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Is the Role of the Small Intestine in First-Pass Metabolism Overemphasized?

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I. Introduction

The primary function of the small intestine is to absorb nutrients and water. This is achieved by mixing food with digestive enzymes to increase the contact of foodstuffs with the absorptive cells of the mucosa. In humans, the small intestine is about 5 to 6 m in length and comprises approximately 1% of body weight (ca. 0.7 kg for adults), which is significantly smaller than the liver (ca. 1.5 kg for adults). Approximately 6 to 12 liters of partially digested foodstuffs, water, and secretions are delivered daily to the small intestine. Of this, only 10 to 20% are passed on to the colon, because most nutrients, electrolytes, and water are absorbed as they are transported through the small intestine. Absorption and movement of the contents are brought about by the activities of the absorptive cells of the mucosa and by coordinated contraction of the smooth muscle cells of the muscularis extern (Weisbradt, 1987; Guyton, 1991). In addition to this fundamental role, a secondary function of the small intestine arises from the fact that it is also a major route of entry into the body for many xenobiotics including drugs.

Although the small intestine is regarded as an absorptive organ in the uptake of orally administered drugs, it also has the ability to metabolize drugs by numerous pathways involving both phase 1 and phase 2 reactions (Caldwell and Marsh, 1982; Renwick and George, 1989; Ilett, 1990; Ilett et al., 1990; Krishna and Klotz, 1994). Anatomically, the small intestine has a serial relationship with the liver relative to the absorption and is the anterior organ. The amount of an orally administered drug that reaches the systemic circulation can be reduced by both intestinal and hepatic metabolism. The metabolism of drugs before entering the systemic circulation is referred to as first-pass metabolism. It has been

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widely believed that the liver is the major site of such first-pass metabolism because of its size and its high content of drug-metabolizing enzymes.

Recent clinical studies, however, have indicated that the small intestine contributes substantially to the overall first-pass metabolism of cyclosporine, nifedipine, midazolam, verapamil, and certain other drugs (Hebert et al., 1992; Wu et al., 1995; Paine et al., 1996; Holtbecker et al., 1996; Fromm et al., 1996). Some studies have even suggested that the role of intestinal metabolism is quantitatively greater than that of hepatic metabolism in the overall first-pass effect (Wu et al., 1995; Holtbecker et al., 1996; Fromm et al., 1996). Much of the evidence for such claims has derived indirectly from comparisons of areas under the plasma concentration curves (AUCs)[2] after i.v. and oral administration, with assumptions that have not yet been tested. In fact, estimates of intestinal metabolism calculated by indirect methods often contradicted those determined from direct measurements. For example, nifedipine, a well absorbed drug, is subject to substantial first-pass metabolism which results in an oral bioavailability of about 30 to 50%. Using an indirect method, Holtbecker et al. (1996) concluded that the contribution of intestinal metabolism was quantitatively as important as that of hepatic metabolism to the overall first-pass metabolism of nifedipine in humans. However, Breimer and his coworkers (Kleinbloesem et al., 1986) have demonstrated that the intestinal metabolism of nifedipine in patients with a portalcaval shunt was absent, because the bioavailability of nifedipine in these patients was complete (100%). In these patients, the portal blood circulation bypassed the liver. Similarly, inconsistencies were noted between the direct and indirect estimation of intestinal metabolism for verapamil in humans (Eichelbaum et al., 1980; Fromm et al., 1996). These findings, therefore, raise the question as to whether intestinal metabolism truly plays such an important role in the first-pass effect, or whether the role of intestinal metabolism is overemphasized (Lin et al., 1997).

The purpose of this review was to examine carefully the physiological, biochemical, and pharmacokinetic factors that influence the extent of intestinal metabolism, with an attempt to address its true importance in first-pass metabolism.

II. Physiological and Biochemical Factors Affecting Intestinal Metabolism

A. Anatomy and Circulation of the Small Intestine

The small intestine is divided arbitrarily into three parts: duodenum, jejunum, and ileum. These regions are not anatomically distinct, although there are differences in their absorptive and secretory capabilities. In humans, the duodenum is the shortest, widest, and least mobile section. It measures 20 to 30 cm in length and 3 to 5 cm in diameter. The rest of the small intestine is about 5-m long; the proximal two-fifths is referred to as the jejunum and the distal three-fifths is called the ileum. The wall of the jejunum is thicker and its lumen is wider than that of the ileum. In general, there is a gradual narrowing of the lumen of the small intestine from the proximal duodenum to the distal ileum (Thomas, 1988; Shiner, 1995).

The three regions of the small intestine share a common histological pattern. Their wall, from inside outward, is composed of the mucosa, the submucosa, the muscle layers, and the serosa (Thomas, 1988; Shiner, 1995). The serosa is an extension of the peritoneum and consists of a single layer of flattened mesothelial cells overlying some loose connective tissues. The muscularis has an outer longitudinal layer and an inner circular layer of muscle. The submucosa is composed of a network of loose connective tissue rich in small blood vessels, lymphatics, and nerve plexus. The mucosa has three components: a superficial lining of epithelium, the lamina propria, and the muscularis mucosa. The epithelium, the innermost layer of mucosa facing the lumen of the bowel, consists of a single layer of columnar epithelial cells (enterocytes) which line both the crypts and the villi. The villi tend to be short and leaf-like in the duodenum and are tall and more broad in the jejunum, where their height ranges from 300 to 780 μm. The villus height decreases from the jejunum to the ileum. The total mucosa thickness varies from 530 to 900 μm (Thomas, 1988; Shiner, 1995).

Unlike hepatocytes which regenerate only when untimely death occurs, epithelial cells of intestinal mucosa have a programmed limited life span. The villous epithelial cells are functionally mature and nondividing, whereas the crypt cells are immature and evolving. The crypt cells continue to mature as they ascend toward the villus and are extruded at its tip. Their life span is estimated at 1 or 2 cells per 100 cells per h. The time required for migration from the base to the tip has been estimated to be 2 to 6 days (Bertalanffy and Nagy, 1961; Lipkin et al., 1963; Creamer, 1967). One consequence of this rapid migration is described in the following example. Intestinal CYP3A4 enzymes have been shown to activate dietary aflatoxin B_1 to reactive metabolites that form macromolecular adducts within enterocytes (Kolars et al., 1994). Consequently, these adducts should pass harmlessly in stool as the enterocytes are shed as a result of rapid migration. The rapidity of enterocyte migration and maturation may provide a protective mechanism against carcinogenic toxins (Morse and Stoner, 1993; Zhang et al., 1997).

[2] Abbreviations: AUC, area under the concentration curve; 3-MC, 3-methylcholanthrene; ISF, isosafrole; BNF, β-naphthoflavone; AhR, arylhydrocarbon receptor; PB, phenobarbital; CL, clearance; AHH, arylhydrocarbon hydroxylase; DX, dexamethasone; EROD, 7-ethoxyresorufin *O*-deethylation; A1254, aroclor-1254; IMM, IMM125; 2-AAF, 2-acetylaminofluorene.

1. Mucosal Blood Flow

The blood (500 ml/min) that courses through the human small intestine flows immediately into the liver by way of the portal vein. In the liver, the blood passes evenly through the hepatocytes and ultimately leaves the liver via hepatic veins that empty into the vena cava of the general circulation. The superior mesenteric arteries supply the small intestine by way of an arching arterial system. Blood flowing through a small artery is distributed in the various layers of the small intestine. In dogs, approximately three-fourths of total resting intestinal blood flow is distributed to the mucosa and the remainder to the submucosa, muscularis, and serosa (Bond and Levitt, 1979; Bond et al., 1979). It has also been demonstrated that approximately 60 to 70% of the intestinal blood flow is distributed to the epithelial mucosal cells of cats and rats (Biber et al., 1973; Gore and Bohlen, 1977). Because only part of the intestinal blood flow reaches the mucosa, the mucosal blood flow, rather than the total intestinal blood flow, should be used when attempts are made to estimate intestinal clearance and extraction (Klippert et al., 1982).

The blood flow in each region of the small intestine, as well as in each layer of the gut wall, is related directly to the metabolic demands of the cells within each region and to the functional activity. During the absorption phase, blood flow in the villi and adjacent regions of the submucosa is increased greatly, whereas blood flow in the muscle layers of the gut wall increases with increased motor activity. After a meal, blood flow increases by 30 to 130% of basal flow, and the hyperemia is confined to the segment of intestine exposed to the chyme. Long-chain fatty acids and glucose are the major stimuli for hyperemia, which is likely mediated by hormones such as cholecystokinin released from mucosal endocrine cells (Bond et al., 1979). In addition, there are a number of factors, operating via neurohormonal and local regulatory mechanisms, which can increase the mucosal blood flow in the small intestine (Bond et al., 1979). Sympathetic stimulation, in contrast, decreases the intestinal blood flow by causing intense vasoconstriction of the blood vessels. During heavy exercise, sympathetic vasoconstriction can shut off the intestinal blood flow for short periods of time when increased flow is needed by the skeletal muscle and heart. The effect of exercise on the absorption of midazolam has been investigated in healthy volunteers following a 15-mg oral dose of midazolam (Strömberg et al., 1992). The rate, but not the extent of midazolam absorption, was affected by exercise (treadmill running for 50 min). The C_{max} was decreased from 112 ng/ml during the control session to 76 ng/ml in the exercise session, while the T_{max} was increased from 73 to 123 min. However, the AUC was not affected significantly by exercise. The alterations in the absorption of midazolam during exercise is likely attributed to a transitory decrease of mucosal blood flow.

After a meal, blood flow to the small intestine is increased, and as a consequence, hepatic blood flow also is increased. As shown in eqs. 8 and 10, the greater the blood flow, the lower will be the hepatic and intestinal first-pass metabolism, resulting in an increased bioavailability. Clinical studies demonstrate convincingly that the bioavailability of drugs subject to significant first-pass metabolism during absorption is increased after a meal (Melander and McLean, 1983; Olanoff et al., 1986). A standardized breakfast increased the bioavailability of propranolol and metoprolol by 40 to 50% in normal volunteers (Melander et al., 1977b). However, the blood flow change is not the sole cause responsible for the increased bioavailability after a meal. For example, it is difficult to explain how a standardized breakfast could increase the bioavailability of hydralazine 2- to 3-fold (Melander et al., 1977a). Other factors, such as food-induced inhibition of hepatic and intestinal drug-metabolizing enzymes, also may be involved in the increased bioavailability.

2. Countercurrent Exchange

The villus is supplied by arterioles that pass to the tip of the villus where they break up into many small capillaries, which then drain into a villus venule (Fig. 1). Because of the close proximity between the arterioles and venules in the intestinal villi, it is possible that some small molecules can diffuse out the ascending arterioles directly into the adjacent descending venules without ever being carried in the blood to the tip of the villus where the majority of the intestinal drug-metabolizing enzymes are located. Studies in animals and humans support the concept of countercurrent exchange in the villi (Hallback et al., 1978; Parks and Jacobson, 1987). Because of this exchange phenomenon, the fraction of a drug metabolized by the small intestine could be quantitatively less important when the drug is delivered from the systemic circulation after i.v. administration or the postabsorptive phase as compared to that which occurs during the absorptive phase. In other words, countercurrent exchange reduces the entry of drugs from the systemic circulation into enterocytes. Thus, the presence of this countercurrent is an important feature in intestinal drug metabolism.

