investigated the role of gut microbiota metabolism in nitrazepam-induced teratogenicity in rats by comparison with antibiotic-treated rats. The results obtained by this study suggested that nitrazepam requires activation by nitroreductase for the teratogenic activity and that the gut microbiota is the primary site for reductive metabolism (Fig. 8(A)).

Nitrazepam's reduction has been found to occur in different environments: rat liver mitochondria, microsomal fractions, bacteria from the rat intestinal tract and bacteria with nitroreductase activity in the human intestinal tract. Its reduction rate in rat caecal contents is approximately seven times higher that that of the liver (Rafii et al., 1997). The proposed mechanism for nitrazepam's teratogenicity starts with its nitroreduction to 7-aminonitrazepam by the intestinal microbiota. 7-Aminonitrazepam is then converted in the liver to 7-acetylaminonitrazepam, which is teratogenic in both rats and mice (Takeno et al., 1993).

4.1.3. Clonazepam

Elmer and Remmel (1984) studied the metabolism of clonazepam *in vitro* and *in vivo* using germ-free and ex-germ-free rats and the incubation of clonazepam with rat intestinal lumen contents gave nearly complete reduction of clonazepam to 7-aminoclonazepam (Fig. 8(B)). A definitive role for the intestinal microbiota in the metabolism and disposition of clonazepam in the rat was established and mammalian tissues play a relatively minor role in clonazepam reduction.

4.1.4. Misonidazole

Misonidazole is a 2-nitroimidazole derivative, which is an effective radiosensitiser of hypoxic cells that is being tested as an adjunct to radiation therapy in the treatment of human cancer. Studies performed by Koch et al. (1980) showed that misonidazole is metabolised to its amino derivative [1-(2-aminoimidazol-1-yl)-3-methoxypropan-2-ol] in pure or mixed cultures of the intestinal microbiota (Fig. 8(C)) and that the metabolite is further metabolised to release CO_2. The metabolite appears in the excreta of conven-

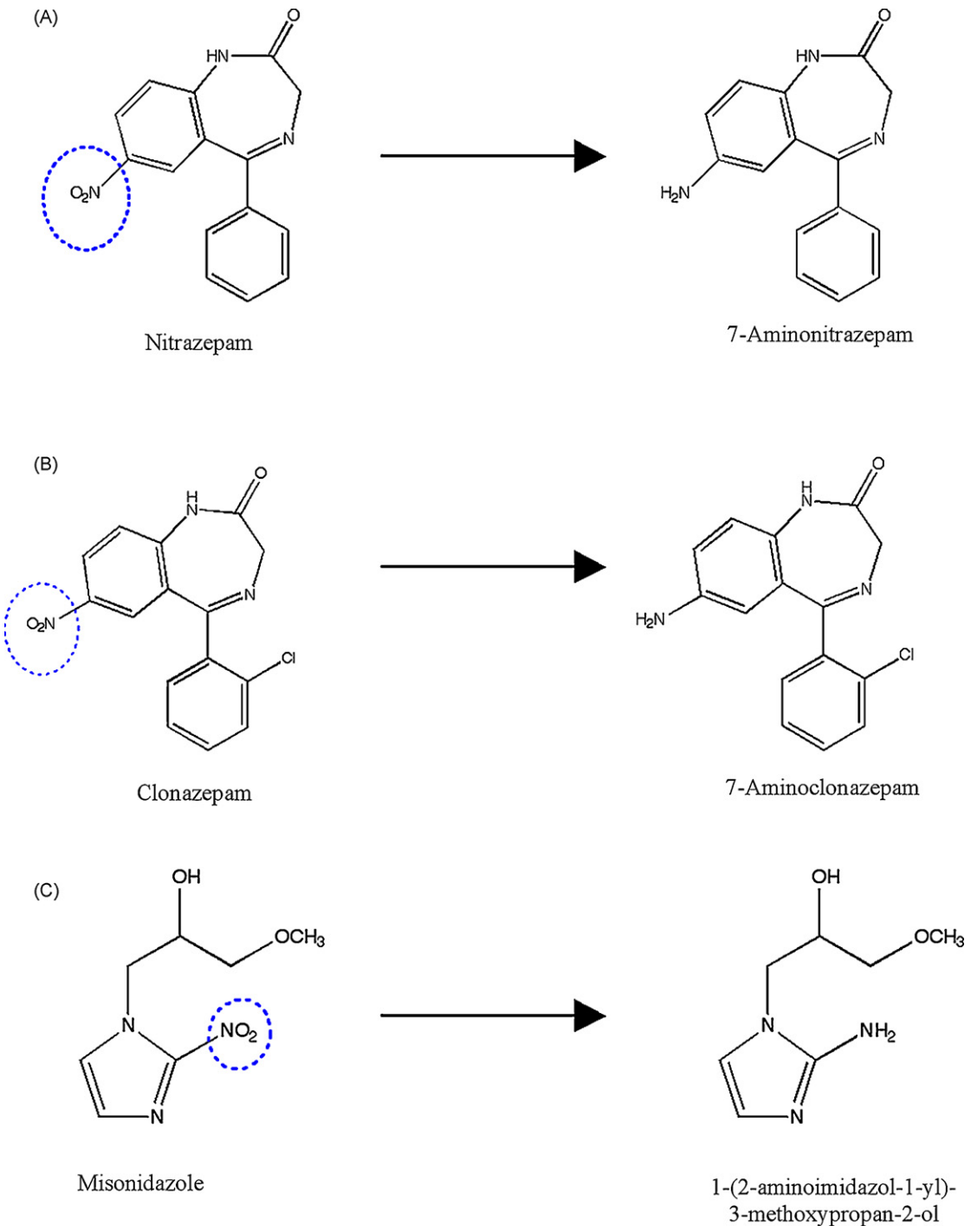

Fig. 8. Proposed reactions for the reduction of nitrazepam (A), clonazepam (B) and misonidazole(C).

tional rats but is not detectable in the excreta of germ-free rats so its formation is due to the intestinal microbiota *in vivo* as well as *in vitro* (Koch et al., 1980).

4.1.5. Omeprazole

Omeprazole is a substituted benzimidazole that effectively suppresses gastric acid secretion by inhibiting H^+/K^+-ATPase in the parietal cell. Watanabe et al. (1995) examined the effect of intestinal microbiota on the pharmacokinetics of omeprazole. The *in vitro* experiments showed that omeprazole is metabolised by the intestinal contents of the rat, being reduced to sulfide metabolites (Fig. 9(A)). In vivo, however, the gut microbiota did not alter the oral pharmacokinetics because the compound was completely absorbed before reaching the hindgut, where microbial activity is most extensive.

4.1.6. Sulfinpyrazone

Sulfinpyrazone is a uricosuric agent with a potential value in thromboembolic disorders. Strong et al. (1987) studied the bacterial reduction of sulfinpyrazone by comparison of the plasma

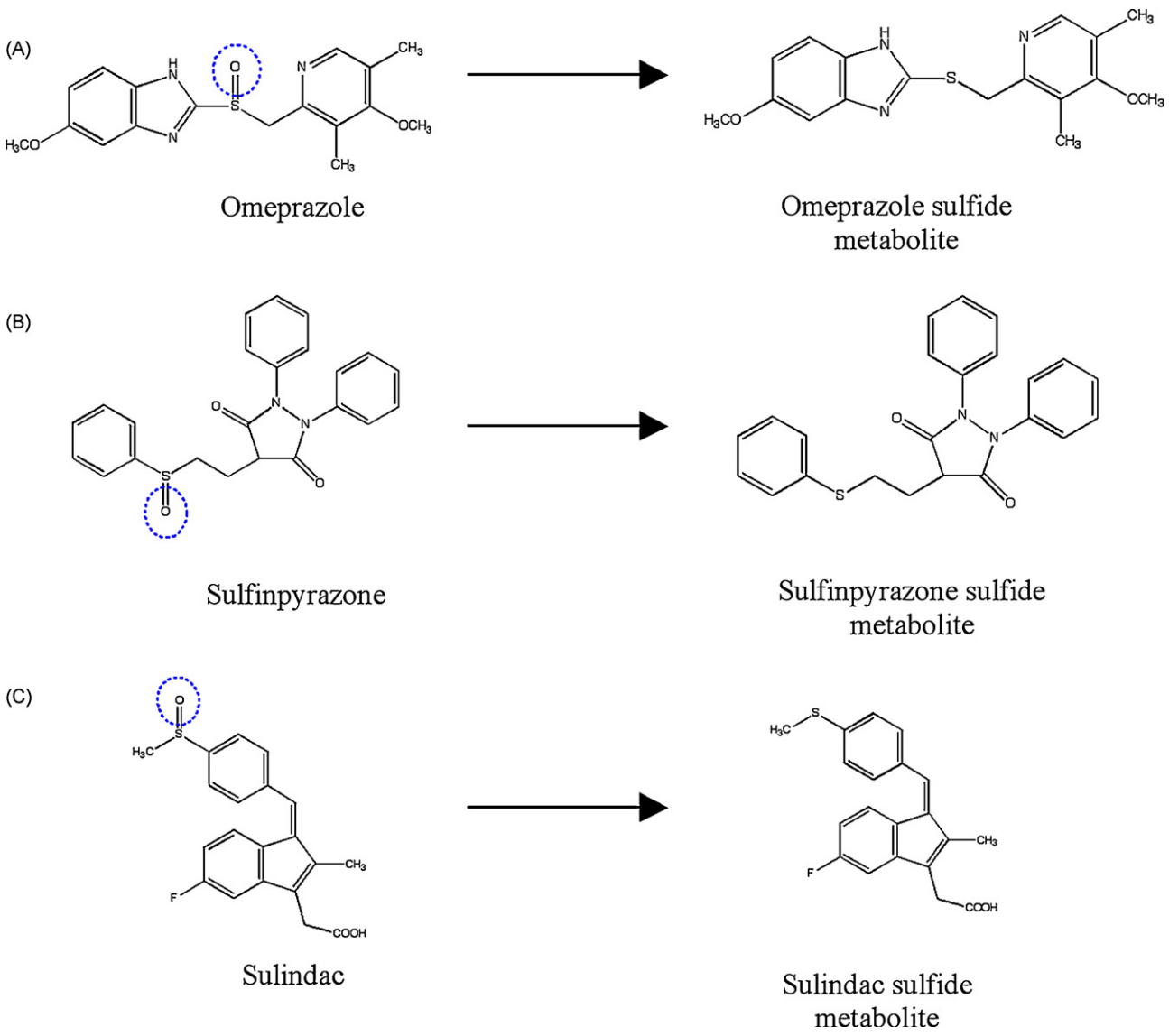

Fig. 9. Proposed reactions for the reduction of omeprazole (A), sulfinpyrazone (B) and sulindac (C).

concentration–time curves after a single oral dose in healthy volunteers and in ileostomy patients. These data indicate that the gut microbiota is the sole site of sulfinpyrazone reduction in man (Fig. 9(B)).