The countercurrent exchange is probably accomplished mainly via simple diffusion created by concentration gradient. The effect of countercurrent exchange may vary between substrates depending on their physicochemical properties, as exemplified by the absorption of four diffusible gases (H_2, He, CH_4, and ^{133}Xe) from the small intestine in dogs (Bond et al., 1977). Although these gases were absorbed efficiently from the intestinal lumen (99.7%, 99.9%, 75.6%, and 36% of the dose for H_2, He, CH_4, and ^{133}Xe, respectively), the absorbed fraction escaping into the systemic blood circulation was only 16.2%, 12.8%, 12.0%, and 15.8%, respectively, indicating

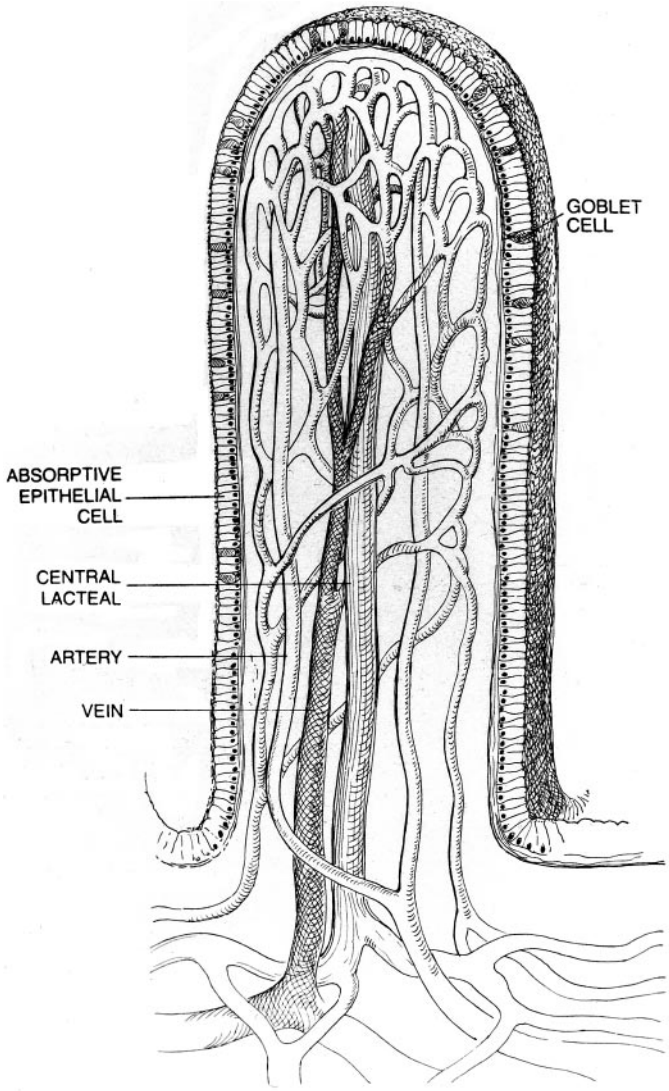

FIG 1. Microcirculation of a human villus.

a substantial countercurrent exchange (83.8%, 87.2%, 84.1%, and 56.1%) of the initially absorbed H_2, He, CH_4, and ^{133}Xe.

To our knowledge, the effect of countercurrent exchange on the intestinal absorption and metabolism of drugs has not yet been investigated. However, Minchin and Ilett (1982) have incorporated the concept of countercurrent exchange into their pharmacokinetic models for the estimation of the intestinal metabolism of drugs. A proportionality constant (α) relating intestinal metabolism after i.v. administration to that after oral administration was proposed to reflect the back-diffusion (countercurrent exchange) of drugs from the intestinal circulation. Theoretically, α can vary from 0 to 1 depending on the physicochemical properties of a given drug. If a compound is not subject to countercurrent exchange, α will be equal to unity. This means that intestinal metabolism of the drug is similar after i.v. and oral administration (Minchin and Ilett, 1982).

B. Localization of Drug-Metabolizing Enzymes in the Small Intestine

Numerous metabolic reactions occur in the gut wall, including those typically referred to as phase 1 and phase 2 processes. Almost all of the drug-metabolizing enzymes present in the liver also are found in the small intestine, although their levels generally are much lower in the latter than in the former. Kinetically, the rate of intestinal metabolism of a drug is determined by the content of a particular catalytic enzyme within the enterocytes and the intracellular residence time of the drug subject to biotransformation. It is, therefore, important to determine the organ content of various intestinal enzymes and their localization to assess the relative contribution of intestinal metabolism to overall metabolism.

1. Cytochromes P-450

The cytochromes P-450 are the principle enzymes involved in the biotransformation of drugs and other foreign compounds. They comprise a superfamily of hemeproteins that contain a single-iron protoporphyrin IX prosthetic group. This superfamily is subdivided into families and subfamilies that are classified solely on the basis of amino acid sequence homology. To date, at least 14 P-450 gene families have been identified in mammals (Nelson et al., 1996). However, only three main P-450 gene families, *CYP1*, *CYP2*, and *CYP3* currently are thought to be responsible for drug metabolism. Approximately 70% of human liver P-450 is accounted for by the CYP1A2, 2A6, 2B6, 2C, 2D6, 2E1, and 3A isoforms. Among these, CYP3A (CYP3A4 and 3A5) and CYP2C (CYP2C8, 2C9, 2C18 and 2C19) are the most abundant subfamilies, accounting for 30% and 20% of total P-450, respectively (Guengerich, 1995).

Unlike the liver in which the distribution of P-450 enzymes is relatively homogeneous (Debri et al., 1995), the distribution of these enzymes is not uniform along the length of the small intestine nor along the villi within a cross-section of mucosa. Longitudinal distribution of total cytochrome P-450 and its functional activity have been measured by CO-binding spectra and aldrin epoxide activity in human small intestinal mucosa. Both the content and activity of cytochrome P-450 was higher in the proximal than that in the distal small intestine (Peters and Kremers, 1989). The average total cytochrome P-450 content in human intestine, about 20 pmol/mg microsomal protein, was found to be much lower than that in the liver (300 pmol/mg microsomal protein) (Peters and Kremers, 1989; Shimada et al., 1994). Immunoblotting studies have shown that CYP3A4 was the dominant cytochrome P-450 in the human small intestine where it accounted for the majority of total microsomal P-450 found in the mucosal epithelium (Watkins et al., 1987; McKinnon et al., 1995). In a recent study, it has been shown that CYP3A4

expression varies along the length of the small intestine. Median values of 31, 23, and 17 pmol/mg microsomal protein were measured in human duodenum, distal jejunum, and distal ileum, respectively (Thummel et al., 1997). The localization and distribution of CYP3A4 along the villi in human small intestine also has been studied by immunoreactivity using a monoclonal antibody to this major form of human hepatic cytochrome P-450. The columnar absorptive epithelial cells of the villi exhibited the strongest immunoreactivity, whereas no immunostaining was detectable in the goblet cells or the epithelial cells in the crypts (Murray et al., 1988).

A similar differential distribution of intestinal P-450 was observed in animals. P-450 content also varied along the villus in rats, where the P-450 content at the villus tip was approximately 10-fold higher than that at the crypts (Hoensch et al., 1976). Furthermore, CYP1A1 has been detected by immunochemical localization in rat duodenum, but was below detectable levels in the jejunum and ileum. CYP2B1/2 and CYP3A1/2, however, were detected in all three regions, again with the highest levels in the duodenum (de Waziers et al., 1990).

The comparative hepatic and intestinal distribution of cytochrome P-450 in humans has been investigated in detail by de Waziers et al. (1990) using immunoblotting techniques. CYP3A4 was found to be the most abundant P-450 in both human liver and small intestine, although the liver exhibited a 2- to 5-fold higher level of the enzyme than the intestine. The levels of CYP3A4 were estimated to be 350 pmol/mg microsomal protein in the liver and 160, 120, and 70 pmol/mg microsomal protein in the duodenum, jejunum, and ileum, respectively (de Waziers et al., 1990). In another study, Watkins et al. (1987) showed that the level of CYP3A4 in the human jejunum was comparable to that in the liver, approximately 70 pmol/mg microsomal protein. Although the concentration of CYP3A4 protein in human intestine is comparable to, or somewhat lower than, that determined in the liver, the estimated total mass of CYP3A4 in the whole intestine was roughly 30 times lower than that in the whole liver (de Waziers et al., 1990). A similar factor (20 times) was reported by Back and Rogers (1987). This difference in total mass of CYP3A4 is related to the very low yield of microsomal protein for the small intestine as compared with the liver because of the localization of the intestinal cytochrome P-450 mainly in villus tip cells, which account for only a very small fraction of the total intestinal cell population (Pacifici et al., 1988). Furthermore, the human liver (ca. 1.5 kg) by weight is about twice as large as the small intestine (ca. 0.7 kg). Other isoforms detected in human intestine are CYP2C and CYP2D6, whereas CYP1A2 and CYP2E1 were not detected (de Waziers et al., 1990). The estimated total mass of CYP2C and CYP2D6 in the whole intestine was 100 to 200 times lower than that in the whole liver (de Waziers et al., 1990).

Depending upon the segment of small intestine used and the method used for enterocyte isolation, a varying population of epithelial cells will be obtained. Indiscriminate scraping of enterocytes from the intestinal mucosa of the small intestine yields a mixture of villus tip, midvillus, and crypt cells which, when combined, exhibit a low content of P-450. This is due to the absence of the enzyme in the crypt and goblet cells. The uneven distribution of enzymes and isolation of enterocytes complicate in vitro studies on intestinal metabolism and probably represent the basis for frequent contradictions in published reports. For example, the mean CYP3A4 content (120 pmol/mg microsomal protein) in human jejunum reported by de Waziers et al. (1990) is much greater than the value (70 pmol/mg microsomal protein) cited by Watkins et al. (1987) and the value (23 pmol/mg microsomal protein) reported by Thummel et al. (1997). It should be noted that there are significant interindividual differences in level of CYP3A4 expression in intestine. The interindividual differences may also contribute to the discrepancy in the intestinal CYP3A4 level reported by different laboratories. Thus, caution should be exercised when comparisons are being drawn between data from different laboratories. With this in mind, in this review comparisons of enzyme protein and catalytic activity between intestinal and hepatic microsomes are made only when data for both organs are available from the same laboratory.

As seen in humans, the levels of P-450 isoforms also are much lower in the rat small intestine than in the rat liver. Although CYP2C11, the major P-450 isoform in male rats, was present at a high concentration in liver (640 pmol/mg microsomal protein), this isoform was not detectable in the small intestine (de Waziers et al., 1990). Rat CYP1A2 and CYP2E1 also were detectable in the liver, but again not in the small intestine. On the other hand, the concentrations of CYP2B1/2 were comparable in both the liver and the intestine, approximately 90 pmol/mg microsomal protein (de Waziers et al., 1990). Taking the yield of microsomal protein into consideration, in terms of total mass, total amounts of CYP2B1/2 in the whole small intestine was about 30 times lower than those in the whole liver (de Waziers et al., 1990).

Consistent with the P-450 enzyme protein levels, the enzyme activities of P-450 isoforms also are higher in the liver than the small intestine. In a study with human hepatic and intestinal microsomes, the CYP3A4 catalytic activities, as measured by erythromycin demethylation, were estimated to be 2.8, 1.6, 1.1, and 0.15 nmol/min/mg microsomal protein in the liver, duodenum, jejunum, and ileum, respectively (de Waziers et al., 1990). Midazolam, a commonly used short-acting benzodiazepine, is metabolized exclusively by CYP3A4 and gives rise to a single major metabolite, 1′-hydroxymidazolam, which is formed by both human hepatic and intestinal microsomes. The metabolic

clearance (V_{max}/K_m) was higher in the liver preparations (540 μl/min/mg microsomal protein) than in the small intestine (135 μl/min/mg microsomal protein) (Thummel et al., 1995). Similarly, tacrolimus, an immunosuppressant, is metabolized by CYP3A4 in human liver and small intestine, where the rate of biotransformation was found to be higher in the liver (96 pmol/min/mg microsomal protein) than in the small intestine (54 pmol/min/mg microsomal protein) (Lampen et al., 1995). The metabolism of (+)-bufuralol, a substrate of CYP2D6, was studied in human hepatic and intestinal microsomes (Prueksaritanont et al., 1995). Although the K_m values (5–10 μM) were similar in both hepatic and intestinal microsomes, the V_{max} values were much higher in human hepatic microsomes (383 pmol/min/mg microsomal protein) than in intestinal microsomes (3–9 pmol/min/mg microsomal protein). Similar results were observed in rhesus monkeys (Prueksaritanont et al., 1995). The K_m values (7–9 μM) for (+)-bufuralol were similar in monkey hepatic and intestinal microsomes, whereas the liver had a much higher V_{max} than the small intestine (860 versus 3 pmol/min/mg microsomal protein). In rats, the rate of 7-ethoxycoumarin *O*-deethylation was 20-fold lower in intestinal microsomes compared to that of hepatic microsomes (Shirkey et al., 1979). The K_m and V_{max} values for 7-ethoxycoumarin were 62 μM and 123 pmol/min/mg microsomal protein for rat intestinal microsomes, and the respective values for hepatic microsomes were 19 μM and 800 pmol/min/mg microsomal protein.

From these data, it is clear that both the total enzyme-to protein and catalytic activities of P-450 isozymes in the whole small intestine are much lower (20- to 300-fold less) than those in the whole liver, when the yield of microsomal protein is taken into consideration. With the limited amount of cytochromes P-450 in the small intestine, it may be expected that the contribution of intestinal oxidative metabolism to the overall first-pass metabolism of a drug would be less likely to be quantitatively as important as that in the liver.

2. UDP Glucosyltransferases and Sulfotransferases

Glucuronidation and sulfation are the most important phase 2 reactions in the biotransformation of many drugs in animals and humans. Both reactions serve to increase the water solubility of lipophilic compounds and, therefore, their renal excretion. Knowledge of the function, biochemistry, and molecular biology of the responsible enzymes, namely, the UDP glycosyltransferases (UGTs) and sulfotransferases (STs), has increased dramatically in recent years (Weinshilboum and Otterness, 1994; Mackenzie et al., 1997). Both enzyme systems are distributed widely in many tissues including the intestine (Pacifici et al., 1988; Cappiello et al., 1989, 1991; Krishna and Klotz, 1997). UGTs are membrane-bound enzymes and are located in the endoplasmic reticulum in cells, while STs are present in the cytosol.

Like cytochromes P-450, UGTs and STs each comprises a superfamily of enzymes. At least 10 rat UGTs and 8 human UGTs have been identified and characterized by cDNA cloning (Clarks and Burchell, 1994; Mackenzie et al., 1997). The cloning of cDNA revealed that there are at least five rat STs and two human STs (Weinshilboum and Otterness, 1994). Although the molecular biology of UGT and ST enzymes has advanced greatly in recent years, information at the protein level of the enzymes expressed in the liver and small intestine is limited, probably due to the lack of specific antibodies for the isozymes.

As with the P-450s, but to a lesser extent, the distribution of UGTs also is not uniform along the length of the intestine, nor along the villi within a specific region of the intestine. In rats, the UGT activities toward 3-hydroxybenzo(*a*)pyrene and 4-hydroxybiphenyl were 4 times lower in crypt cells than in upper villus cells (Dubey and Singh, 1988). The bilirubin UGT activity decreased significantly from duodenum to ileum, whereas the UGT activity toward 4-nitrophenol was roughly similar in human duodenum, jejunum, and ileum (Peters et al., 1991). In addition, a dramatic fall in activity for 4-nitrophenol, as well as for bilirubin UGT, was observed in the large intestine (Peters et al., 1991).

In a recent study, hepatic and intestinal UGT activities in rats and rabbits have been investigated by measuring the glucuronidation of 1-naphthol, 2-naphthol, 4-methylumbelliferone, 4-nitrophenol, 2-hydroxybiphenyl, and 4-hydroxybiphenyl (Vargas and Franklin, 1997). Generally, intestinal UGT activities were higher in rabbits when compared with rats, whereas hepatic activities were much higher in rats than in rabbits. In rats, the activities (nanomoles per minute per miiligram of microsomal protein) in the small intestinal mucosa were much lower than those in the liver, with the activities in the intestine representing 5 to 15% of hepatic levels. On the other hand, the intestinal activities were comparable (70–100%) to the hepatic activities for most aglycones in rabbits. In another study, the UGT activities toward benzo(*a*)pyrene-3,6-quinol (BP-3,6-quinol), bilirubin, 4-hydroxybiphenyl, and morphine were higher in rat liver than in the intestine, whereas the intestinal activities toward 1-naphthol and fenoterol were comparable to the corresponding hepatic values (Koster et al., 1986).

The activity of UGTs also has been measured in human liver and intestinal mucosa using 1-naphthol, morphine, and ethynylestradiol as marker substrates (Cappiello et al., 1991). The liver had much higher UGT activity than the intestine for all substrates studied. The UGT activity in liver microsomes for 1-naphthol, morphine, and ethynylestradiol was 5.86, 0.38, and 0.11 nmol/min/mg microsomal protein, respectively, whereas the corresponding values in intestinal microsomes were

1.3, 0.03, and 0.025 nmol/min/mg microsomal protein, respectively. In addition, the UGT activities toward bilirubin, 4-nitrophenol, and 4-methylumbelliferone were investigated using human hepatic and intestinal microsomes (Peters and Jansen, 1988). The UGT activities toward bilirubin and 4-nitrophenol were much higher in the liver than the intestine, whereas the UGT activity toward 4-methylumbelliferone in the intestine was comparable to that in the liver. Overall, the UGT activities in human intestine were lower than, or comparable to, the hepatic values when the activities were expressed on a per milligram microsomal protein basis. However, the total UGT activities in whole liver would be much greater (at least 30-fold) than those in whole small intestine when the yield of microsomal protein is taken into consideration.

In addition to the UGT activity, the distribution of the cosubstrate UDP-glucuronic acid has been studied in human liver and intestine, where it was found to be 280 nmol/g tissue in liver and 19 nmol/g tissue in the intestinal mucosa (Cappiello et al., 1991). In rats, the UDP-glucuronic acid concentrations in the liver and small intestine were 400 and 100 nmol/g tissue, respectively (Goon and Klassen, 1992). These results provide additional evidence that UGT activity is greater overall in the liver than in the intestine.

The activity of ST toward 2-naphthol and the concentration of its cosubstrate, 3′-phosphoadenosine-5′-phosphosulfate, have been measured in human liver and the mucosa from ileum and colon (Cappiello et al., 1989). The ST activity in the liver also was higher than that in the small intestine. The mean ST activity of 2-naphthol sulfotransferase in human liver, ileum, and sigmoid colon was 1.82, 0.64, and 0.40 nmol/min/mg protein, respectively. The concentration of 3′-phosphoadenosine-5′-phosphosulfate followed the same rank order as did ST activity, namely, liver (23 nmol/g tissue) > ileum (13 nmol/g tissue) > sigmoid colon (6 nmol/g tissue). Sulfation also can occur at amino group of drugs and is mediated by amino sulfotransferase (N-ST). The N-ST activity toward desipramine in human liver, ileum, and sigmoid colon has been measured and found to be 47, 22, and 2.6 nmol/min/mg protein, respectively (Romiti et al., 1992). Thus, the same general trend also is observed for ST activity where it is lower in the small intestine than in the liver.

Despite the many examples in which enzyme activity in the liver is greater than that in the intestine, this is not always the case. The sulfation of (+)- and (−)-terbutaline, a β_2-sympathomimetic, has been studied in human intestinal mucosa isolated from the duodenum, ileum, ascending colon, and sigmoid colon and in human liver cytosol (Pacifici et al., 1993). Terbutaline ST was more active in the small and large intestine than in the liver. The rates of sulfation (picamoles per minute per milligram protein) of (+)- and (−)-terbutaline were 1195 and 948 (duodenum), 415 and 317 (ileum), 268 and 166 (ascending colon), 263 and 193 (sigmoid colon), and 45 and 34 (liver), respectively. Similarly, the ST activity toward isoproterenol, a nonspecific β-sympathomimetic which is structurally related to terbutaline, was much higher in human small intestine than in liver (Pesola and Walle, 1993). Thus, the rates of conjugation of the sulfation of (+)- and (−)-isoproterenol were 1400 and 700 pmol/min/mg protein in human jejunum and 30 and 10 pmol/min/mg protein in the liver, respectively. Similar results were observed in dogs, where the sulfation of isoproterenol was higher in small intestine than in liver (George et al., 1974; Ilett et al., 1980). Similar to the yield of microsomal protein, the yield of cytosolic protein is about 50-fold lower in the small intestine as compared to that of the liver (Pacifici et al., 1988). Taking the protein yield into consideration, the total ST capacity for both terbutaline and isoproterenol in the whole small intestine would be comparable to that in the liver. These results suggest that the small intestine could be the major site for first-pass metabolism with these drugs.

In general, the metabolic activities of P-450s, UGTs, and STs in the whole small intestine are considerably lower than the corresponding values in the whole liver. In some cases, however, the capacity of ST reactions in the whole small intestine appear to be comparable to that in the whole liver. For these exceptions, the role of small intestine in first-pass metabolism could be important.

C. Intestinal Enzyme Induction

One of the many important aspects of mammalian drug-metabolizing enzymes is that some are inducible. Because both liver and small intestine are involved in first-pass metabolism, and because both organs are subject to exposure to orally administered inducers, it is not surprising that intestinal and hepatic enzymes can be induced. Such induction may lead to an increased first-pass effect and, in turn, to a decreased oral bioavailability (Kaminsky and Fasco, 1992).

1. Cytochromes P-450

Using specific monoclonal antibodies, the induction of CYP1A1 and 1A2 has been studied in the liver and intestine before and after treatment of rats with 3-methylcholanthrene (3-MC) and isosafrole (ISF) (Sesardic et al., 1990). Only CYP1A1 was inducible in rat intestine by 3-MC and ISF, whereas in the liver both CYP1A1 and 1A2 were inducible by these compounds (Table 1). As shown in Table 1, both total P-450 and CYP1A enzyme protein were much higher in rat liver than in the intestine, either before or after induction. After induction, phenacetin *O*-deethylase activity in the liver (1600 pmol/min/mg microsomal protein) of 3-MC-treated rats was about 100 times that in the small intestine (15.4 pmol/min/mg microsomal protein). These results suggested that the inducers had differential effects on hepatic and intestinal microsomes, both qualitatively and quantita-

TABLE 1
Cytochromes P-450 and phenacetin O-deethylase activity in rat liver and intestine after induction

Tissue	Treatment	Total Cytochrome P-450	CYP1A1	CYP1A2	Phenacetin O-Deethylase Activity
		pmol/mg microsomal protein			*pmol/min/mg protein*
Liver	Untreated	722 ± 5	<0.5	9.0 ± 0.6	59.7 ± 6.4
	3-MC	984 ± 4	356 ± 60	124 ± 22	1600 ± 154
	ISF	1030 ± 4	106 ± 32	183 ± 42	
Intestine	Untreated	45.7 ± 8.3	<0.5	<2	<0.2
	3-MC	116 ± 18	107 ± 14.5	<2	15.4 ± 4.57
	ISF	ND	9 ± 0.6	<2	

Data were adapted from Sesardic et al. (1990). Rats were treated with 3-MC (80 mg/kg i.p.) for 48 h or ISF (160 mg/kg/day i.p.) for 4 days before sacrifice. ND, not determined.

tively. The response to the inducers appeared to be more pronounced in the liver than in the intestine. In another study in rats, the time courses of hepatic and intestinal CYP1A1 induction were compared quantitatively at the protein and mRNA levels after a single dose of β-naphthoflavone (BNF; 40 mg/kg i.p.). CYP1A1 mRNA levels in both organs increased sharply and peaked at about 6 h, after which they returned to near basal levels within 12 h after BNF treatment (Zhang et al., 1997). In association with the increase in mRNA, the level of CYP1A1 protein was increased after BNF treatment. Maximum protein levels were attained between 12 and 24 h in the intestine and 24 and 48 h in the liver. Again, the maximum protein level was 2- to 3-fold higher in the liver than in the intestine (Zhang et al., 1997). The extent of CYP1A1 induction by BNF decreased markedly along the small intestine from the duodenum to the ileum (Fig. 2). This gradient of CYP1A1 induction is due, at least in part, to the gradient distribution of the arylhydrocarbon receptor (AhR). As shown in Fig. 2, the gradient distribution of the AhR along the length of the small intestine correlated very well with the pattern of CYP1A1 induction (Zhang et al., 1997).

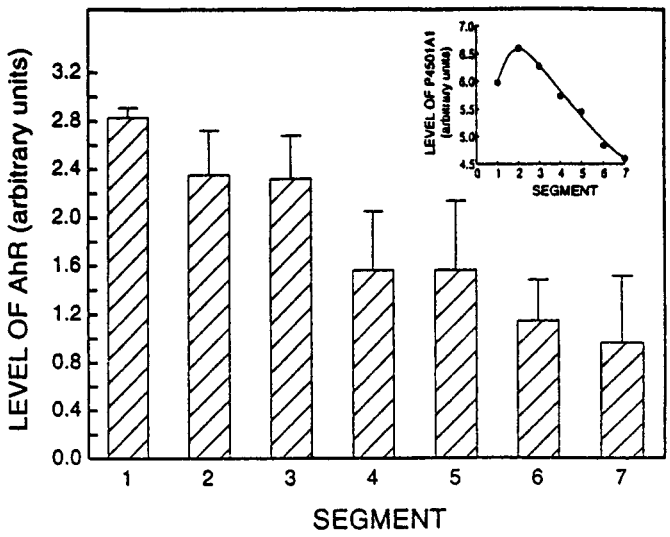

FIG. 2. Distribution of CYP1A1 protein (inset) and AhR protein along the length of the rat small intestine. Data adapted from Zhang et al., 1997.