4.1.7. Sulindac

Simultaneously with the study of sulfinpyrazone, Strong et al. (1987) examined the bacterial reduction of sulindac, a non-steroidal anti-inflammatory analgesic used in the treatment of rheumatoid arthritis and similar conditions. The study using healthy volunteers and ileostomy patients indicates that gut microbiota contributes significantly to the formation of sulindac sulfide in man, probably by reduction of sulindac which is excreted in bile (Fig. 9(C)).

4.1.8. Digoxin

Some patients treated with digoxin may convert the cardiac glycoside to inactive metabolites. If the lactone ring on the digoxin molecule is saturated (Fig. 10(A)), metabolites such as dihydrodigoxin or its aglycone, dihydrodigoxigenin are formed. These reduced derivatives have markedly decreased cardiac activity, possibly because they bind poorly to the Na-K-ATPase of cardiac cells and are taken up less avidly by cardiac muscle.

Lindenbaum et al. (1981) suggested that the formation of reduced metabolites from digoxin occurs in the gastrointestinal tract, conceivably by colonic bacteria. This was suggested after the observation that the maximal production of reduced derivatives from digoxin happens when the most poorly absorbed tablets are taken. On the other hand, the lowest percentage of reduction products was excreted after digoxin was given intravenously. Lindenbaum et al. (1981) further hypothesised that variation in the activity of the gut microbiota explains why only a minority (10%) of patients on prolonged digoxin therapy excrete reduced derivatives. In many other subjects, such metabolites are not detectable. As aforementioned, the reduction products possess markedly decreased cardiac activity therefore reducing digoxin's effectiveness if the reaction occurs.

The antibiotics erythromycin and tetracycline were also proven to block the reduction of digoxin *in vitro* and *in vivo* (Lindenbaum et al., 1981). In addition, to further confirm that the gut microbiota is responsible for this reaction, the ability to metabolise digoxin to dihydrodigoxin in four volunteers was shown when a prepara-

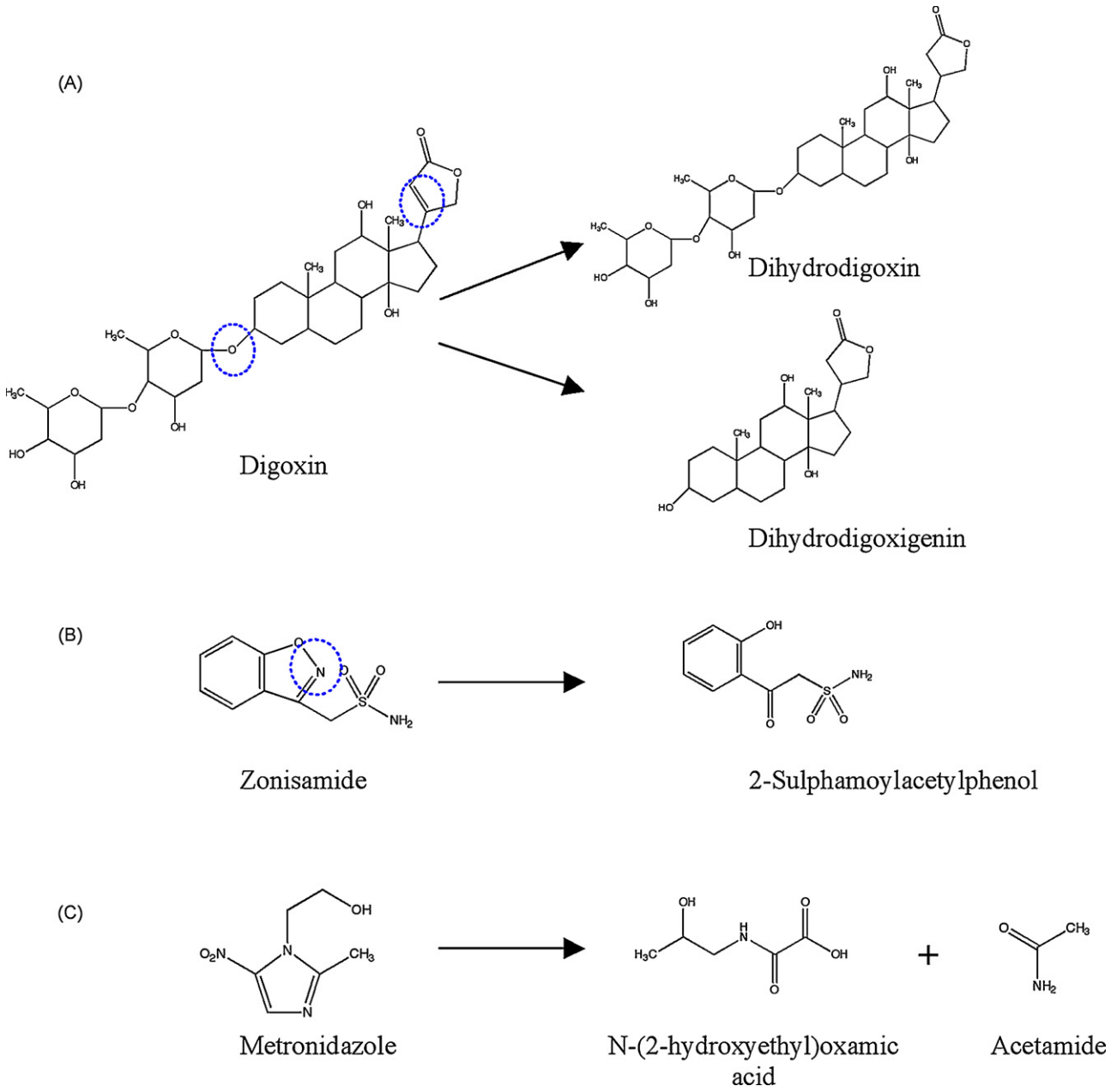

Fig. 10. Proposed reactions for the reduction of digoxin (A), zonisamide (B) and metronidazole (C).

tion releasing digoxin in the more distal small intestine was given (Magnusson et al., 1982).

4.1.9. Zonisamide

Zonisamide is an anticonvulsant clinically used for the treatment of epilepsy. It is primarily metabolised to 2-sulfamoyacetylphenol by reduction of the benzisoxazole ring and the studies performed by Kitamura et al. (1997) concluded that intestinal bacteria play a major role in this reductive metabolism of zonisamide to 2-sulfamoylacetylphenol *in vivo* (Fig. 10(B)).

The relative contribution of intestinal bacteria to the *in vivo* metabolism of zonisamide was examined in rats. The treatment with antibiotics caused a significant decrease in the urinary and faecal excretion of 2-sulfamoylacetylphenol, re-contamination of the antibiotic-treated rats with microbiota restored the excretion of the metabolite (Kitamura et al., 1997). In the same study the caecal contents of mice, hamsters, rabbits and guinea-pigs, like those of rats, had the ability to catalyse the reduction of zonisamide to its metabolite under anaerobic conditions, the highest activity was observed with mice and the lowest with guinea-pigs.

4.1.10. Metronidazole

Metronidazole is a 5-nitroimidazole derivative with activity against protozoa and anaerobic bacteria. Besides the biotransformation in the liver by side-chain oxidation and glucuronide formation the drug can undergo reductive metabolism producing small amounts of reduced metabolites.

N-(2-hydroxyethyl)-oxamic acid and acetamide are formed when metronidazole is reduced as the result of the activity of anaerobic bacteria (Fig. 10(C)); it decomposes with loss of the imidazole

ring (Koch and Goldman, 1979). Metronidazole's metabolism in conventional rats was compared with germ-free rats and the metabolites N-(2-hydroxyethyl)-oxamic acid and acetamide were only found in excreta of conventional rats and not in the urine or faeces of germ-free rats (Koch et al., 1979; Koch and Goldman, 1979). Those metabolites are also formed after incubation of metronidazole with either *C. perfringens* or rat caecal contents (Koch et al., 1979). In addition, both metabolites were found in small amounts in the urine of human patients taking the drug, presumably, as in the rat, they represent metabolites formed by the intestinal microbiota (Koch et al., 1981).

4.2. Hydrolysis

4.2.1. Lactulose

Lactulose, the keto analogue of lactose (4-(β-D-galactopyranosyl)-D-fructose) appears to depend on metabolism by the intestinal bacteria for its therapeutic activity. The drug is hydrolyzed by several kinds of intestinal bacteria (lactobacillus, bacteroides and *E. coli*) to form lactic and acetic acids, which lower the pH of the intestinal contents (Elkington et al., 1969). At the lower pH, ammonia and the other amines present in the gastrointestinal tract become protonated and tend to be excreted in faeces, the accentuated elimination of these amines is responsible for the laxative effect of lactulose (Peppercorn and Goldman, 1976).

4.2.2. Sorivudine

Sorivudine was released into the Japanese market in 1993 as an antiviral drug. In a toxicokinetic study performed by Okuda et al. (1998) to investigate 18 acute deaths associated with interactions between sorivudine and oral 5-fluorouracil (5-FU) prodrugs, one of the major metabolites of sorivudine was found to be involved in this lethal toxicity. The authors found that sorivudine's metabolite (E)-5-(2-bromovinyl)uracil (BVU) inactivates a key liver enzyme (DPD) regulating the systemic 5-FU level (Fig. 11), the marked increase in 5-FU tissue levels led to death in rats and humans. BVU is generated from sorivudine by gut microbiota. *Bacteroides* species, such as *Bacteroides eggerthii* and *Bacteroides vulgatus*, which abundantly exist in human intestines, were identified as the major bacteria, which generate BVU from sorivudine (Nakayama et al., 1997). Little BVU was detected in the plasma of germ-free rats, and oral or i.v. administration gave similar levels of BVU in the urine of germ-free rats (Ashida et al., 1993). These results indicate that the formation of BVU is predominantly due to the action of enterobacteria.

4.3. Removal of succinate group: succinylsulfathiazole

The sulfa drug succinylsulfathiazole is itself inactive and poorly absorbed from the gastrointestinal tract, but is converted by intestinal bacteria to sulfathiazole (Fig. 12(A)), which has strong antibacterial action (Poth et al., 1942). The reaction involves the removal of the succinate group.

4.4. Dehydroxylation: L-dopa

L-Dopa is effective in the therapy of Parkinson's disease where dopamine depletion within the central nervous system is responsible for the disease's clinical abnormalities. It is believed that L-dopa undergoes decarboxylation within the central nervous system and exerts its beneficial effect by restoring the level of dopamine. Most of the decarboxylation of L-dopa, however, occurs outside the CNS and the intestinal bacteria may be capable of this reaction (Fig. 12(B)) (Peppercorn and Goldman, 1976).

The metabolites *m*-tyramine and *m*-hydroxyphenylacetic acid are found in the urine of conventional, but not germ-free, rats. The dehydroxylation reactions at the para position of the catechol ring which are required to form these metabolites can be demonstrated in preparations of rat caecal contents indicating the importance of the microbiota in this aspect of L-dopa metabolism (Goldin et al., 1973).

4.5. Acetylation: 5-aminosalicylic acid

5-Aminosalicylic acid (5-ASA, mesalazine) is used in the treatment of inflammatory bowel disease and is also the active component of the prodrugs sulfasalazine, balsalazide and olsalazine mentioned earlier in this review. 5-ASA efficacy is correlated with its delivery to the diseased site and intestinal metabolism may be important in determining drug efficacy.