Inducibility of CYP2B1/2 in the intestine and liver has been studied in rats following phenobarbital (PB) treatment (Bonkovsky et al., 1985). The concentration of CYP2B1/2 was below the limit of detection in both organs of untreated rats, whereas the enzymes were readily measurable after PB treatment, accounting for about 50% of the total cytochrome P-450 (Table 2). An increase of CYP2B1/2 enzyme protein was associated with an increase in the catalytic activity measured by 7-ethoxycoumarin *O*-deethylation (Table 2). As in the case of untreated rats, concentrations of CYP2B1/2 in the intestine of PB-treated rats varied along the length and between villus and crypt cells. The concentrations in the proximal two-thirds and distal one-third of the small intestine were 127 and 50 pmol/mg microsomal protein, respectively, in PB-treated rats. The CYP2B1/2 concentration in the upper villus was 137 pmol/mg microsomal protein, whereas it was below detection limits in crypt cells (Bonkovsky et al., 1985). After induction, both CYP2B1/2 enzyme protein and 7-ethoxycoumarin *O*-deethylase activity were higher in the liver than in the intestine by approximately 10- to 15-fold (Table 2). Based on the ethoxycoumarin *O*-deethylase activity, the extent of enzyme induction caused by PB appeared to be similar between liver and intestine, namely, about 3-fold (73). In another study, however, Shirkey et al. (1979) reported that rat liver was more responsive to both PB and 3-MC than rat intestine, as measured by 7-ethoxycoumarin *O*-deethylation in rat hepatic and intestinal microsomes. The rate of the hepatic deethylation was increased 4- and 11-fold, respectively, after PB- and 3-MC treatment, whereas the values were only 2- and 4-fold for intestinal deethylation. It should be noted that both rat CYP 1A1/2 and 2B1/2 are known to be involved in 7-ethoxycoumarin *O*-deethylation (Correia, 1995).

Hepatic and intestinal induction caused by PB and 3-MC have been investigated in rats, mice, guinea pigs, and rabbits by measuring arylhydrocarbon hydroxylase (AHH) and 7-ethoxycoumarin deethylase activities (Miranda and Chhabra, 1979). PB treatment induced both hepatic and intestinal 7-ethoxycoumarin deethylase activity in rats, mice, and guinea pigs, whereas PB treatment enhanced only hepatic activity in rabbits (Table 3). Similarly, treatment with 3-MC resulted in sig-

TABLE 2
Cytochromes P-450 and ethoxycoumarin O-deethylase activity in rat liver and intestine after PB treatment

Tissue	Treatment	Total Cytochrome P-450	CYP2B1/2	Ethoxycoumarin O-Deethylase Activity
		pmol/mg microsomal protein		*pmol/min/mg protein*
Liver	Untreated	735 ± 143	Undetectable	862 ± 83.9
	PB	2200 ± 273	1056 ± 133	2550 ± 332
Intestine	Untreated	84.3 ± 16.8	Undetectable	57.9 ± 6.6
	PB	199 ± 17.4	107 ± 9.3	173 ± 36.9

Data were adapted from Bonkovsky et al. (1985). Rats were treated with PB (80 mg/kg i.p.) for 5 days before sacrifice.

TABLE 3
Activities of AHH and 7-ethoxycoumarin deethylase in the liver and small intestine of various animal species treated with PB and 3-MC

Species	Tissue	AHH		7-Ethoxycoumarin Deethylase	
		Control	3-MC	Control	PB
		relative fluorescence unit/min/mg microsomal protein		*pmol/min/mg protein*	
Rat	Liver	7,164 ± 719	11,199 ± 609	500 ± 110	2,790 ± 150
	Small intestine	40 ± 13	646 ± 137	49 ± 8	123 ± 19
Mouse	Liver	919 ± 84	4,716 ± 602	1,600 ± 190	6,190 ± 250
	Small intestine	50 ± 14	258 ± 64	64 ± 12	260 ± 23
Guinea pig	Liver	1,266 ± 62	3,601 ± 65	1,050 ± 110	5,300 ± 610
	Small intestine	408 ± 43	980 ± 207	92 ± 10	150 ± 30
Rabbit	Liver	2,743 ± 478	1,635 ± 480	670 ± 100	3,700 ± 500
	Small intestine	383 ± 95	316 ± 66	59 ± 20	41 ± 7

Values are means ± S.E. (n = 4). Data were adapted from Miranda and Chhabra (1979). 3-MC was injected i.p. daily for 3 days in mice at a dose level of 100 mg/kg; in rats and guinea pigs, 25 mg/kg; and in rabbits, 25 mg/kg. PB was injected i.p. daily for 3 days in mice at a dose of 100 mg/kg; in rats and guinea pigs, 80 mg/kg; and in rabbits, 25 mg/kg.

nificant increases in hepatic and intestinal AHH activity in rats, mice, and guinea pigs, but not in rabbits (Table 3). As shown in Table 3, although there were differences in the degree of induction of hepatic and intestinal enzyme activities, depending on the type of inducing agents and animal species used, overall the hepatic enzyme activities were much greater than those in the small intestine both before and after induction.

Treatment of rats with dexamethasone (DX) resulted in an increase of CYP3A1/2 enzyme protein in both liver and intestine (Watkins et al., 1987, 1989) where the concentration of enzyme was increased 6- to 7-fold in the liver and 3- to 4-fold in the intestine (Table 4). In parallel with the increase in the enzyme protein, the activity of erythromycin *N*-demethylation, a reaction highly characteristic of CYP3A1/2, was increased after induction (Table 4). The erythromycin *N*-demethylase activity in the small intestine appeared to be somewhat higher than that in the liver before and after induction (Table 4).

In humans, CYP3A4 in the small intestine and liver also is inducible (Ged et al., 1989; Kolars et al., 1992). Rifampin treatment (300 mg b.i.d. for 7 days) resulted in a 5- to 8-fold increase in the concentration of CYP3A4 mRNA in human small intestinal enterocytes. This increase in the concentration of mRNA was accompanied by a similar increase in CYP3A4 enzyme protein levels as well as catalytic activity measured by erythromycin *N*-demethylation. In one subject, erythromycin *N*-demethylase activity in intestinal microsomes was increased from 100 pmol/min/mg microsomal protein before rifampin treatment to 1000 pmol/min/mg microsomal protein after induction (Kolars et al., 1992). In another study conducted by other investigators, treatment with rifampin (600 mg daily for 4 days) resulted in a 5- to 6-fold increase of CYP3A4 enzyme protein and catalytic activity in liver microsomes measured by erythromycin *N*-demethylation. The enzyme protein and catalytic activity in liver were increased, respectively, from 33 pmol/mg microsomal protein and 500 pmol/min/mg

TABLE 4
Cytochromes P-450 and erythromycin demethylase activity in rat liver and intestine after DX treatment

Tissue	Treatment	CYP3A1/2	Erythromycin Demethylase
		pmol/mg microsomal protein	*pmol/min/mg protein*
Liver[a]	Untreated	<50	880 ± 260
	DX	330	2,220 ± 470
Intestine[b]	Untreated	1[c]	1,750
	DX	3–4[c]	3,300

[a] Data adapted from Watkins et al. (1989). Rats were treated with DX (20 mg/kg p.o.) for 2 days before sacrifice.
[b] Data adapted from Watkins et al. (1987). Rats were treated with DX (20 mg/kg p.o.) for 2 days before sacrifice.
[c] Arbitrary units.

microsomal protein before rifampin treatment to 161 pmol/mg microsomal protein and 2908 pmol/min/mg microsomal protein after treatment (Ged et al., 1989). From these two studies, the extent of induction caused by rifampin at a similar dose appeared to be quantitatively similar between human liver and small intestine.

CYP1A1 also is inducible in human small intestine and liver (Pelkonen et al., 1986; Sesardic et al., 1988; Buchthal et al., 1995). CYP1A1 activity in human duodenal mucosa was determined by measuring 7-ethoxyresorufin *O*-deethylation (EROD) in biopsies from 20 smokers (3–30 cigarettes/day), 10 nonsmokers receiving omeprazole treatment (20–60 mg/day for at least 1 week), and 21 nonsmokers (Buchthal et al., 1995). The intestinal EROD activity was found to be induced significantly in smokers and in omeprazole-treated patients, with medians of 2.1 and 1.1 pmol/min/mg microsomal protein, respectively, compared to 0.5 pmol/min/mg microsomal protein in the nonsmokers. Immunoblot analysis revealed that EROD activity correlated well with CYP1A enzyme protein in these patients (Buchthal et al., 1995). As was the case with intestinal CYP1A1, other investigators reported that cigarette smoking induced CYP1A1 in human liver as measured by immunoblotting and EROD activity (Pelkonen et al., 1986; Sesardic et al., 1988). The concentration of CYP1A1 (16.3 pmol/mg microsomal protein) in the liver biopsies from the smokers was significantly higher than in those (4.7 pmol/mg microsomal protein) from the nonsmokers. Consistent with this increase in enzyme protein, the hepatic activity of phenacetin *O*-deethylation also was increased from 54 pmol/min/mg microsomal protein in nonsmokers to 230 pmol/min/mg microsomal protein in smokers (Sesardic et al., 1988). Similarly, cigarette smoking induced hepatic EROD activity in liver biopsies from smokers and nonsmokers (ex-smokers and never-smokers) which were 1045 and 330 pmol/min/g liver, respectively (Pelkonen et al., 1986). Taken together, these results suggested that the extent of hepatic and intestinal induction caused by cigarette smoking were quantitatively similar at approximately 3- to 4-fold.

2. UDP Glucosyltransferases

Both hepatic and intestinal UGTs are inducible. Hepatic and intestinal UGT activity toward 1-naphthol, BP-3,6-quinol, morphine, 4-hydroxybiphenyl, bilirubin, and fenoterol have been determined in rats before and after pretreatment with PB, 3-MC, and aroclor-1254 (A1254) (Koster et al., 1986). The hepatic enzymes were more responsive to the inducers than were the corresponding intestinal enzymes. As shown in Table 5, the induction factors, determined as the ratio of enzyme activity after induction to that before treatment, generally were higher in the liver than in the intestine. These results suggested that the inducers exhibited differential effects on hepatic and intestinal UGTs. Similar results were reported by Shirkey et al. (1979). No induction of 1-naphthol glucuronidation was observed in rat intestinal microsomes after PB or 3-MC treatment, whereas the hepatic enzyme was induced significantly by these agents (approximately 3-fold increase by 3-MC and 2-fold increase by PB). In another study, the effects of various inducers on rat hepatic and intestinal UGTs were studied using acetaminophen, harmol, and 1-naphthol as the aglycones (Goon and Klassen, 1992). The hepatic UGT activities toward these aglycones were 2- to 3-fold higher than that in the intestine before induction. Overall, the inductive response of UGT was more sensitive in the liver than in the intestine.

Although induction of UGTs has been studied thoroughly in animals, little is known about the inducibility of human UGTs. Bock et al. (1984) reported that liver microsomes from patients treated with PB and phenytoin exhibited significantly higher UGT activity toward 1-naphthol, 4-methylumbelliferone, and bilirubin. In addition, Bock and Bock-Hennig (1987) showed that PB and phenytoin treatment resulted in increased glucuronidation of 1-naphthol, acetaminophen, BP-3,6-quinol, and 4-methylumbelliferone, whereas the conjugation of morphine and 4-hydroxybiphenyl were unaffected by these agents. These results suggest that PB and phenytoin exert differential inductive effects on human hepatic UGT isozymes. Cigarette smoking also has been known

TABLE 5
Induction factors for glucuronidation in microsomes from rat liver and intestine after treatment with PB, 3-MC, and A1254

Substrate	Liver			Intestine		
	PB	3-MC	A1254	PB	3-MC	A1254
1-Naphthol	1.5	4.3	6.5	0.8	0.6	2.1
BP-3,6-quinol	1.8	10.4	12.9	1.1	1.3	2.1
BP-3,6-quinol-monoglucuronide	2.3	38.2	72.0	1.1	1.1	2.8
Morphine	4.3	1.3	3.5	1.0	0.6	1.8
4-Hydroxybiphenyl	3.3	1.5	3.1	0.8	0.4	1.3
Bilirubin	1.5	0.8	0.6	0.8	0.6	1.3
Fenoterol	1.1	1.0	1.2	0.8	0.8	0.9

Data given represent the fold induction with respect to control activities before treatment. The mean control activities in rat liver for 1-naphthol, BP-3,6-quinol, BP-3,6-quinol-monoglucuronide, morphine, 4-hydroxybiphenyl, bilirubin, and fenoterol were 39.6, 4.4, 0.20, 5.8, 13.8, 1.4, and 7.2 nmol/min/mg microsomal protein, respectively. These respective intestinal activities were 21.4, 0.7, 0.2, 0.07, 2.4, 0.4, and 6.6 nmol/min/mg microsomal protein. Pretreatment with PB, 3-MC, and A1254 was performed as follows. PB: An initial dose of 100 mg/kg was given once i.p. and was followed up by 0.1% (w/v) in drinking water; animals were sacrificed 4 days after treatment. 3-MC: A dose of 40 mg/kg, dissolved in olive oil, was given once i.p.; animals were sacrificed 4 days after treatment. A1253: A dose of 500 mg/kg, dissolved in olive oil, was given once i.p.; animals were sacrificed 6 days after treatment. Data were adapted from Koster et al. (1986).

to induce hepatic UGT activities toward acetaminophen and propranolol in humans (Bock et al., 1987; Walle et al., 1987). In contrast to the hepatic UGT activity, Buchthal et al. (1995) have shown that cigarette smoking had little effect on UGT activity toward 4-methylumbelliferone in human duodenal mucosa.