Acetylated 5-ASA has been found in the faeces of animals dosed with 5-ASA (or its prodrugs). Dull et al. (1987) proved that less than 3.4% of 5-ASA was acetylated in *in vitro* suspensions of either guinea-pig caecal contents or faeces from rats, dogs or humans. Furthermore, 5-ASA was incubated with faecal suspensions obtained from germ-free rats finding no acetylating activity, which supported the conclusion that intestinal bacteria mediate this acetylation reaction. 5-ASA is acetylated in human faecal suspensions under both aerobic and anaerobic conditions as well as by individual bacteria (Fig. 12(C)) (Van Hogezand et al., 1992; Deloménie et al., 2001).

4.6. Deacetylation: phenacetin

The analgesic phenacetin is rapidly and almost completely absorbed from the gastrointestinal tract and metabolised mostly to acetaminophen in the liver. However, a small amount of phenacetin is converted to *p*-phenetidin (Fig. 12(D)). The deacetylation reaction involved in forming this metabolite can be demonstrated when rat caecal contents are incubated with phenacetin under anaerobic conditions (Smith and Griffiths, 1974). The formation of *p*-phenetidin correlates with methemoglobinemia, one of the complications of the use of phenacetin and with nephritis that accompanies chronic phenacetin abuse (Smith and Griffiths, 1974).

4.7. Cleavage of N-oxide bond: ranitidine and nizatidine

Basit and Lacey (2001) used a batch culture fermentation system simulating the conditions of the colon to assess the *in vitro* stability of ranitidine. In this study a marked decline in ranitidine concentration was noted over time and further analysis indicated that metabolism occurred via cleavage of an N-oxide bond within the molecule with the resultant loss of an oxygen atom (Fig. 13(A)). Such metabolism may in part be responsible for the poor absorption of ranitidine from the colon (Basit et al., 2004).

The other H_2-receptor antagonists, cimetidine, famotidine and nizatidine were studied also to assess the *in vitro* stability to colonic bacteria (Basit et al., 2002). That study noted a marked decline in nizatidine concentration over time and no such decline was noted for cimetidine or famotidine. The metabolic reaction pathway for nizatidine is complex, although the metabolism is initiated via cleavage of an N-oxide bond within the molecule (Fig. 13(B)).

4.8. Proteolysis: insulin and calcitonin

The possibility of delivering peptide and protein drugs to the colon has been investigated: however peptide drugs such as insulin and calcitonin are degraded by some proteolytic enzymes in rat caecal contents (Fig. 14) (Tozaki et al., 1997). Further to this, calcitonin was more susceptible to proteolysis in rat caecal contents

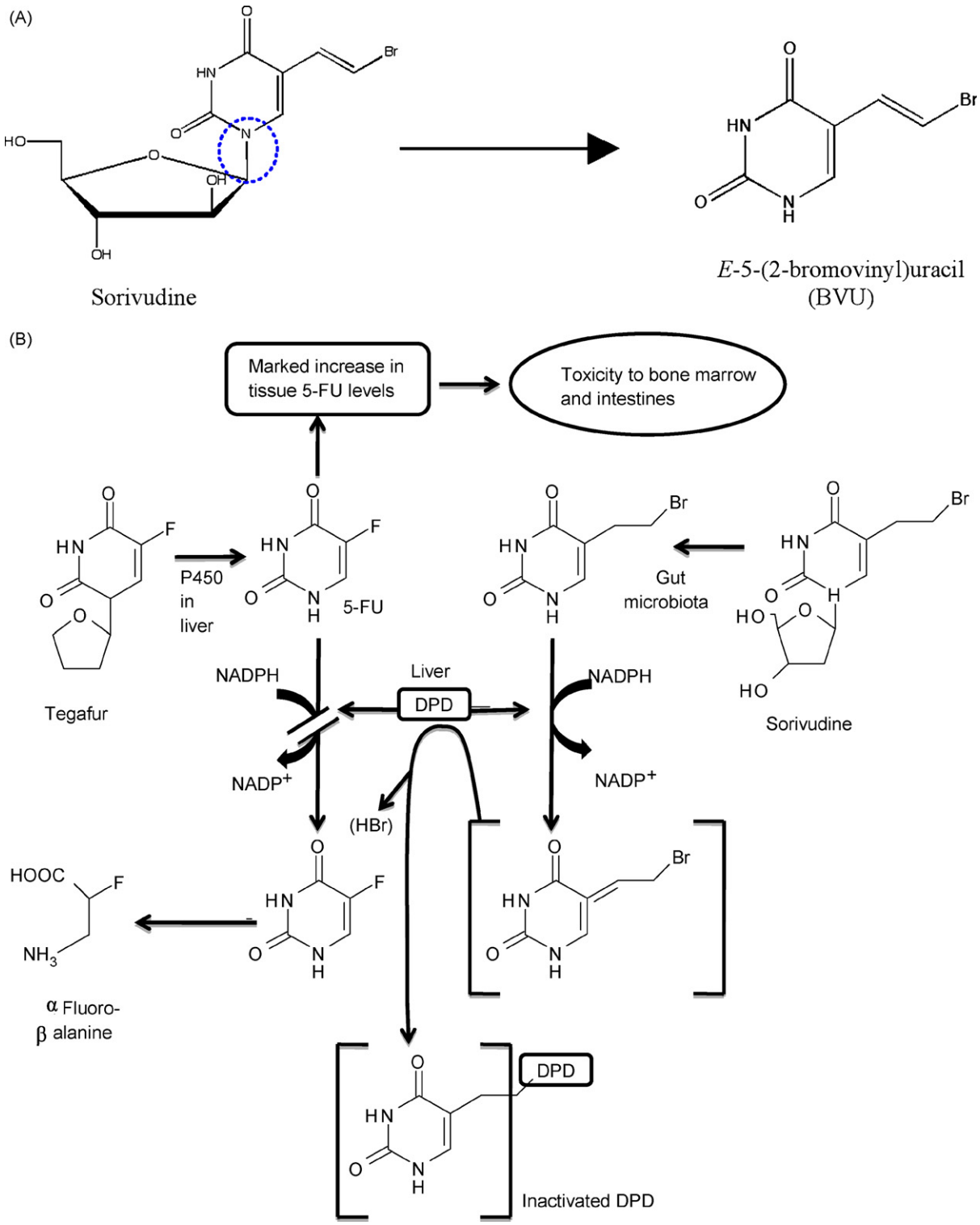

Fig. 11. Proposed reactions for the hydrolysis of sorivudine (A) and proposed mechanism for the lethal toxicity of sorivudine and 5-fluorouracil in humans and rats (B) (adapted permission from Okuda et al., 1998).

than insulin (Tozaki et al., 1995). Calcitonin was rapidly degraded in the supernatant and insulin was mainly metabolised by microorganism's membrane enzymes (Tozaki et al., 1995). Some protease inhibitors could inhibit the degradation of peptides in rat caecal contents, suggesting that these protease inhibitors might be useful for improving the large intestinal absorption of peptides by the systemic circulation (Tozaki et al., 1997).

4.9. Denitration: glyceryl trinitrate and isosorbide dinitrate

Glyceryl trinitrate and isosorbide dinitrate are among the most commonly used organic nitrates for the treatment of angina pectoris. The metabolism of glyceryl trinitrate by a mixed culture of rat caecal contents has been investigated by Abu Shamat and Beckett (1983). In their studies, following combination of glyceryl

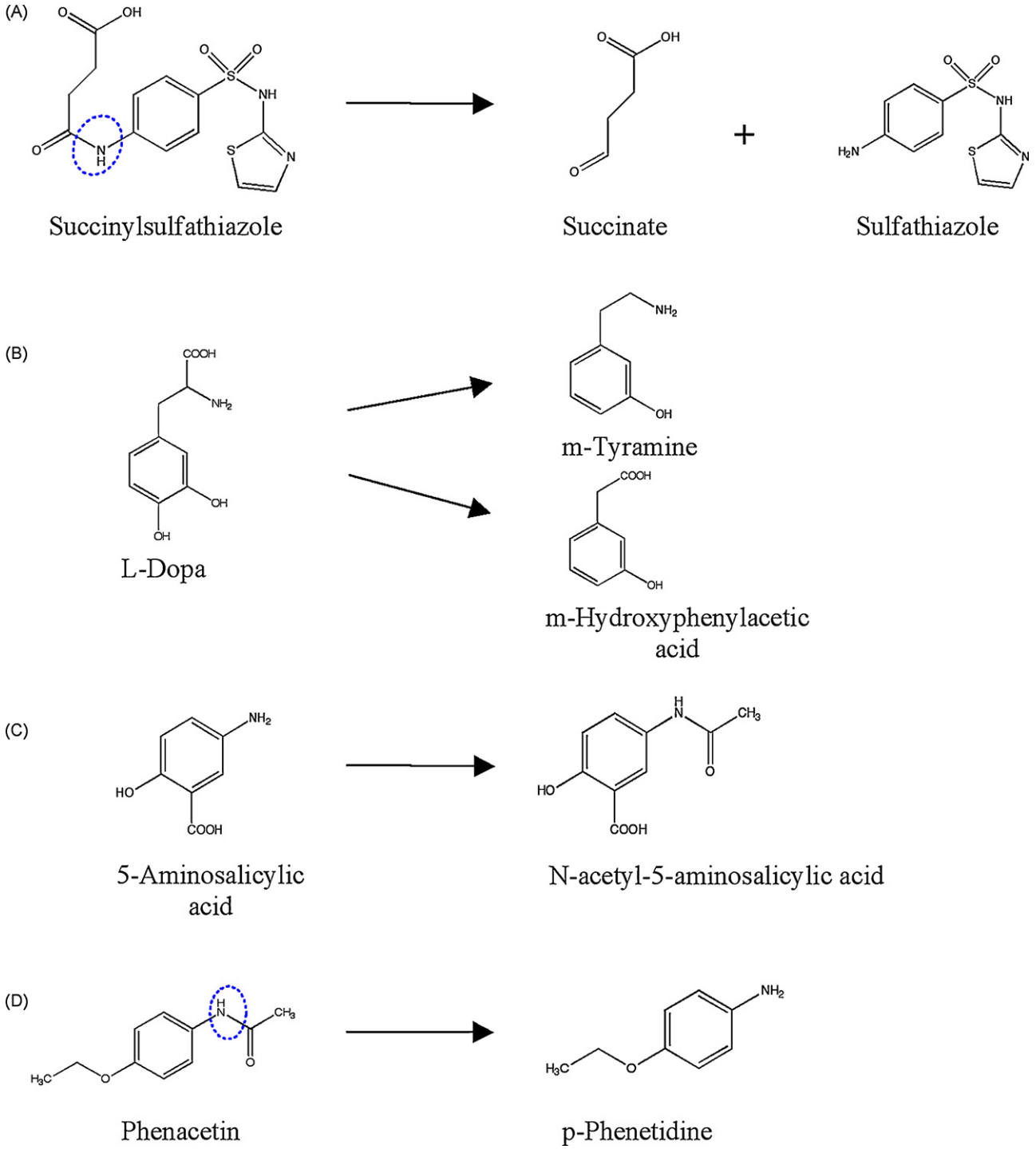

Fig. 12. Proposed reactions for the removal of the succinate group in succinylsulfathiazole (A), the dehydroxylation of L-dopa (B), the N-acetylation of 5-aminosalicylic acid (C) and N-deacetylation of phenacetin (D).

trinitrate with rat caecal contents, the drug disappeared exponentially from the anaerobic incubation mixture (Fig. 15(A)) to give the metabolites glyceryl-1,3-dinitrate, glyceryl-1,2-dinitrate, glyceryl-1-mononitrate and glyceryl-2-mononitrate (Fig. 15(B)). A comparison with the rate of *in vitro* metabolism in human faecal microbiota was also performed.