3. Effect of Route and Dose on Enzyme Induction

Because induction is a dose- and time-dependent phenomenon, the dose of inducing agents and the route of administration are important in determining the extent of intestinal and hepatic enzyme induction. BNF (0.2–100 mg/kg diet, equivalent to 0.01–10 mg/kg b.wt./day) administered in the feed of rats for 7 days produced dose-dependent increases in intestinal CYP1A1 activity as measured by 7-ethoxyresorufin and 7-ethoxycoumarin deethylase activities, whereas a significant increase in hepatic deethylation was seen only at the highest dose of 10 mg/kg b.wt. (McDanell and McLean, 1984). On the other hand, CYP1A1 mRNA and enzyme protein in both liver and small intestine were increased markedly after a single i.p. dose of BNF (40 mg/kg) to rats, and the maximum protein level of CYP1A1 was 2- to 3-fold higher in the liver than in the intestine (Zhang et al., 1997). These results strongly suggest that the degree of intestinal and hepatic induction may vary, depending on the oral dose of inducers.

Although it is widely believed that intestinal enzymes respond to a greater extent than hepatic enzymes to orally administered inducers because of more direct availability of the inducers in the intestine, the above data indicate that this belief is valid only when a small dose of an inducer is given orally. This is because, at a low dose, the inducer may be metabolized significantly by the small intestine, and only a very small fraction of the inducer will reach the liver intact. In fact, as indicated in Tables 1 to 5, the extent of hepatic induction generally is much higher than intestinal induction when high doses of inducers were given. These results also suggest that expression of drug-metabolizing enzymes in the intestinal epithelial cells and the hepatocytes may be independently and noncoordinately regulated. The hypothesis of independent regulation of hepatic and intestinal enzymes is supported further by the work of Watkins and coworkers (Lown et al., 1994; Lown et al., 1997). Although the CYP3A4 present in human enterocytes appears to be functionally and structurally identical with the CYP3A4 present in human hepatocytes, there was no significant correlation between protein levels (or catalytic activity) of intestinal CYP3A4 and hepatic CYP3A4 activities.

The intestinal induction appears to be independent of the route of administration when the inducer is given at high doses. There were no differences in intestinal induction following i.p. or oral administration of PB and 3-MC when the inducing agents were given at high doses (75–100 mg/kg) (Miranda and Chhabra, 1979). Similarly, the expression of CYP2B1 and CYP2B2 mRNA in rat intestinal mucosa was not dependent on the route of administration of inducers when a high dose of PB (80 mg/kg) was given either i.p. or p.o. (Traber et al., 1988). However, it is likely that a route-dependent intestinal induction will be observed when inducers are administered at low doses.

D. p-Glycoprotein

Two types of p-glycoprotein have been found in mammals: the drug-transporting p-glycoproteins and phospholipid-transporting p-glycoproteins. The former is encoded by human *MDR*1 and rodent *mdr1a*/*b* genes (Higgins, 1992). p-Glycoprotein was identified initially through its ability to confer multidrug resistance in mammalian tumor cells (Juliano and Ling, 1976). This observation led to the finding that p-glycoprotein is involved in a drug efflux transport that lowers the intracellular concentration of cytotoxic drugs. The tissue-specific expression of the *mdr* genes has been investigated in humans, mice, rats, and hamsters (Silverman and Schrenk, 1997). In humans, p-glycoprotein is localized on the bile canalicular surface of hepatocytes, apical surface of proximal tubules in kidneys and columnar epithelial cells of intestine, and capillary endothelial cells of brain and testis (Thiebaut et al., 1987). The localization suggests that p-glycoprotein functionally can protect the body against toxic xenobiotics by excreting these compounds into bile, urine, and the intestinal lumen, and by preventing their accumulation in brain and testis. Thus, p-glycoprotein may play a significant role in drug absorption and disposition in animals and humans.

Recent studies using the *mdr1a* knockout mice have demonstrated a role for p-glycoprotein in the blood-brain barrier. Thus, when ivermectin, a potent antiparasitic agent, was given at a dose which is innocuous to wild-type animals, the *mdr1a* knockout mice developed a fatal neurotoxicity resulting from accumulation of the drug in their central nervous system (Schinkel et al., 1994). Similarly, marked drug accumulation in brain of *mdr1a* (−/−) mice compared to wild-type, *mdr1a* (+/+), mice was observed when ^{3}H-labeled digoxin and cyclosporin A were given i.v. (Schinkel et al., 1995). At 4 h after administration, the ratios of brain levels in *mdr1a* (−/−) mice to that in *mdr1a* (+/+) mice were 35 and 17 for digoxin and cyclosporin A, respectively. At the same time, the drug concentration in plasma and in most tissues was roughly 2-fold higher in *mdr1a* (−/−) mice. These results clearly indicated that p-glycoprotein played a significant role not only in brain penetration, but also in the overall elimination of digoxin and cyclosporin A.

1. Intestinal p-Glycoprotein

Immunohistological studies with human small intestine indicated that p-glycoprotein is located on the apical

brush border membrane of the mature epithelium (Thiebaut et al., 1987). Therefore, p-glycoprotein may play a role in limiting the absorption of p.o. administered drugs by extruding the drugs from the epithelial cells into the intestinal lumen. Most evidence supporting this role for intestinal p-glycoprotein has been derived from in vitro studies. The functionality of intestinal p-glycoprotein was demonstrated in a study with rat mucosal brush-border membrane vesicles and everted rat small intestine (Hsing et al., 1992). In this study, the release of [^{3}H]daunomycin from brush-border membrane vesicles and the release of rhodamine 123 from everted rat small intestine were inhibited by the *p*-glycoprotein substrates diltiazem, colchicine, and verapamil. In another study, Leu and Huang (1995) showed that the efflux of etoposide, an anticancer drug, from everted rat small intestine was inhibited by the addition of C219, a monoclonal antibody of p-glycoprotein. A number of studies aimed at elucidating the role of p-glycoprotein on intestinal drug absorption have used Caco-2 cells as a model system. Using Caco-2 cells, both vinblastine and docetaxel were shown to be substrate of p-glycoprotein. The basolateral-to-apical flux (J_{B-A}) of vinblastine and docetaxel were 10- and 22-fold greater, respectively, than the apical-to-basolateral transport (J_{A-B}) (Hunter et al., 1993). Studies with Caco-2 cells also indicated the involvement of p-glycoprotein in the absorption of cyclosporin A. The J_{A-B} of cyclosporin A was increased significantly in the presence of the p-glycoprotein inhibitors chlorpromazine and progesterone (Augustijns et al., 1993). The involvement of p-glycoprotein in limiting the absorption of celiprolol also was demonstrated with the Caco-2 cell system (Karlson et al., 1993).

However, more direct evidence for a role of intestinal p-glycoprotein in limiting drug absorption was derived from in vivo studies with *mdr1a* (−/−) mice. The pharmacokinetics of paclitaxel (Taxol) were studied in *mdr1a* (−/−) and *mdr1a* (+/+) mice (Sparreboom et al., 1997). The plasma AUC of paclitaxel was 2- and 6-fold higher in *mdr1a* (−/−) mice than in *mdr1a* (+/+) mice after i.v. and oral administration, respectively. Consequently, the oral bioavailability increased from 11% in *mdr1a* (+/+) mice to 35% in *mdr1a* (−/−) mice after an oral dose (10 mg/kg). Although there were no significant differences in biliary excretion between *mdr1a* (−/−) and *mdr1a* (+/+) mice, the cumulative fecal excretion (0–96 h) was reduced from 40% in *mdr1a* (+/+) mice to <2% in *mdr1a* (−/−) mice after i.v. dosing of [^{3}H]paclitaxel. Collectively, these results indicated that p-glycoprotein limited the oral absorption of paclitaxel by excreting the drug from the epithelial cells into the intestinal lumen.

The involvement of p-glycoprotein in the absorption of digoxin was demonstrated in vivo with *mdr1a* (−/−) mice (Mayer et al., 1996). After i.v. administration of [^{3}H]digoxin, there were no significant differences in the biliary excretion of [^{3}H]digoxin in *mdr1a* (+/+) and (−/−) mice with a cannulated gallbladder, which accounted for approximately 20% of the administered dose in each case. Intestinal excretion was measured after interruption of bile flow. Approximately 2% of the dose was excreted into the intestine of the *mdr1a* (−/−), and 16% in the *mdr1a* (+/+) over 90 min after i.v. dosing, suggesting the role of p-glycoprotein in transporting the drug from the systemic circulation into the intestinal lumen. Indirectly, these results suggest that p-glycoprotein would be expected to inhibit the intestinal absorption of digoxin in mice. Similar to mice, the involvement of p-glycoprotein in the intestinal absorption of the digoxin also was observed in rats using the digoxin-quinidine interaction approach (Su and Huang, 1996).

The distribution of p-glycoprotein is not uniform along the length of intestine nor along the villi within a cross-section of mucosa. Thiebaut et al. (1987) used monoclonal antibody MRK16 to study the distribution of p-glycoprotein in human jejunum and colon. Both tissues showed high levels of p-glycoprotein on the apical surface of superficial columnar epithelial cells, but not of crypt cells (Thiebaut et al., 1987). In a recent study, the content of mRNA expression of p-glycoprotein was measured over the total length of the human gastrointestinal tract. The levels of mRNA appeared to increase progressively from the stomach to the colon with low levels in the stomach (5 arbitrary units), intermediate in the jejunum (20 arbitrary units) and high levels in colon (30 arbitrary units) (Fricker et al., 1996). A similar observation was found by other investigators, wherein levels of MDR1 mRNA were measured in normal human tissues (Fojo et al., 1987). The level of mRNA was higher in the colon than in the jejunum by a factor of 2 (Fojo et al., 1987). These results suggest that the expression of p-glycoprotein, contrary to that of cytochrome P-450, increases progressively along the length of intestine. This reciprocal protein concentration gradient of P-450 and p-glycoprotein expression reflects the perfection of Mother Nature in designing defense systems to protect the body against toxic xenobiotics.

2. Cytochromes P-450 and p-Glycoprotein

As described above, both cytochromes P-450 and p-glycoprotein function to protect the body from toxic accumulation of hydrophobic xenobiotics via metabolism and excretion. Interestingly, literature surveys reveal a striking overlap between substrates for CYP3A4 and p-glycoprotein, including cyclosporin, FK506, diltiazem, verapamil, etoposide, and pactaxol (Schuetz et al., 1995a; Wacher et al., 1995; Benet et al., 1996; Kusuhara et al., 1997). Furthermore, a significant overlap also has been identified between inhibitors of CYP3A4 and p-glycoprotein. For example, ketoconazole, itraconazole, and erythromycin, well known CYP3A4 inhibitors, significantly inhibit the activity of p-glycoprotein (Hofsli and Nissen-Meyer, 1989; Gupta et al., 1991; Siegsmund et al., 1994).