Isosorbide dinitrate in addition to other metabolic pathways can be metabolised *in vitro* by anaerobic intestinal microorganisms of both rat and man (Fig. 15(C)) (Abu Shamat, 1993). The metabolic pathway involves step-wise denitration to produce isomeric mononitrates and isosorbide. In the same study a comparison with the rate of *in vitro* metabolism in human faecal microbiota was performed.

4.10. Amine formation and hydrolysis of an amide linkage: chloramphenicol

Chloramphenicol, an agent used in treating typhoid fever, rickettsial diseases, brucellosis, and anaerobic infections, contains a nitrobenzene group and an amide of dichloroacetic acid. When

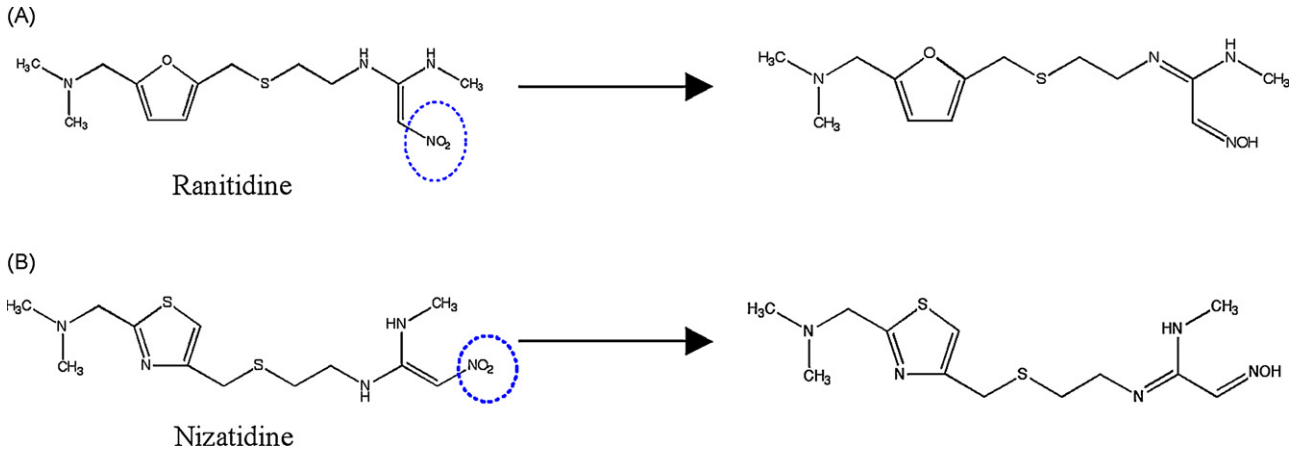

Fig. 13. Proposed reactions for the metabolism of ranitidine (A) and nizatidine (B).

incubated with bacteria commonly found in human faeces a number of reactions can be detected and these include the conversion of a nitro group to a primary aromatic amine (Fig. 16(A)) and hydrolysis of the amide linkage (Holt, 1967).

It has been suggested that the most serious complication of chloramphenicol, aplasia of the marrow, is due to the activity of the intestinal microbiota (Holt, 1967). This is supported by the fact that marrow aplasia has only been reported in patients taking chloramphenicol by the oral route. Furthermore, patients susceptible to this complication belong to the few percent of the population whose microbiota contains coliform organisms capable of metabolizing chloramphenicol to the critical metabolites.

4.11. Deconjugation: drugs excreted in bile as inactive conjugates

Bacteria have evolved to metabolise conjugated bile acids that have escaped absorption from the ileum and reach the colonic region (Narushima et al., 2006). Bile acids are synthesized in the liver from cholesterol and conjugated to either glycine or taurine before being secreted into the small intestine through the bile. Bacteria have a role in their deconjugation by removing the amino acid molecule on the carboxyl group (Thomas et al., 2001; Nagengast et al., 1995; Schiller, 2007). This activity takes place mainly in the caecum and ascending colon where deconjugated bile acids are then absorbed and enter the enterohepatic circulation.

The metabolism of the sex steroid hormones also involves an enterohepatic circulation that is dependent upon a biologically active microbiota. These hormones undergo a cycle of biliary excretion, mucosal and bacterial deconjugation and intestinal reabsorption. Approximately 60% of circulating estrogens are conjugated in the form of glucuronides or sulfates, and are excreted in the bile (Simon and Gorbach, 1984; Orme and Back, 1990). Deconjugation, the prerequisite step for mucosal cell reabsorption, is catalysed by enzymes produced by both the intestinal wall (glucuronidases) and bacteria (glucuronidases and sulfatases); their activity increases from the upper to the lower small intestine and colon (Winter and Bokkenheuser, 1987; Adlercreutz and Martin, 1980).

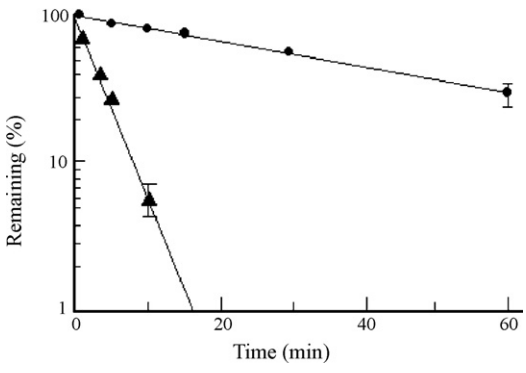

Fig. 14. Insulin and calcitonin degradation in rat caecal contents. Results are expressed as the mean ± S.D. of three experiments. (●) Insulin suspension; (▲) calcitonin suspension (reproduced with permission from Tozaki et al., 1997).

The microbiota also play a role in maintaining the enterohepatic circulation of drugs other than estrogens, which are excreted in the bile as inactive conjugates of sulfate or glucuronic acid. The removal of the polar conjugating group by the bacterial microbiota not only restores the active drug but also allows it to be reabsorbed and returned to its site of action. Thus, the microbiota may prolong the action of drugs by allowing their enterohepatic circulation to continue. Evidence from studies with digitoxin in the dog is consistent with an enterohepatic circulation of digitoxin, which depends on the hydrolysis of the glucuronide in the gastrointestinal tract (Peppercorn and Goldman, 1976).

The drug indomethacin is widely used as an anti-inflammatory agent. The studies performed by Smith (1978) suggested that indomethacin, although in man is rapidly eliminated in urine as its glucuronide, in dogs and monkeys is initially metabolised to its acyl-glucuronide, which is excreted in the bile. The intestinal microorganisms then free the drug from its glucuronide metabolite so that it may cycle back through the liver, forming metabolites.

Morphine also undergoes conjugation and excretion in the bile and thus its half-life could be influenced by the deconjugating activity of the microbiota (Peppercorn and Goldman, 1976).

4.12. Thiazole ring-opening: levamisole

Levamisole has been extensively used as an anthelmintic drug in veterinary and human medicine and recently some anti-colon cancer activity has been attributed to this compound (Shu et al., 1991).

Shu et al. (1991) showed that anaerobic incubation of levamisole with human intestinal microbiota resulted in the formation of three thiazole ring-opened metabolites, namely levametabol-I, II and III (Fig. 16(B)). The study demonstrated that strongest metabolisers

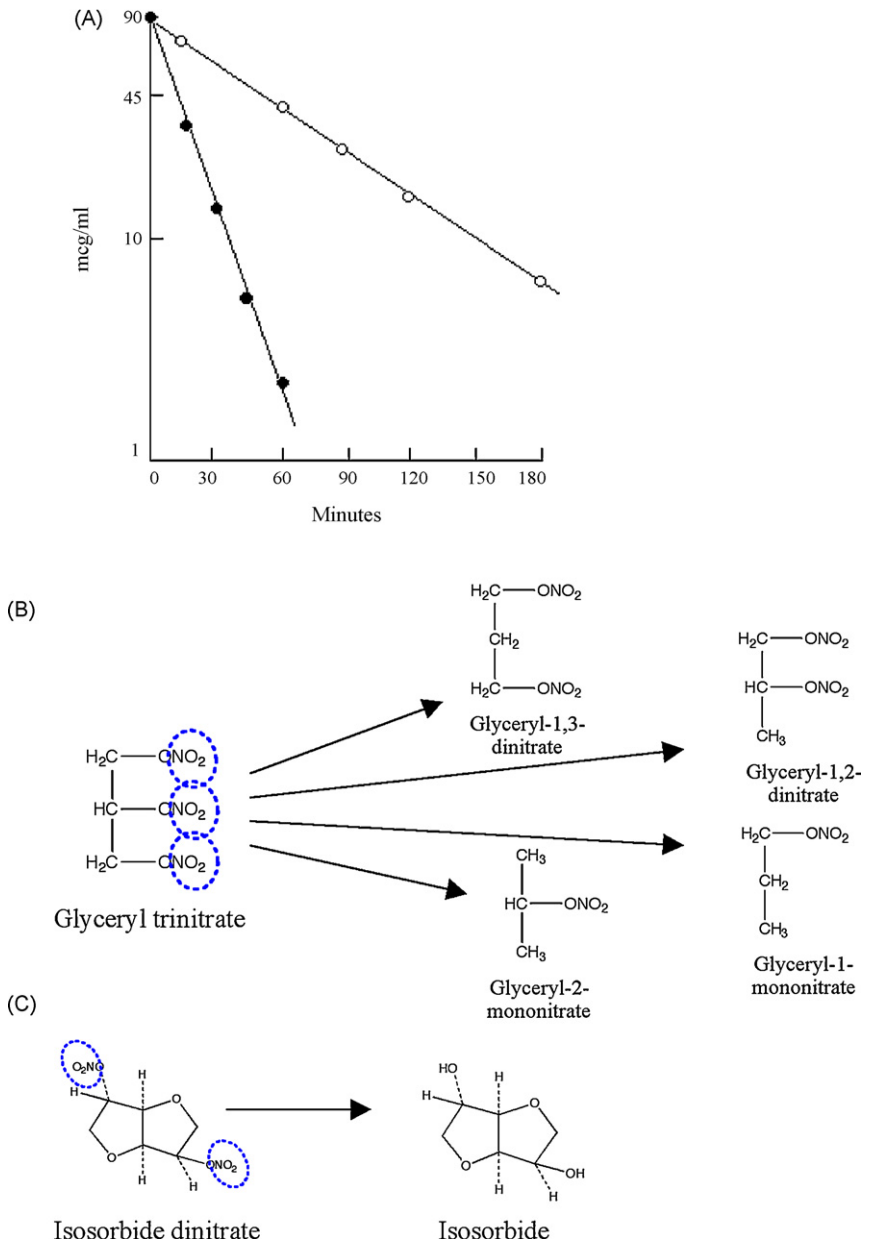

Fig. 15. Disappearance of glyceryl trinitrate (●) and isosorbide dinitrate (○) in rat caecal contents (A) (Reproduced with permission from Abu Shamat and Beckett, 1983); proposed reactions for the denitration of glyceryl trinitrate (B) and isosorbide dinitrate (C).

include *Bacteroides* and *Clostridium* spp. and it has been suggested that levametabol-I is responsible for anti-colon cancer activity (Shu et al., 1991).

4.13. Isoxazole scission: risperidone

Risperidone is an antipsychotic drug with very potent serotonin-5HT$_2$ and potent dopamine-D$_2$ antagonist properties. Meuldermans et al. (1994) studied the metabolism of risperidone and among major metabolic pathways was the scission of the isoxazole in the benzisoxazole ring system which appeared to be a effected primarily by the intestinal microbiota (Fig. 16(C)). In this study incubation of risperidone with intestinal contents from rats showed that the benzisoxazole of risperidone was easily cleaved in the presence of caecal and colonic contents to a metabolite with a phenol and a carbonyl function under aerobic as well as under anaerobic conditions. *In vivo* studies in rats contributed for this evidence by detecting bacterial metabolites in the rat's faecal excreta and not in the bile.

4.14. Deglycosylation: quercetin-3-glucoside

Quercetin is a flavonoid that is widely distributed in plants, usually linked to sugars such as glucose (quercetin-3-glucoside) or rutinose (rutin). Evidence suggests that quercetin has sparing effects on the cardiovascular system, possibly due to its antioxidative properties and inhibitory effects on cyclic phosphodiesterase and cyclooxygenase, which are involved in platelet aggregation (Schneider et al., 2000). Orally ingested quercetin and other flavonoids are partially degraded by bacteria in the human intestinal tract, resulting in the formation of 3,4-dihydroxyphenylacetic acid (Schneider et al., 2000). Studies using germ-free rats asso-

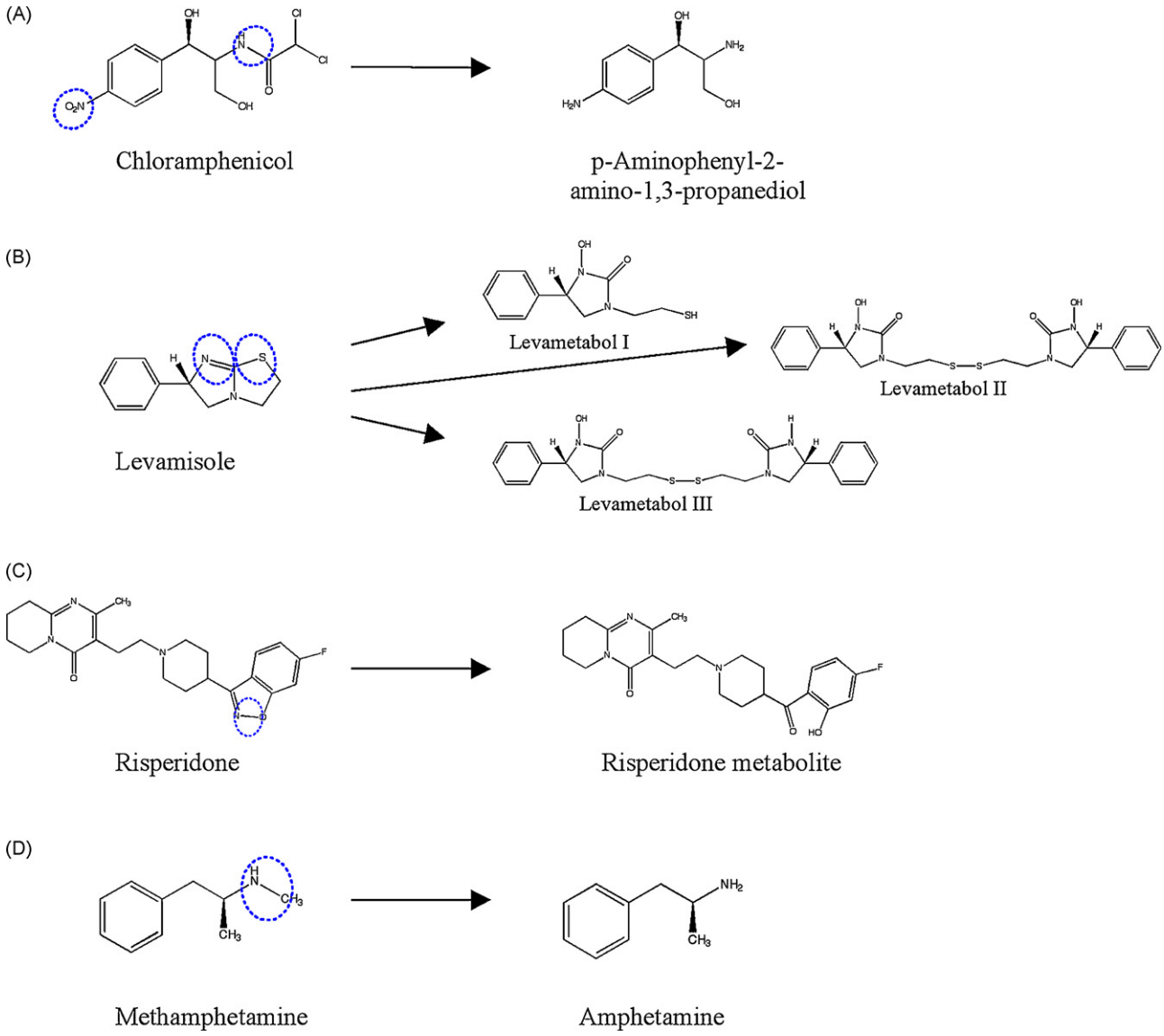

Fig. 16. Proposed reactions for the metabolism of chloramphenicol (A), levamisole (B), risperidone (C), N-demethylation of methamphetamine (D).

ciated with *Eubacterium ramulus* and *Enterococcus casseliflavus* showed that both species are capable of metabolizing quercetin-3-glucoside. Based on the wide occurrence of *Eu. Ramulus* in the human intestinal tract it is believed that this species plays a dominant role in bacterial transformation of flavonoids (Schneider et al., 2000).

4.15. N-demethylation: methamphetamine

Methamphetamine is a sympathomimetic drug liable to serious abuse. The detection of metabolites of this drug in the faeces raised the possibility that it was being metabolised by intestinal microbiota.

A study performed by Caldwell and Hawksworth (1973) showed that the intestinal contents of guinea-pigs possess the ability to biotransform methamphetamine by N-demethylation (Fig. 16(D)) and probably one other pathway. It was found that when methamphetamine is incubated with guinea-pig caecal contents, 47% is converted to amphetamine while a 76% conversion was observed following incubation with rectal contents from the same animal (Caldwell and Hawksworth, 1973). The differences seen in the extent of metabolism between caecal and rectal contents are probably a reflection of the differences in the amount of material from these two regions used in the incubations, rather than a fundamental difference in the activity of their microbiota. This transformation is probably of little consequence in man since methamphetamine is quite efficiently absorbed in the upper portion of the gut.

4.16. Other chemical reactions

4.16.1. Azetirelin

Azetirelin is a novel thyrotropin-releasing hormone (TRH) analogue which shows relative selectivity for action on the central nervous system (CNS) and less thyrotropin (TSH)-releasing activity than TRH (Sasaki et al., 1997).

Sasaki et al. (1997) evaluated the effect of luminal bacterial metabolism on intestinal absorption of azetirelin in antibiotic-treated rats and the results of *in vitro* incubation studies with rat luminal contents showed that azetirelin is metabolised by an anaerobic bacteria localised mainly in the large intestine. Faecal suspensions from rats, dogs, and humans showed comparable

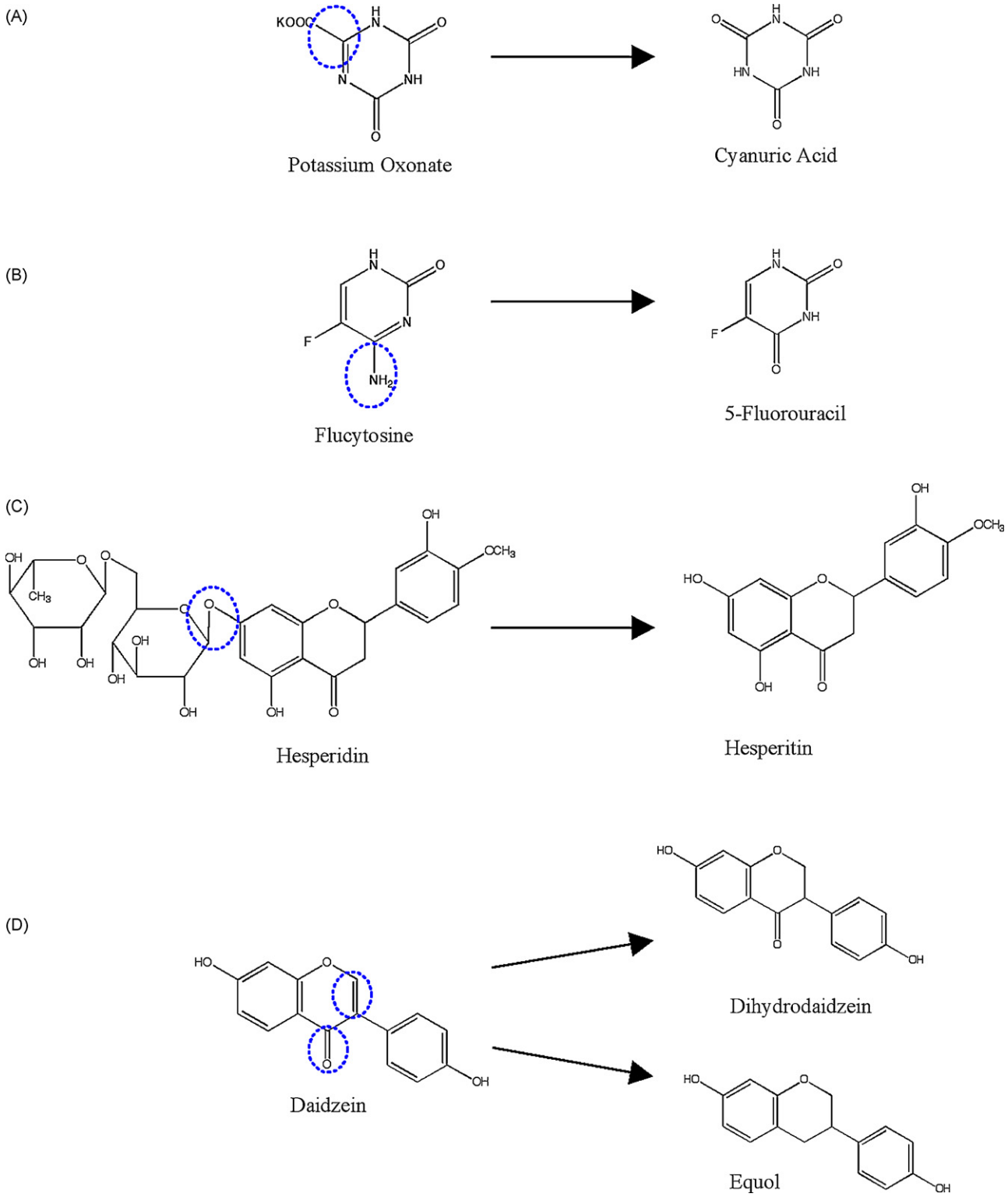

Fig. 17. Proposed reactions for the metabolism of potassium oxonate (A), flucytosine (B), hesperidin (C) and daidzein (D).

metabolic activity, azetirelin breakdown in the bacterial suspension was pH-dependent and was inhibited in the presence of bacitracin or puromycin (Sasaki et al., 1997).