In addition to similarities in substrates and inhibitors, CYP3A4 and *p*-glycoprotein appear to be induced by the similar inducers. In a cell line derived from human colon adenocarcinoma LS 180/WT and its Adriamycin-resistant subline (LS 180/AD 50), both p-glycoprotein and CYP3A4 were induced after treatment with many drugs, including phenobarbital, isosafrole, rifampin, clotrimazole, and reserpine (Schuetz et al., 1996). Similarly, dexamethasone, which is a potent inducer of CYP3A4 (Pichard et al., 1992), has been shown to induce p-glycoprotein in human hepatoma cells and rat hepatocytes (Fardel et al., 1993; Zhao et al., 1995). In vivo induction of p-glycoprotein by rifampin, a potent CYP3A4 inducer, was observed in monkeys (Gant et al., 1995). Thus, treatment of monkeys with rifampin (15 mg/kg p.o., twice daily for 7 days) resulted in a 2- to 4-fold increase in hepatic p-glycoprotein mRNA and a 4- to 13-fold increase in liver p-glycoprotein. Similarly, pretreatment of rats with dexamethasone resulted in a 5-fold increase in liver p-glycoprotein (Salphati and Benet, 1998). Coinduction of *mdr1* and *P-450* genes in rat liver by the administration of inducers also has been reported by Burt and Thorgeirsson (1988). Moreover, the human multidrug resistance gene *MDR1* has been reported to be located at chromosome locus 7q21.1, while the gene for CYP3A4 is located at 7q22.1 (Callen et al., 1987; Inoue et al., 1992). Collectively, these observations have led to speculation on possible coordinate regulation of CYP3A4 and p-glycoprotein gene expression in tissues.

However, some evidence suggests that the expression of CYP3A4 and p-glycoprotein is independently and noncoordinately regulated. Lown et al. (1997) found no correlation between intestinal p-glycoprotein and CYP3A4 content in 25 kidney transplant patients who underwent small bowel biopsy for measurement of CYP3A4 and p-glycoprotein. Similarly, Schuetz et al. (1995a) reported that there was no significant correlation between expression of p-glycoprotein and CYP3A4 proteins in livers from 41 patients, although large variations in the levels of expression of p-glycoprotein (55-fold) and CYP3A4 (37-fold) were noted. Moreover, in a recent study in rats, Vickers et al. (1996) have shown that SDZ IMM 125 (IMM), a new immunosuppressant, increased or decreased liver CYP3A and p-glycoprotein levels, depending on the dose and duration of exposure. Regardless of the dose, the modulation of p-glycoprotein levels by IMM did not parallel the changes in CYP3A levels. For example, IMM treatment for 26 weeks at an oral dose of 10 mg/kg/day resulted in a significant decrease (30%) in liver p-glycoprotein in rats, but an increase (56%) in liver CYP3A levels. These results strongly suggest that although p-glycoprotein and CYP3A4 may cooperate to minimize exposure to toxic xenobiotics, they appear to be regulated separately.

Independent regulation also was observed between p-glycoprotein and CYP1A proteins. Doxorubicin was shown to increase *mdr* mRNA and p-glycoprotein levels in a dose-dependent manner in both rat liver epithelial cells and primary rat hepatocytes (Fardel et al., 1997). This induction was detected as early as 4 h after exposure to doxorubicin at 0.5 μg/ml. In contrast to its effect on p-glycoprotein, doxorubicin did not induce CYP1A levels in rat epithelial cells and hepatocytes (Fardel et al., 1997). Expression of p-glycoprotein and CYP1A1 and 1A2 also was investigated in primary cultured human hepatocytes exposed to 2-acetylaminofluorene (2-AAF) (Lecureur et al., 1996). Human hepatocytes obtained from 10 individuals exhibited no change in either *MDR*1 or *MDR*2 mRNA levels, or in doxorubicin intracellular retention, in response to 2-AAF treatment, whereas both CYP1A1 and 1A2 were induced significantly (Lecureur et al., 1996). In another study, Schuetz et al. (1995b) studied the induction of p-glycoprotein and CYP1A1 by aromatic hydrocarbons in human hepatocytes obtained from 15 individuals and concluded that aromatic hydrocarbons regulate p-glycoprotein in humans by a novel mechanism distinct from the classical AhR pathway of CYP1A1 induction. In addition, Schuetz et al. (1995a) reported that there was no significant correlation between expression of CYP1A1 and p-glycoprotein in liver biopsies from 41 patients.

3. p-Glycoprotein and Intracellular Residence Time

Because of its anatomical location, p-glycoprotein can act as a countertransporter that extrudes foreign compounds from inside the enterocytes into the intestinal lumen as they begin to be absorbed across the epithelial cells. A portion of the extruded xenobiotics then can be reabsorbed into the enterocytes. Thus, it is possible that p-glycoprotein increases the exposure of drugs to drug-metabolizing enzymes and hence enhances intestinal metabolism of drugs by prolonging their intracellular residence time through the repetitive process of extrusion and reabsorption.

The effect of p-glycoprotein on intracellular residence time and intestinal metabolism has been investigated by Gan et al. (1996) in Caco-2 cells using cyclosporin A as a model compound. Cyclosporin A is a substrate for both p-glycoprotein and CYP3A4. In the Caco-2 cell system, η-hydroxy cyclosporin A (**AM1**) was the major metabolite. The formation of the **AM1** (**M-17**) metabolite during apical to basolateral transport of cyclosporin A was greater than that during basolateral to apical transport. At a substrate concentration of 0.76 μM, the respective formation rate of AM1 from cyclosporin A was 13 and 5 pmol/h. These results suggest an increase in the metabolism of cyclosporine. Another possible explanation is that by pumping primary metabolite **AM1** from the enterocyte by p-glycoprotein, and the secondary metabolism of **AM1** is diminished. This concept has been proposed by Watkins (1997).

However, it should be noted that the effect of p-glycoprotein on intestinal metabolism in vivo during drug

absorption might not be as great as in the case of the in vitro Caco-2 cell system. This is because a portion of the extruded drugs would be moved from the proximal small intestine to more distal segments where the CYP3A4 content of the mucosa falls significantly. Therefore, gastrointestinal transit of drugs to more distal regions in vivo would be expected to result in less intestinal first-pass metabolism. Furthermore, transport of drugs by p-glycoprotein becomes saturated when the local drug concentration exceeds the K_m value for p-glycoprotein. Thus, at high doses, the effect of p-glycoprotein on the intestinal metabolism and absorption of foreign compounds is expected to be quantitatively less important than with low doses. Most substrates of p-glycoprotein appear to have relatively low K_m values. For example, the apparent K_m values for cyclosporin A and vinblastine for transport across Caco-2 cells (basolateral-to-apical) are 4 and 18 μM, respectively (Hunter et al., 1993; Fricker et al., 1996).

Although our understanding of the molecular biology of p-glycoprotein has advanced greatly in recent years, much less is known about the quantitative contribution of p-glycoprotein to the intestinal metabolism and absorption of foreign compounds. More kinetic studies are required before we can properly assess the relative contribution of p-glycoprotein and P-450 enzymes to intestinal drug metabolism and absorption.

III. Drug Absorption and Intestinal First-Pass Metabolism

Although oral administration is the most convenient and widely used route for medication, there are many disadvantages and shortcomings of oral dosing. One of those shortcomings is that both the rate and extent of absorption vary considerably among individuals, and even within the same individual during chronic or multiple dosing. Interindividual and intraindividual variations in oral absorption are best exemplified by studies on verapamil (Eichelbaum et al., 1981a,b). Using a stable isotope-labeling technique, the kinetics of verapamil were studied in six healthy volunteers on two separate occasions (10 days apart). In addition to large interindividual differences in the verapamil plasma AUC (~5-fold), profound day-to-day intraindividual variations in plasma AUC were observed after oral administration. In one subject, the AUC deviated from one study to another by as much as 3-fold.

There are many factors that influence the rate and extent of drug absorption, which can be categorized as biological and physiochemical factors. The former include gastric and intestinal transit time, membrane permeability, lumen pH, blood flow rate and first-pass metabolism, and the latter comprise the drug's intrinsic properties such as pK_a, molecular size, lipophilicity, and solubility (Higuchi et al., 1981; Ho et al., 1983). In addition, unstirred water layer also plays a significant role in drug absorption. There is convincing evidence that an unstirred water layer is adjacent to the luminal surface of the intestinal membrane (Ho et al., 1983).

A. Drug Absorption and Concentration Gradient

After oral administration, drug absorption occurs predominantly within the small intestine, because of the large surface area provided by epithelial folding and the villous structures of the absorptive cells. In humans, the mucosa of the small intestine has a large surface area which is increased greatly by the folds of Kercking, villi, and microvilli and is approximately 200 m^2 in adults (Wilson and Washington, 1989). Drug absorption across the gut wall can be mediated by either transcellular or paracellular transport, or a combination of both. For transcellular transport, drugs are transported into and through the epithelial cells, and then into the blood circulation, whereas for paracellular transport, drugs reach the blood circulation via the tight junctions between epithelial cells. The relative contribution of the transcellular and paracellular pathway to overall absorption is highly dependent on the lipophilicity of drugs. In vitro studies with Caco-2 cells revealed that the relative contribution of the transcellular pathway was 25%, 45%, 85%, and 99% for chlorothiazide, furosemide, cimetidine, and propranolol, respectively. These values correlated well with the lipophilicity of the compounds in question, the log P values of which were $-.2$, $-.08$, .4, and 3.6, respectively (Pade and Stavchansky, 1997). From these data, it is clear that the uptake of drug into epithelial cells is not obligatory during absorption. Obviously, only those drugs that are absorbed via the transcellular, but not the paracellular, pathway are subject to intestinal first-pass metabolism.

Regardless of whether the transcellular or paracellular pathway operates, absorption rates of most drugs can be described by Fick's law of simple diffusion. The rate (amount) of drug absorbed is determined by the coefficient of diffusion, the surface are of the mucosa, and the concentration gradient of the drug (Macey, 1978). The concentration gradient across the unstirred water layer and epithelial plasma membrane leads to reduced drug concentration in the enterocytes. The magnitude of the lumen-enterocyte concentration gradient is dependent on the drug's lipophilicity and the thickness of the unstirred water layer (Ho et al., 1983). Drug in the intracellular space will continue to diffuse along a concentration gradient into capillary blood, and the mucosal capillary blood drains into the mesenteric veins which converge to form the hepatic partial vein. In addition to the mucosal blood flow, there is significant contribution from the splanchnic area, such as the splenic vein and gateric vein, to the portal blood flow. Again, this leads to reduced drug concentration in the portal vein before entering the liver. The drug concentration in the portal vein will be diluted further by intimate mixing with hepatic arterial blood before it passes evenly through the hepatocytes. Because of the mixing and dilution

effects, the drug concentration in hepatocytes would be significantly lower than that in enterocytes. As will be discussed in the next section (eqs. 2 and 5), the rate of biotransformation and the intrinsic clearance are concentration-dependent parameters. The higher the drug concentration the lower its intrinsic clearance will be. This means that saturable metabolism would be more likely to occur in the intestine than in the liver during drug absorption. Thus, the extent of intestinal first-pass metabolism is highly dependent on the oral dose. At high dose of a given drug, intestinal first-pass metabolism is expected to be appreciably less than following low doses of the same agent.

B. Saturable First-Pass Metabolism

The general equation for an enzyme-substrate interaction is as follows:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} E + P \quad (1)$$

where S and P are substrate and product concentrations, and E and ES represent unoccupied and complexed enzyme concentrations, respectively. In addition, k_1 and k_{-1} are, respectively, the kinetic constants for substrate-enzyme affinity and dissociation of the ES complex, while k_{cat} is the rate constant for product formation.

For simple enzymatic reactions, the rate (v) of product formation for a single enzyme can be described by the Michaelis-Menten equation (Segel, 1975):

$$v = \frac{V_{max}[s]}{K_m + [s]} \quad (2)$$

where V_{max} is the maximum product formation rate and K_m is the Michaelis-Menten constant. Kinetically, these parameters can be expressed as:

$$V_{max} = k_{cat} \cdot E_{total} \quad (3)$$

and

$$K_m = k_{cat} + k_{-1}/k_1 \quad (4)$$

As shown in eqs. 2 to 4, the rate of biotransformation (v) is determined by total enzyme content (E_{total}), k_1, k_{-1}, k_{cat}, and substrate concentration [s].

Metabolism by an organ, such as the small intestine or the liver, can be expressed as intrinsic clearance (CL_{int}) according to Michaelis-Menten kinetics. The CL_{int} is a measure of the summed Michaelis-Menten equation for each individual enzymatic pathway (Wilkinson, 1987).

$$CL_{int} = \sum_{i=1}^{n} \frac{V_{maxi}}{K_{mi} + [s]} \quad (5)$$

As shown in eq. 5, drugs exhibit linear elimination kinetics when the K_m for overall metabolism is substantially greater than the drug concentration [s] at the site of metabolism. In this situation, the CL_{int} can be approximated by the summed ratio of V_{max}/K_m, and is constant. On the other hand, drugs have nonlinear kinetics when the K_m is not appreciably greater than the [s], and the CL_{int} will decrease as the drug concentration increases (Lin, 1994). A drug with high hepatic and/or intestinal CL_{int} will be subject to significant first-pass metabolism. A high intrinsic clearance normally is associated with a relatively low K_m value. Midazolam, nifedipine, felodipine, quinidine, saquinavir, indinavir, tacrolimus, cyclosporine, and terfenidine have a high intrinsic clearance and are subject to a significant first-pass effect. The corresponding K_m values for these drugs are 3.7, 9, 6.9, 4, 0.35, 1.3, 6.2, 4, and 9.6 μM, respectively (Thummel et al., 1997). Thus, depending on the oral dose administered, both intestinal and hepatic first-pass metabolism may be saturated during oral absorption when drug concentrations in the enterocytes and hepatocytes exceed the K_m value. As mentioned above, saturable first-pass metabolism is more likely to occur in the small intestine than in the liver because of the substantial drug concentration gradient which exists during absorption. However, intestinal first-pass metabolism could be substantial when a very low oral dose is given.

C. Hepatic and Intestinal Organ Clearance

When a single eliminating organ is considered, the rate of drug elimination from that organ is determined not only by its intrinsic clearance, but also by the rate of drug delivery to the organ. Mathematically, the rate of organ elimination is equal to the difference between the rate of drug delivery to the organ in the inflow and the rate of its exit in the outflow. Accordingly, organ clearance (CL_{organ}) is equal to the product of blood flow to the organ (Q) and the arteriovenous concentration difference ($C_{in} - C_{out}$) normalized to the drug concentration (C_{in}) in the inflow, as defined by eq. 6 (Wilkinson, 1987):

$$CL_{organ} = \frac{Q(C_{in} - C_{out})}{C_{in}} = Q \cdot E \quad (6)$$

where E, the extraction ratio, is defined as the fraction of the drug entering the organ that is metabolized during its transit through the organ. Therefore, only a portion (1-E) of the dose passed through the organ will escape metabolism. During drug absorption, the extraction ratio (E) also is termed "first-pass" or "presystemic" elimination.

Several kinetic models of hepatic clearance that relate intrinsic enzymatic activity and hepatic blood flow have been proposed (Wilkinson, 1987). Despite some differences in describing the elimination characteristics of the liver, in all models the hepatic clearance (CL_H) is determined by the hepatic intrinsic clearance ($CL_{int,h}$), the unbound fraction of drug in the blood (f_B), and the liver blood flow (Q_h). Among the various models, the well stirred model probably is the most widely used because

of its simplicity. In the well stirred model, the liver is considered to be a single, well mixed compartment with intimate mixing between portal and hepatic arterial blood in the sinusoids. It is considered that only unbound drug can diffuse across hepatocytes. Furthermore, the unbound drug in the blood is assumed to be in equilibrium with that in the hepatocytes. Accordingly, the hepatic clearance (CL_H) and hepatic extraction ratio (E_H) for the well stirred model can be expressed in terms of eqs. 7 and 8, respectively (Wilkinson, 1987).

$$CL_H = \frac{Q_h \cdot f_B \cdot CL_{int,h}}{Q_h + f_B \cdot CL_{int,h}} \quad (7)$$

$$E_H = \frac{f_B \cdot CL_{int,h}}{Q_h + f_B \cdot CL_{int,h}} \quad (8)$$

In principle, the well stirred model also can be applied to the small intestine, when this eliminating organ is considered as a single homogenous compartment. In fact, the well stirred model has been adopted for studies on intestinal metabolism by many investigators (Gillette and Pang, 1977; Colburn, 1979; Klippert et al., 1982; Ilett et al., 1990; Thummel et al., 1997; Chiba et al., 1997), despite the anatomical and biochemical differences between the intestine and liver. The intestinal clearance (CL_G) and extraction ratio (E_G) may be expressed in terms of eqs. 9 and 10, respectively:

$$CL_G = \frac{Q_g \cdot f_B \cdot CL_{int,g}}{Q_g + f_B \cdot CL_{int,g}} \quad (9)$$

$$E_G = \frac{f_B \cdot CL_{int,g}}{Q_g + f_B \cdot CL_{int,g}} \quad (10)$$

where Q_g is the mucosal blood flow in the small intestine, and $CL_{int,\ g}$ is the intestinal intrinsic clearance. There are several anatomical and biochemical aspects of the small intestine that need special consideration. First, the distribution of drug-metabolizing enzymes is not uniform along the length of the small intestine, nor along the villi from the villous tip to the crypt. Therefore, prediction of drug elimination by the well stirred model will be less accurate for the small intestine than for the liver which has a more uniform distribution of enzymes. Another complicating factor to be considered is that the efficiency of intestinal metabolism may vary during the absorptive and postabsorptive phase. During drug absorption, the movement of drug to the site of intestinal metabolism is mediated by simple diffusion from the intestinal lumen as a result of sequential thermodynamic events. On the other hand, drugs are delivered to the site of intestinal metabolism from the systemic circulation of the mucosal capillaries during the postabsorptive phase and after i.v. administration. Thus, protein binding of drugs in the blood will limit diffusion out of the capillary when drugs are delivered from the systemic circulation, while simultaneously enhancing the "sink" effect of capillary flow by removing drugs from the site of intestinal metabolism during the absorptive phase.

Although the validity of the well stirred model with respect to intestinal metabolism has not yet been tested carefully, the model appears to predict the intestinal extraction ratio (E_G) reasonably well for low protein-binding drugs, but not for high protein-binding drugs. Using the intestinal intrinsic clearance ($V_{max}/K_{m)}$ obtained from rat mucosal cells and a literature value for mucosal blood flow, Klippert et al. (1982) successfully predicted the intestinal first-pass metabolism of phenacetin, a low protein-binding drug in control and 3-MC-treated rats. Based on in vitro data, the intestinal extraction ratio (E_G) was estimated to be in the range of 0.3 to 0.5 for 3-MC-treated rats, a value which was in good agreement with the measured in vivo extraction ratio of 0.5. Similarly, Chiba et al. (1997) predicted the in vivo intestinal extraction ratio (E_G) of indinavir (a low protein-binding drug) reasonably well from in vitro data. However, the intestinal well stirred model did not accurately predict the extraction ratio (E_G) of midazolam, a high protein-binding drug (more than 96% bound to plasma proteins). Thus, using an intestinal intrinsic clearance (V_{max}/K_m) value of 154 ml/min (Paine et al., 1997) and the reported mucosal blood flow of 250 ml/min (Hulten et al., 1977), the intestinal extraction ratio (E_G) for midazolam in humans was estimated to be only 0.06 when protein binding ($f_B = 0.04$) was taken into account (Thummel et al., 1997). This in vitro E_G was much lower than the in vivo E_G (0.43) determined in liver transplant patients during the anhepatic phase (Paine et al., 1996). However, a better estimate of the in vitro E_G (0.35) of midazolam was obtained when protein binding was not taken into consideration (Paine et al., 1997). These results led to speculation by Thummel et al. (1997) that protein binding is not an important factor in intestinal first-pass metabolism. Whether or not plasma protein binding influences the vectoral movement of drug from the intestinal lumen and the extent of intestinal first-pass metabolism remains to be carefully examined.

The intestine epithelium is considered to be much less permeable to drugs than hepatocytes, making permeability more important when dealing with quantitative aspects of intestinal first-pass metabolism. In addition, because the intracellular residence time of a drug during absorption is determined by two competing processes, the rate of drug absorption and the rate of drug removal from the enterocytes by circulation blood flow, Noordhoek and his coworkers (Klippert et al., 1983; Borm et al., 1985) have proposed a new concept of intestinal absorption clearance (CL_{abs}) for the intestinal model for the estimation of the intestinal extraction ratio (E_G). Thus, eq. 10 can be modified as following

$$E_G = \frac{f_B \cdot CL_{int,g}}{CL_{abs} + f_B \cdot CL_{int,g}} \quad (11)$$

Although this model is quite attractive, particularly for those drugs where CL_{abs} is smaller than mucosal blood flow (Q_g), the validity of the model requires more studies. Using the $CL_{int,\ g}$ and CL_{abs} obtained from rat isolated mucosal cells and intestinal perfused segments, this model predicted the in vivo E_G for hexamethylmelaine reasonably well, but not for pentamethylmelaine (Klippert et al., 1983; Borm et al., 1985).

IV. Relative Contribution of Hepatic and Intestinal First-Pass Metabolism

Oral bioavailability of a drug is defined as the fraction of an oral dose of the drug that reaches the systemic circulation. Normally, the bioavailability (F) is calculated by comparing the AUCs after p.o and i.v. administration and normalized for their doses.

$$F(\%) = \left(\frac{AUC_{p.o.}}{AUC_{i.v.}}\right) \times \left(\frac{DOSE_{i.v.}}{DOSE_{p.o.}}\right) \times 100 \qquad (12)$$

Because of the sites of administration (oral versus i.v.), the F measured at the peripheral sampling site can be viewed as the continuous mathematical product of the fractions of drug that escape loss in each successive organ (Wilkinson, 1987).

$$F = f_{abs} \cdot F_G \cdot F_H \cdot F_L \qquad (13)$$

Where f_{abs} is the fraction of drug absorbed from the intestinal lumen and F_G, $F_{H,}$ and F_L are the fractions of drug not metabolized by the intestine, liver, and lung, respectively. Assuming that first-pass metabolism by the lung is negligible ($F_L = 1$), eq. 13 can be simplified as:

$$\begin{aligned} F &= f_{abs} \cdot F_G \cdot F_H \\ &= f_{abs} \cdot (1 - E_G)(1 - E_H) \end{aligned} \qquad (14)$$

Pharmacokinetic models used to describe the absorption and disposition profiles of drugs that undergo both intestinal and hepatic metabolism have been developed by Gillette and Pang (1977) and by Klippert and Noordhoek (1983). The total body clearance (CL_{total}) and the AUC after i.v. and oral administration can be expressed as:

$$CL_{total} = CL_H + F_H \cdot CL_G \qquad (15)$$

$$AUC_{i.v.} = \frac{Dose}{CL_H + F_H \cdot CL_G} \qquad (16)$$

$$\begin{aligned} AUC_{p.o.} &= \frac{F \cdot Dose}{CL_H + F_H \cdot CL_G} \\ &= \frac{f_{abs} \cdot F_G \cdot F_H \cdot Dose}{CL_H + F_H \cdot CL_G} \end{aligned} \qquad (17)$$

By substitution of eqs. 8, 10, and 14, eqs. 16 and 17 can be rearranged as:

$$AUC_{i.v.} = \frac{Dose}{f_B(F_H \cdot CL_{int,h} + F_H \cdot F_G \cdot CL_{int,g})} \qquad (18)$$

$$AUC_{p.o.} = \frac{f_{abs} \cdot Dose}{f_B(CL_{int,h}/F_G + CL_{int,g})} \qquad (19)$$

As shown in eqs. 18 and 19, based only on measurements of AUC i.v. and AUC p.o., there are no exact solutions for the parameters, $Cl_{int,h}$, $CL_{int,\ g}$, F_H, and F_G. These parameters can be assessed only by comparing AUCs after drug administration and blood sampling at a number of sites relative to the intestine or liver using specialized surgical procedures (Ilett et al., 1990). These techniques are often used in experimental animals (Mistry and Houston, 1987; Wang et al., 1989; Lin et al., 1996) but not in humans.

V. Conceptions and Misconceptions

Although the potential importance of intestinal metabolism to the overall first-pass effect has been recognized for more than 30 years, efforts to quantify the contribution of intestinal metabolism only began in the late 1980s. Most of the evidence supporting a role for intestinal metabolism in humans has been derived from studies conducted in vitro. Because of the ethical and technical limitations, in vivo intestinal first-pass metabolism in humans often is determined indirectly by comparing the plasma AUCs after i.v. and oral administration, with assumptions which are generally untested and may be invalid.