4.16.2. Potassium oxonate

Potassium oxonate has recently been added to tegafur and to 5-chloro-2,4-dihydropyridine to form S-1, a new oral 5-fluorouracil (5-FU)-derivative antitumor agent, with the purpose of inhibiting the phosphorylation of 5-FU and reducing the gastrointestinal toxicity of the agent (Yoshisue et al., 2000). In a study on the biotransformation of potassium oxonate, Yoshisue et al. (2000) found that potassium oxonate converts to cyanuric acid and that one of the routes is the direct conversion to cyanuric acid by gut microbiota in the caecum (Fig. 17(A)).

4.16.3. Flucytosine

Flucytosine has anti-fungal properties and Vermes et al. (2003) investigated the rate of active conversion of flucytosine to 5-fluorouracil by microorganisms in the intestinal microbiota (Fig. 17(B)). In *in vitro* experiments, the human intestinal microbiota was capable of converting flucytosine to 5-fluorouracil (Harris et al., 1986). This metabolism was also strongly reduced in patients receiving antimicrobial agents (Vermes et al., 2003).

4.16.4. Hesperidin

Lee et al. (2004) studied the metabolism of hesperidin (a natural product with anti-allergy activity) by intestinal bacteria and incubated hesperidin with fresh human and rat faecal suspensions. During the incubation Lee et al. (2004) found that hesperidin was metabolised to hesperitin (Fig. 17(C)) via hesperetin-7-O-glucopyranoside. Hesperitin was also found to have anti-allergic activity so the authors suggested that hesperidin may be a prodrug, which is metabolised to hesperetin by intestinal bacteria.

4.16.5. Daidzein

Daidzein (4,7-dihydroxyisoflavone), a principal soy isoflavonoid, is a weakly estrogenic compound with potential health benefits for several conditions, including hypercholesterolemia and osteoporosis (Rafii et al., 2004).

The conversion of daidzein to metabolites by the intestinal microbiota is essential for absorption, bioavailability and estrogen activity of this compound. In a study performed by Rafii et al. (2004) the ability of faecal bacteria from rhesus monkeys to metabolize daidzein was documented; dihydrodaidzein and equol were the two principal metabolites produced (Fig. 17(D)).

5. Conclusions

The human intestinal microbiota can have a major impact on drug metabolism and ultimately on oral bioavailability. More than 30 drugs that made it onto the market were subsequently identified as substrates for colonic bacteria. However, these examples are merely the tip of the iceberg as the next generation of drugs have a higher probability of being presented to the microbiota in the lower gut, either through poor solubility/permeability properties or formulation development strategies. The numbers of potential substrates are expected to increase. Given the potentially serious implications of bacterial metabolism on drug performance or toxicity, an assessment of the action of the microbiota should form an integral part of the drug development process.

References

Abu Shamat, M., 1993. The role of the gastrointestinal microflora in the metabolism of drugs. Int. J. Pharm. 97, 1–13.

Abu Shamat, M.S., Beckett, A.H., 1983. Glyceryl trinitrate: metabolism by the intestinal flora. J. Pharm. Pharmacol. 35, 71P.

Adlercreutz, H., Martin, F., 1980. Biliary excretion and intestinal metabolism of progesterone and estrogens in man. J. Steroid Biochem. 13, 231–244.

Allison, C., McFarlan, C., MacFarlane, G.T., 1989. Studies on mixed populations of human intestinal bacteria grown in single-stage and multistage continuous culture systems. Appl. Environ. Microbiol. 55, 672–678.

Amidon, G.L., Lennernäs, H., Shah, V.P., Crison, J.R., 1995. A theoretical basis for a biopharmaceutics drug classification: The correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm. Res. 12, 413–420.

Ashida, N., Ijichi, K., Watanabe, Y., Machida, H., 1993. Metabolism of 5′-ether prodrugs of 1-β-d-arabinofuranosyl-e-5(2-bromovinyl)uracil in rats. Biochem. Pharmacol. 46, 2201–2207.

Bachrach, W.H., 1988. Sulfasalazine: I. An historical perspective. Am. J. Gastroenterol. 83, 487–496.

Basit, A.W., 2005. Advances in colonic drug delivery. Drugs 65, 1991–2007.

Basit, A.W., Lacey, L.F., 2001. Colonic metabolism of ranitidine: implications for its delivery and absorption. Int. J. Pharm. 227, 157–165.

Basit, A.W., Newton, J.M., Lacey, L.F., 2002. Susceptibility of the H_2-receptor antagonists cimetidine, famotidine and nizatidine, to metabolism by the gastrointestinal microflora. Int. J. Pharm. 237, 23–33.

Basit, A.W., Podczeck, F., Newton, J.M., Waddington, W.A., Ell, P.J., Lacey, L.F., 2004. The use of formulation technology to assess regional gastrointestinal drug absorption in humans. Eur. J. Pharm. Sci. 21, 179–189.

Burkitt, D.P., Walker, A.R., Painter, N.S., 1972. Effect of dietary fibre on stools and the transit-times, and its role in the causation of disease. Lancet 2, 1408–1412.

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

Celesk, R.A., Asano, T., Wagner, M., 1976. The size, pH and redox potential of the cecum in mice associated with various microbial floras. Proc. Soc. Exp. Biol. Med. 151, 260–263.

Chan, R.P., Pope, D.J., Gilbert, A.P., Sacra, P.J., Baron, J.H., Lennard-Jones, J.E., 1983. Studies of two novel sulfasalazine analogs, ipsalazide and balsalazide. Dig. Dis. Sci. 28, 609–615.

Cinquin, C., Le Blay, G., Fliss, I., Lacroix, C., 2004. Immobilization of infant fecal microbiota and utilization in an in vitro colonic fermentation model. Microb. Ecol. 48, 128–138.

Coates, M.E., Drasar, B.S., Mallett, A.K., Rowland, I.R., 1988. Methodological considerations for the study of bacterial metabolism. In: Rowland, I.R. (Ed.), Role of the Gut Flora in Toxicity and Cancer. Academic Press, London.

Cummings, J.H., Antoine, J.-M., Azpiroz, F., Bourdet-Sicard, R., Brandtzaeg, P., Calder, P.C., Gibson, G.R., Guarner, F., Isolauri, E., Pannemans, D., Shortt, C., Tuijtelaars, S., Watzl, B., 2004. Passclaim—gut health and immunity. Eur. J. Nutr. 43, 118–173.

Cummings, J.H., Bingham, S.A., Heaton, W., Eastwood, M.A., 1992. Fecal weight, colon cancer risk, and dietary intake of nonstarch polysaccharides (dietary fiber). Gastroenterology 103, 1783–1789.

Cummings, J.H., Hill, M.J., Bone, E.S., Branch, W.J., Jenkins, D.J., 1979. The effect of meat protein and dietary fiber on colonic function and metabolism. II. Bacterial metabolites in feces and urine. Am. J. Clin. Nutr. 32, 2094–2101.

Cummings, J.H., Macfarlane, G.T., 1997. Role of intestinal bacteria in nutrient metabolism. JPEN J. Parenter. Enteral Nutr. 21, 357–365.

Cummings, J.H., Macfarlane, G.T., Drasar, B.S., 1989. The gut microflora and its significance. In: Whitehead, R. (Ed.), Gastrointestinal and Oesophageal Pathology. Churchill Livingstone, London.

Davis, S.S., 2005. Formulation strategies for absorption windows. Drug Discov. Today 10, 249–257.

De Boever, P., Deplancke, B., Verstraete, W., 2000. Fermentation by gut microbiota cultured in a simulator of the human intestinal microbial ecosystem is improved by supplementing a soygerm powder. J. Nutr. 130, 2599–2606.

Deloménie, C., Fouix, S., Longuemaux, S., Brahimi, N., Bizet, C., Picard, B., Denamur, E., Dupret, J.-M., 2001. Identification and functional characterization of arylamine *n*-acetyltransferases in eubacteria: evidence for highly selective acetylation of 5-aminosalicylic acid. J. Bacteriol. 183, 3417–3427.

Drasar, B.S., Barrow, P.A., 1985. The bacterial flora of the normal intestine. In: Barrow, P.A. (Ed.), Intestinal Microbiology. Van Nostrand Reinhold, Wokingham.

Drasar, B.S., Hill, M.J., Williams, R.E.O., 1970. The significance of the gut flora in safety testing of food additives. In: Roe, F.J.C. (Ed.), Metabolic Aspects of Food Safety. Academic, New York.

Dressman, J.B., Amidon, G.L., Reppas, C., Shah, V.P., 1998. Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms. Pharm. Res. 15, 11–22.

Dull, B.J., Salata, K., Goldman, P., 1987. Role of the intestinal flora in the acetylation of sulphasalazine metabolites. Biochem. Pharmacol. 36, 3772–3774.

Eckburg, P.B., Bik, E.M., Bernstein, C.N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S.R., Nelson, K.E., Relman, D.A., 2005. Diversity of the human intestinal microbial flora. Science 308, 1635–1638.

Edwards, C.A., Parrett, A.M., 1999. Colonic fermentation—in vitro and in vivo approaches to measurement. Sci. Aliment. 19, 291–300.

Egert, M., de Graaf, A.A., Smidt, H., de Vos, W.M., Venema, K., 2006. Beyond diversity: functional microbiomics of the human colon. Trends Microbiol. 14, 86–91.

Elkington, S.G., Floch, M.H., Conn, H.O., 1969. Lactulose in the treatment of chronic portal-systemic encephalopathy. N. Engl. J. Med. 281, 408–412.

Elmer, G.W., Remmel, R.P., 1984. Role of intestinal microflora in clonazepam metabolism in the rat. Xenobiotica 14, 829–840.

Escherich, T., 1885. Die darmbakterien des neugeborenen und sauglings. Fortschr. Med. 3, 447–547.

Evans, D.F., Pye, G., Bramley, R., Clark, A.G., Dyson, T.J., Hardcastle, J.D., 1988. Measurement of gastrointestinal ph profiles in normal ambulant human subjects. Gut 29, 1035–1041.

Fanaro, S., Chierici, R., Guerrini, P., Vigi, V., 2003. Intestinal microflora in early infancy: composition and development. Acta Paediatr. 92, 48–55.

Finegold, S.M., Sutter, V.L., Mathisen, G.E., 1983. Normal and indigenous flora. In: Hentges, D.J. (Ed.), Human Intestinal Microflora in Health and Disease. Academic Press, New York.

Fouts, J.R., Kamm, J.J., Brodie, B.B., 1957. Enzymatic reduction of prontosil and other azo dyes. J. Pharmacol. Exp. Ther. 120, 291–300.

Fuller, A.T., 1937. Is *p*-aminobenzenesulphonamide the active agent in prontosil therapy? Lancet 1, 194–198.

Gibson, G.R., Cummings, J.H., Macfarlane, G.T., 1988. Use of a three-stage continuous culture system to study the effect of mucin on dissimilatory sulfate reduction and methanogenesis by mixed populations of human gut bacteria. Appl. Environ. Microbiol. 54, 2750–2755.

Gibson, G.R., Fuller, R., 2000. Aspects of in vitro and in vivo research approaches directed toward identifying probiotics and prebiotics for human use. J. Nutr. 130, 391S–395S.
Gibson, G.R., Wang, X., 1994. Regulatory effects of bifidobacteria on the growth of other colonic bacteria. J. Appl. Bacteriol. 77, 412–420.
Gingell, R., Bridges, J.W., Williams, R.T., 1971. The role of the gut flora in the metabolism of prontosil and neoprontosil in the rat. Xenobiotica 1, 143–156.
Goldin, B.R., Peppercorn, M.A., Goldman, P., 1973. Contributions of host and intestinal microflora in the metabolism of L-dopa by the rat. J. Pharmacol. Exp. Ther. 186, 160.
Goldman, P., 1984. Gnotobiotic rats in studies of drug metabolism. In: Coates, M.E., Gustafsson, B.E. (Eds.), The Germ-free Animal in Biomedical Research. Laboratory Animals Ltd., London.
Gorbach, S.L., Nahas, L., Weinstein, L., Levitan, R., Patterson, J.F., 1967. Studies of intestinal microflora. IV. The microflora of ileostomy effluent: a unique microbial ecology. Gastroenterology 53, 874–880.
Harris, B.E., Manning, B.W., Federle, T.W., Diasio, R.B., 1986. Conversion of 5-fluorocytosine to 5-fluorouracil by human intestinal microflora. Antimicrob. Agents Chemother. 29, 44–48.
Hayllar, J., Bjarnason, I., 1991. Sulphasalazine in ulcerative colitis: in memoriam? Gut 32, 462–463.
Heneghan, J.B., 1984. Physiology of the alimentary tract. In: Coates, M.E., Gustafsson, B.E. (Eds.), The Germ-free Animal in Biomedical Research. Laboratory Animals Ltd., London.
Hill, M.J., 1997. Intestinal flora and endogenous vitamin synthesis. Eur. J. Cancer Prev. 6, S43–S45.
Holt, R., 1967. The bacterial degradation of chloramphenicol. Lancet 1, 1259.
Ibekwe, V.C., Liu, F., Fadda, H.M., Khela, M.K., Evans, D.F., Parsons, G.E., Basit, A.W., 2006. An investigation into the in vivo performance variability of pH responsive polymers for ileo-colonic drug delivery using gamma scintigraphy in humans. J. Pharm. Sci. 95, 2760–2766.
Ibekwe, V.C., Fadda, H.M., McConnell, E.L., Khela, M.K., Evans, D.F., Basit, A.W., 2008. Interplay between intestinal pH, transit time and feed status on the in vivo performance of ph responsive ileo-colonic release systems. Pharm. Res. 25, 1828–1835.
Ilett, K.F., Tee, L.B.G., Reeves, P.T., Minchin, R.F., 1990. Metabolism of drugs and other xenobiotics in the gut lumen and wall. Pharmacol. Ther. 46, 67–93.
Khoshini, R., Dai, S.-C., Lezcano, S., Pimentel, M., 2007. A systematic review of diagnostic tests for small intestinal bacterial overgrowth. Dig. Dis. Sci. (November) (online version).
Kitamura, S., Sugihara, K., Kuwasako, M., Tatsumi, K., 1997. The role of mammalian intestinal bacteria in the reductive metabolism of zonisamide. J. Pharm. Pharmacol. 49, 253–256.
Koch, R.L., Beaulieu, B.B., Goldman, P., 1980. Role of the intestinal flora in the metabolism of misonidazole. Biochem. Pharmacol. 29, 3281–3284.
Koch, R.L., Beaulieu Jr., B.B., Chrystal, E.J.T., Goldman, P., 1981. A metronidazole metabolite in human urine and its risk. Science 211, 398–400.
Koch, R.L., Chrystal, E.J.T., Beaulieu Jr., B.B., Goldman, P., 1979. Acetamide—a metabolite of metronidazole formed by the intestinal flora. Biochem. Pharmacol. 28, 3611–3615.
Koch, R.L., Goldman, P., 1979. The anaerobic metabolism of metronidazole forms *n*-(2-hydroxyethyl)-oxamic acid. J. Pharmacol. Exp. Ther. 208, 406–410.
Lee, N.K., Choi, S.H., Park, S.H., Park, E.K., Kim, D.H., 2004. Antiallergic activity of hesperidin is activated by intestinal microflora. Pharmacology 71, 174–180.
Lennernäs, H., Abrahamsson, B., 2005. The use of biopharmaceutic classification of drugs in drug discovery and development: current status and future extension. J. Pharm. Pharmacol. 57, 273–285.
Ley, R.E., Peterson, D.A., Gordon, J.I., 2006. Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell 124, 837–848.
Lindahl, A., Ungell, A.-L., Knutson, L., Lennernas, H., 1997. Characterization of fluids from the stomach and proximal jejunum in men and women. Pharm. Res. 14, 497–502.
Lindenbaum, J., Rund, D.G., Butler Jr., V.P., Tse-Eng, D., Saha, J.R., 1981. Inactivation of digoxin by the gut flora: reversal by antibiotic therapy. N. Engl. J. Med. 305, 789–794.
Macfarlane, G.T., Cummings, J.H., Macfarlane, S., Gibson, G.R., 1989. Influence of retention time on degradation of pancreatic enzymes by human colonic bacteria grown in a 3-stage continuous culture system. J. Appl. Bacteriol. 67, 520–527.
Macfarlane, G.T., Macfarlane, S., 1997. Human colonic microbiota: ecology, physiology and metabolic potential of intestinal bacteria. Scand. J. Gastroenterol. 222 (Suppl.), 3–9.
Macfarlane, G.T., Macfarlane, S., Gibson, G.R., 1998. Validation of a three-stage compound continuous culture system for investigating the effect of retention time on the ecology and metabolism of bacteria in the human colon. Microb. Ecol. 35, 180–187.
Macfarlane, S., Macfarlane, G.T., 2004. Bacterial diversity in the human gut. Adv. Appl. Microbiol. 54, 261–289.
Magnusson, J.O., Bergdahl, B., Bogentoft, C., Johnsson, U.E., 1982. Metabolism of digoxin and absorption site. Br. J. Clin. Pharmacol. 14, 284–285.
Manning, B.W., Campbell, W.L., Franklin, W., Delclos, K.B., Cerniglia, C.E., 1988. Metabolism of 6-nitrochrysene by intestinal microflora. Appl. Environ. Microbiol. 54, 197–203.
Marsh, P.D., 1995. The role of continuous culture in modelling the human microflora. J. Chem. Technol. Biotechnol. 64, 1–9.
McBain, A.J., Macfarlane, G.T., 1998. Ecological and physiological studies on large intestinal bacteria in relation to production of hydrolytic and reductive enzymes involved in formation of genotoxic metabolites. J. Med. Microbiol. 47, 407–416.
McConnell, E.L., Fadda, H.M., Basit, A.W., 2008a Gut instincts: explorations in intestinal physiology and drug delivery. Int. J. Pharm., doi:10.1016/j.ijpharm.2008.05.012.
McConnell, E.L., Tutas, J., Mohamed, M.A.M., Banning, D., Basit, A.W., 2007. Colonic drug delivery using amylose films: the role of aqueous ethylcellulose dispersions in controlling drug release. Cellulose 14, 25–34.
McConnell, E.L., Short, M.D., Basit, A.W., 2008b. An in vivo comparison of intestinal pH and bacteria as physiological trigger mechanisms for colonic targeting in man. J. Control Release 130, 154–160.
McConnell, E.L., Basit, A.W., Murdan, S., 2008c. Measurements of rat and mouse gastrointestinal pH, fluid and lymphoid tissue, and implications for in-vivo experiments. J. Pharm. Pharmacol. 60, 63–70.
Meuldermans, W., Hendrickx, J., Mannens, G., Lavrijsen, K., Janssen, C., Bracke, J., Le Jeune, L., Lauwers, W., Heykants, J., 1994. The metabolism and excretion of risperidone after oral administration in rats and dogs. Drug Metab. Dispos. 22, 129–138.
Mikov, M., 1994. The metabolism of drugs by the gut flora. Eur. J. Drug Metab. Pharmacokinet. 19, 201–207.
Minekus, M., Smeets-Peeters, M., Bernalier, A., Marol-Bonnin, S., Havenaar, R., Marteau, P., Alric, M., Fonty, G., Huis in't Veld, J.H.J., 1999. A computer-controlled system to simulate conditions of the large intestine with peristaltic mixing, water absorption and absorption of fermentation products. Appl. Microbiol. Biotechnol. 53, 108–114.
Molly, K., Vande Woestyne, M., De Smet, I., Verstraete, W., 1994. Validation of the simulator of the human intestinal microbial ecosystem (shime) reactor using microorganism-associated activities. Microb. Ecol. Health Dis. 7, 191–200.
Molly, K., Vande Woestyne, M., Verstraete, W., 1993. Development of a 5-step multi-chamber reactor as a simulation of the human intestinal microbial ecosystem. Appl. Microbiol. Biotechnol. 39, 254–258.
Nagengast, F.M., Grubben, M.J., van Munster, I.P., 1995. Role of bile acids in colorectal carcinogenesis. Eur. J. Cancer 31A, 1067–1070.
Nakayama, H., Kinouchi, T., Kataoka, K., Akimoto, S., Matsuda, Y., Ohnishi, Y., 1997. Intestinal anaerobic bacteria hydrolyse sorivudine, producing the high blood concentration of 5-(e)-(2-bromovinyl)uracil that increases the level and toxicity of 5-fluorouracil. Pharmacogenetics 7, 35–43.
Narushima, S., Itoha, K., Miyamoto, Y., Park, S.H., Nagata, K., Kuruma, K., Uchida, K., 2006. Deoxycholic acid formation in gnotobiotic mice associated with human intestinal bacteria. Lipids 41, 835–843.
Nicholson, J.K., Wilson, I.D., 2005. Gut microorganisms, mammalian metabolism and personalized health care. Nat. Rev. Microbiol. 3, 431–438.
Oktyabrsky, O.N., Smirnova, G.V., 1989. Dynamics of redox potential in bacterial cultures growing on media containing different sources of carbon, energy and nitrogen. Acta Biotechnol. 9, 203–209.
Okuda, H., Ogura, K., Kato, A., Takubo, H., Watabe, T., 1998. A possible mechanism of eighteen patient deaths caused by interactions of sorivudine, a new antiviral drug, with oral 5-fluorouracil prodrugs. J. Pharmacol. Exp. Ther. 287, 791–799.
Orme, M.L.E., Back, D.J., 1990. Factors affecting the enterohepatic circulation of oral contraceptive steroids. Am. J. Obstet. Gynecol. 163, 2146–2152.
Peppercorn, M.A., Goldman, P., 1972. The role of intestinal bacteria in the metabolism of salicylazosulfapyridine. J. Pharmacol. Exp. Ther. 181, 555–562.
Peppercorn, M.A., Goldman, P., 1973. Distribution studies of salicylazosulfapyridine and its metabolites. Gastroenterology 64, 240–245.
Peppercorn, M.A., Goldman, P., 1976. Drug–bacteria interactions. Rev. Drug Inter. II, 75–88.
Possemiers, S., Verthe, K., Uyttendaele, S., Verstraete, W., 2004. Pcr-dgge-based quantification of stability of the microbial community in a simulator of the human intestinal microbial ecosystem. FEMS Microbiol. Ecol. 49, 495–507.
Poth, E.J., Knotts, F.L., Lee, J.T., Inui, F., 1942. Bacteriostatic properties of sulfanilamide and some of its derivatives. Arch. Surg. 44, 187–207.
Priebe, M.G., Vonk, R.J., Sun, X., He, T., Harmsen, H.J.M., Welling, G.W., 2002. The physiology of colonic metabolism. Possibilities for interventions with pre- and probiotics. Eur. J. Nutr. 41, 102–110.
Rafii, F., Hotchkiss, C., Heinze, T.M., Park, M., 2004. Metabolism of daidzein by intestinal bacteria from rhesus monkeys (macaca mulatta). Comparat. Med. 54, 165–169.
Rafii, F., Sutherland, J.B., Hansen, E.B., Cerniglia, C.E., 1997. Reduction of nitrazepam by clostridium leptum, a nitroreductase-producing bacterium isolated from the human intestinal tract. Clin. Infect. Dis. 25, S121–S122.
Rajilic-Stojanovic, M., Smidt, H., de Vos, W.M., 2007. Diversity of the human gastrointestinal tract microbiota revisited. Environ. Microbiol. 9, 2125–2136.
Rowland, I.R., Mallett, A.K., Bearne, C.A., Farthing, M.J.G., 1986. Enzyme activities of the hindgut microflora of laboratory animals and man. Xenobiotica 16, 519–523.
Rubinstein, A., 2005. Colonic drug delivery. Drug Discov. Today: Technol. 2, 33–37.
Rumney, C.J., Rowland, I.R., 1992. In vivo and in vitro models of the human colonic flora. Crit. Rev. Food Sci. Nutr. 31, 299–331.
Sasaki, I., Tamura, T., Shibakawa, T., Fujita, T., Murakami, M., Yamamoto, A., Muranishi, S., 1997. Metabolism of azetirelin, a new thyrotropin-releasing hormone (trh) analogue, by intestinal microorganisms. Pharm. Res. 14, 1004–1007.
Savage, D.C., 2001. Microbial biota of the human intestine: A tribute to some pioneering scientists. Curr. Issues Intest. Microbiol. 2, 1–15.
Scheline, R.R., 1973. Metabolism of foreign compounds by gastrointestinal microorganisms. Pharmacol. Rev. 25, 451–523.

Schiller, L.R., 2007. Evaluation of small bowel bacterial overgrowth. Curr. Gastroenterol. Rep. 9, 373–377.
Schneider, H., Simmering, R., Hartmann, L., Pforte, H., Blaut, M., 2000. Degradation of quercetin-3-glucoside in gnotobiotic rats, associated with human intestinal bacteria. J. Appl. Microbiol. 89, 1027–1037.
Schröder, H., Campbell, D.E.S., 1972. Absorption, metabolism, and excretion of salicylazosulfapyridine in man. Clin. Pharmacol. Ther. 13, 539–551.
Shu, Y.Z., Kingston, D.G.I., Van Tassell, R.L., Wilkins, T.D., 1991. Metabolism of levamisole, an anti-colon cancer drug, by human intestinal bacteria. Xenobiotica 21, 737–750.
Siew, L.F., Man, S.M., Newton, J.M., Basit, A.W., 2004. Amylose formulations for drug delivery to the colon: a comparison of two fermentation models to assess colonic targeting performance in vitro. Int. J. Pharm. 273, 129–134.
Simon, G.L., Gorbach, S.L., 1984. Intestinal flora in health and disease. Gastroenterology 86, 174–193.
Sirotek, K., Slovákova, L., Kopečný, J., Marounek, M., 2004. Fermentation of pectin and glucose, and activity of pectin-degrading enzymes in the rabbit caecal bacterium bacteroides caccae. Lett. Appl. Microbiol. 38, 327–332.
Smith, G.E., Griffiths, L.A., 1974. Metabolism of *n*-acetylated and o-alkylated drugs by the intestinal microflora during anaerobic incubation in vitro. Xenobiotica 4, 477–487.
Smith, H.W., 1965. Observations on the flora of the alimentary tract of animals and factorsaffecting its composition. J. Pathol. Bacteriol. 89, 95–122.
Smith, R.V., 1978. Metabolism of drugs and other foreign compounds by intestinal microorganisms. World Rev. Nutr. Diet. 29, 60–76.
Stirrup, V., Ledingham, S.J., Thomas, M., Pye, G., Evans, D.F., 1990. Redox potential measurement in the gastrointestinal tract in man. Gut 31, A1171.
Strong, H.A., Renwick, A.G., George, C.F., Liu, Y.F., Hill, M.J., 1987. The reduction of sulphinpyrazone and sulindac by intestinal bacteria. Xenobiotica 17, 685–696.
Svartz, N., 1988. Sulfasalazine: II. Some notes on the discovery and development of salazopyrin. Am. J. Gastroenterol. 83, 497–503.
Takeno, S., Hirano, Y., Kitamura, A., Sakai, T., 1993. Comparative development toxicity and metabolism of nitrazepam in rats and mice. Toxicol. Appl. Pharmacol. 121, 233–238.
Takeno, S., Sakai, T., 1990. The role of gut flora metabolism in nitrazepam-induced teratogenicity in rats. Eur. J. Pharmacol. 183, 2439–2440.
Tannock, G.W., 1999. Analysis of the intestinal microflora: a renaissance. Antonie Van Leeuwenhoek 76, 265–278.
Thomas, L.A., Veysey, M.J., French, G., Hylemon, P.B., Dowling, R.H., 2001. Bile acid metabolism by fresh human colonic contents: a comparison of caecal versus faecal samples. Gut 49, 835–842.
Tiwari, S.B., Rajabi-Siahboomi, A.R., 2008. Extended-release oral drug delivery technologies: monolithic matrix systems. Methods Mol. Biol. 437, 217–243.
Tozaki, H., Emi, Y., Horisaka, E., Fujita, T., Yamamoto, A., Muranishi, S., 1995. Metabolism of peptide drugs by the microorganisms in rat cecal contents. Biol. Pharm. Bull. 18, 929–931.
Tozaki, H., Emi, Y., Horisaka, E., Fujita, T., Yamamoto, A., Muranishi, S., 1997. Degradation of insulin and calcitonin and their protection by various protease inhibitors in rat caecal contents: implications in peptide delivery to the colon. J. Pharm. Pharmacol. 49, 164–168.
Tuleu, C., Basit, A.W., Waddington, W.A., Ell, P.J., Newton, J.M., 2002. Colonic delivery of 4-aminosalicylic acid using amylose-ethylcellulose-coated hydroxypropylmethylcellulose capsules. Aliment. Pharmacol. Ther. 16, 1771–1779.
Van Hogezand, R.A., Kennis, H.M., Van Schaik, A., Koopman, J.P., van Hees, P.A.M., 1992. Bacterial acetylation of 5-aminosalicylic acid in faecal suspensions cultured under aerobic and anaerobic conditions. Eur. J. Clin. Pharmacol. 43, 189–192.
Varum, F.J., McConnell, E.L., Sousa, J.J., Veiga, F., Basit, A.W., 2008. Mucoadhesion and the gastrointestinal tract. Crit. Rev. Ther. Drug Carrier Syst. 25, 207–258.
Vaughan, E.E., Schut, F., Heilig, H.G., Zoetendal, E.G., de Vos, W.M., Akkermans, A.D., 2000. A molecular view of the intestinal ecosystem. Curr. Issues Intest. Microbiol. 1, 1–12.
Vermes, A., Kuijper, E.J., Guchelaar, H.J., Dankert, J., 2003. An in vitro study on the active conversion of flucytosine to fluorouracil by microorganisms in the human intestinal microflora. Chemotherapy 49, 17–23.
Wadworth, A.N., Fitton, A., 1991. Olsalazine—a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in inflammatory bowel disease. Drugs 41, 647–664.
Wang, M., Ahrne, S., Jeppsson, B., Molin, G., 2005. Comparison of bacterial diversity along the human intestinal tract by direct cloning and sequencing of 16S rRNA genes. FEMS Microbiol. Ecol. 54, 219–231.
Watanabe, K., Yamashita, S., Furuno, K., Kawasaki, H., Gomita, Y., 1995. Metabolism of omeprazole by gut flora in rats. J. Pharm. Sci. 84, 516–517.
Whitman, W.B., Coleman, D.C., Wiebe, W.J., 1998. Prokaryotes: the unseen majority. Proc. Natl. Acad. Sci. U.S.A. 95, 6578–6583.
Wilding, I.R., 2001. The enterion capsule: a novel technology for undersanding the biopharmaceutical complexicity of new molecular entities (NMEs). Drug Del. Technol. 1, 8–11.
Williams, B.A., Bosch, M.W., Awati, A., Konstantinov, S.R., Smidt, H., Akkermans, A.D.L., Verstegen, M.W.A., Tamminga, S., 2005. In vitro assessment of gastrointestinal tract (git) fermentation in pigs: fermentable substrates and microbial activity. Anim. Res. 54, 191–201.
Wilson, P.J., Basit, A.W., 2005. Exploiting gastrointestinal bacteria to target drugs to the colon: an in vitro study using amylose coated tablets. Int. J. Pharm. 300, 89–94.
Winter, J., Bokkenheuser, V.D., 1987. Bacterial metabolism of natural and synthetic sex hormones undergoing enterohepatic circulation. J. Steroid Biochem. 27, 1149–1154.
Yang, L., 2008. Biorelevant dissolution testing of colon-specific delivery systems activated by colonic microflora. J. Control Release 125, 77–86.
Yoshisue, K., Masuda, H., Matsushima, E., Ikeda, K., Nagayama, S., Kawaguchi, Y., 2000. Tissue distribution and biotransformation of potassium oxonate after oral administration of a novel antitumor agent (drug combination of tegafur, 5-chloro-2,4-dihydroxypyridine, and potassium oxonate) to rats. Drug Metab. Dispos. 28, 1162–1167.