One of these assumptions is that the systemic clearance of a drug after an i.v. dose reflects only hepatic elimination and that metabolism of the systemically available drug by intestinal enzymes is negligible. With this assumption, the hepatic extraction ratio (E_H) can be calculated directly from the observed total systemic clearance (CL_{total}) after i.v. administration and the reported value of hepatic blood flow (Q_h) according to the relationship $E_H = CL_{total}/Q_h$. The intestinal extraction ratio (E_G) then is estimated indirectly from eq. 14 by substituting the values of the calculated E_H and the observed bioavailability (F). From an anatomical point of view, this assumption is not necessarily valid. As mentioned earlier, due to the potential countercurrent exchange and plasma protein binding, the fraction of the systemically available drug metabolized by the intestine may not be as great as that which occurs during absorption. However, it is unrealistic to neglect completely the intestinal metabolism of drugs when delivered from the systemic circulation. In fact, there is mounting evidence that intestinal metabolism may contribute appreciably to the overall clearance of certain drugs following systemic administration. In a recent study, 4-methylumbelliferone metabolism by the rat intestine and liver was

investigated simultaneously in a vascularly perfused rat intestine-liver preparation in which the drug was delivered systemically (Chen and Pang, 1997). At an input concentration of 4 μM, the systemic E_G of 4-methylumbelliferone was estimated directly to be 0.57. With the same rat intestine-liver preparation, a substantial systemic E_G (0.3–0.4) was observed for salicylamide (Xu et al., 1989), gentisamide (Hirayama and Pang, 1990), and 1-naphthol (de Vries et al., 1989) when these drugs were delivered systemically. Furthermore, a significant and variable intestinal component to the metabolism of midazolam, a high plasma protein-binding drug, was observed in patients. The mean value of midazolam E_G was estimated to be 0.08, ranging from 0 to 0.25, after i.v. administration in hepatic patients during liver transplantation (Paine et al., 1996). These examples demonstrate clearly that the extent of intestinal metabolism after i.v. administration cannot be neglected. The systemic E_G not only depends on the particular drug, but also varies between individuals. Thus, it is naive to assume that intestinal metabolism will not contribute to the overall clearance of drugs after i.v. administration. As shown in eq. 15, failure to take into account the intestinal metabolism component after i.v. administration may lead to miscalculation of E_H. When this erroneous E_H then is used in eq. 14, the result is an underestimation of E_G.

Another problem in estimating E_H is the use of hepatic blood flow (Q_h). Again, it is unrealistic to assume that the Q_h is the same for all individuals. As often seen in the literature, the Q_h values in humans vary appreciably from 1200 to 1600 ml/min (Campa and Reynolds, 1988). Therefore, unless a reliable estimate of Q_h can be obtained for each individual studied, estimates of E_H will be subject to significant errors by using the average value of Q_h in the relationship $E_H = CL_{total}/Q_h$. For example, in the case of a patient whose Q_h is 1200 ml/min at the time of experiment, the E_H will be underestimated by 30% if the reported value of 1600 ml/min is used in this equation. An underestimation of E_H will result in an almost proportional overestimation of E_G, using eq. 14. This could lead to an inappropriate conclusion. Thus, for a hypothetical drug with E_G and E_H values of 0.1 and 0.5, respectively, a 30% underestimation of E_H (0.35) will result in a 3-fold overestimation of E_G (0.31). This, in turn, leads to the erroneous conclusion that the intestinal first-pass metabolism is quantitatively as important as that due to the liver, when in fact it may be much less so. Of course, if Q_H at the time of experiment is greater than the reported value, there will be an underestimation of E_G.

Another invalid assumption in estimating E_G is that the fraction of a given drug absorbed (f_{abs}) is the same for all individuals. As indicated earlier, a large number of factors can influence drug absorption. Thus, it is expected that the f_{abs} of any given drug may vary significantly within or between individuals. Equation 14 has been used to estimate E_G for midazolam (Thummel et al., 1995), verapamil (Fromm et al., 1996), and nifedipine (Holtbecker et al., 1996) using the observed F and calculated E_H. All three of these drugs are known to be well absorbed after oral dosing, and the f_{abs} was, therefore, assumed to be 1.0 (100%) for the estimation of E_G in all individuals studied. As discussed earlier, interindividual and day-to-day intraindividual variations in the absorption of verapamil can be as much as 3- to 5-fold (Eichelbaum et al., 1981a,b). Thus, it is unrealistic to assume the f_{abs} is 100% for all individuals at all times. Clearly, failure to recognize incomplete absorption will result in an overestimation of the E_G when the observed F and calculated E_H are used in eq. 14. For example, the F of a hypothetical drug ($E_H = 0.5$ and $E_G = 0.1$) will be 0.45 or 0.36 when the drug is absorbed completely ($f_{abs} = 1$) or incompletely ($f_{abs} = 0.8$). In the case of incomplete absorption (f_{abs}=0.8), if one erroneously assumes that f_{abs} is equal to 1.0, the E_G will be overestimated at 0.28 by using the observed F (0.36) and E_H (0.5). Recently, Thummel et al. (1995) have shown that the E_G of midazolam exhibits considerable interindividual variation in normal healthy subjects. In some individuals, the E_G was as high as 0.75, whereas in others, the intestinal activity was essentially absent. Because the f_{abs} was assumed to be 1.0 for all individuals in the study, the large interindividual variability in the E_G could be attributed, at least in part, to incomplete absorption of the drug after oral dosing.

The above indirect approach also has been used to study differential intestinal and hepatic induction for nifedipine and verapamil by comparing the calculated E_G and E_H before and after treatment with rifampin (Holtbecker et al., 1996; Fromm et al., 1996). The pharmacokinetics of nifedipine were investigated in six healthy volunteers before and after 7 days of rifampin treatment (600 mg/day). By comparing the plasma AUCs after i.v. and oral administration, the absolute bioavailability of nifedipine decreased from 41.3% before rifampin treatment to 5.3% after treatment, and the E_G and E_H increased from 0.218 and 0.474 before rifampin treatment to 0.758 and 0.674 after rifampin induction, respectively (Holtbecker et al., 1996). The investigators concluded that the reduction of nifedipine bioavailability during rifampin treatment was due mainly to preferential induction of intestinal enzymes. Similarly, with the same indirect approach, other investigators concluded that the intestinal metabolism of verapamil was induced preferentially by rifampin as compared to hepatic metabolism (Fromm et al., 1996). The estimates of E_G and E_H in these two studies were performed with the assumption of complete absorption which, as discussed above, may not be correct. In fact, in vitro data showed that the magnitude of intestinal and hepatic induction by rifampin was quite similar as measured by direct comparison of both CYP3A4 protein levels and catalytic activities in human intestinal and hepatic microsomes

before and after rifampin treatment (600 mg/day for 4–7 days) (Ged et al., 1989; Kolars et al., 1992).

The same indirect approach was used to differentiate the inhibitory effect of ketoconazole on the intestinal and hepatic first-pass metabolism of cyclosporine (Wu et al., 1995) and tacrolimus (Floren et al., 1997) in healthy volunteers. Based on their analysis, the investigators concluded that metabolism of cyclosporine and tacrolimus is more significant in the small intestine than in the liver after oral dosing, and that the inhibitory effect of ketoconazole is more effective in the small intestine than in the liver. With the same potential pitfalls as the other works, the assessment of E_G and E_H may be subject to errors, and the interpretation of the importance of intestinal metabolism for cyclosporine and tacrolimus may be overemphasized.

There is another widespread misconception that the intestinal metabolism of foreign compounds is induced preferentially by orally administered inducers compared with hepatic metabolism. This misconception arises in part from the general belief that there is more direct availability of the orally administered inducers in the intestine and in part from misinterpretation of the observation that the changes in the $AUC_{p.o.}$ are greater than those in the $AUC_{i.v.}$ after enzyme induction. However, mere observation of a greater change in the $AUC_{p.o.}$ than the $AUC_{i.v.}$ after enzyme induction does not necessarily reflect a greater degree of induction in the small intestine. Upon close examination of eq. 18, increases in the $CL_{int,h}$ and $CL_{int,g}$ due to enzyme induction will be offset by the multipliers F_H and F_G, which are <1 and will decrease due to induction. On the other hand, as indicated in eq. 19, the increase in the $CL_{int,h}$ upon induction will be amplified by dividing by F_G. Thus, changes in the $AUC_{p.o.}$ always will be greater than those in the $AUC_{i.v.}$ after enzyme induction.

To illustrate this point, computer simulations of the effect of intestinal and hepatic enzyme induction on the $AUC_{p.o.}$ and $AUC_{i.v.}$ were conducted. Using eqs. 18 and 19, the effect of enzyme induction on the AUCs after i.v. and oral dosing were computed for high-, intermediate-, and low-clearance drugs (Figs. 3-5). The literature values for hepatic blood flow (Q_h=1500 ml/min) and mucosal blood flow (Q_g=300 ml/min) were used for the simulations, while the factor, $f_B CL_{int,h}$, was assumed to be 6000 ml/min for the high-clearance, 2000 ml/min for the intermediate-clearance, and 200 ml/min for the low-clearance drug. The values of $f_B CL_{int,g}$ were fixed at 50%, 10%, or 0% of $f_B CL_{int,h}$ for all three classes of drugs. Furthermore, the degree of enzyme induction was assumed to be equal in the intestine and liver. As shown in Figs. 3 to 5, enzyme induction had a less profound effect on the $AUC_{i.v.}$ than on the $AUC_{p.o.}$, regardless of whether the compound was a high- or low-clearance drug. However, the differences between the percentage of change in the $AUC_{i.v.}$ and $AUC_{p.o.}$ were more pronounced for high-clearance, compared to low-clearance drugs (Fig. 3 versus Fig. 5), even when intestinal metabolism was absent (Fig. 3C versus Fig. 5C). From these computer simulations, it is clear that the increases in $CL_{int,h}$ and $CL_{int,g}$ caused by enzyme induction will have minimal effect on the AUC of high-clearance drugs after i.v. administration. In contrast, the $AUC_{p.o.}$ is sensitive to changes in the $CL_{int,h}$ and $CL_{int,g}$, regardless of whether the drug is a high- or low-clearance compound.

VI. Conclusions

Over the last 10 years, our knowledge on the subject of intestinal metabolism has increased enormously as a result of major advances in the fields of molecular biology and biochemistry. Although in vitro and in vivo data have clearly demonstrated that the small intestine plays a significant role in first-pass metabolism in certain situations, it is clear from in vitro data that both the protein level and catalytic activity of drug-metabolizing enzymes in the small intestine are generally lower than those in the liver, and that this is particularly true for cytochrome P-450 enzymes. With the limited drug-metabolizing capacity in the small intestine, the contribution of this organ to the overall metabolism of a drug is less likely to be quantitatively as important as that of the liver, unless a very small oral dose is given. Furthermore, it should be noted that extrapolation of in vitro data to in vivo situations is far from straightforward and

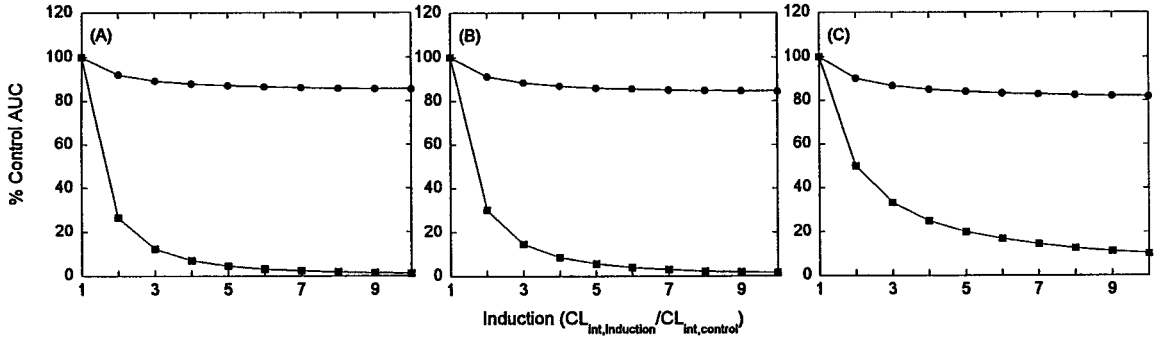

FIG. 3. Simulated effect of enzyme induction on the $AUC_{i.v.}$ (●) and $AUC_{p.o.}$ (■) of a high-clearance drug. The changes in the AUCs are expressed as percentage of the AUC before induction. The value of $f_B \cdot CL_{int,h}$ (6000 ml/min) is constant for A–C, whereas the $f_B \cdot CL_{int,g}$ is 3000 ml/min for A, 600 ml/min for B, and 0 ml/min for C.

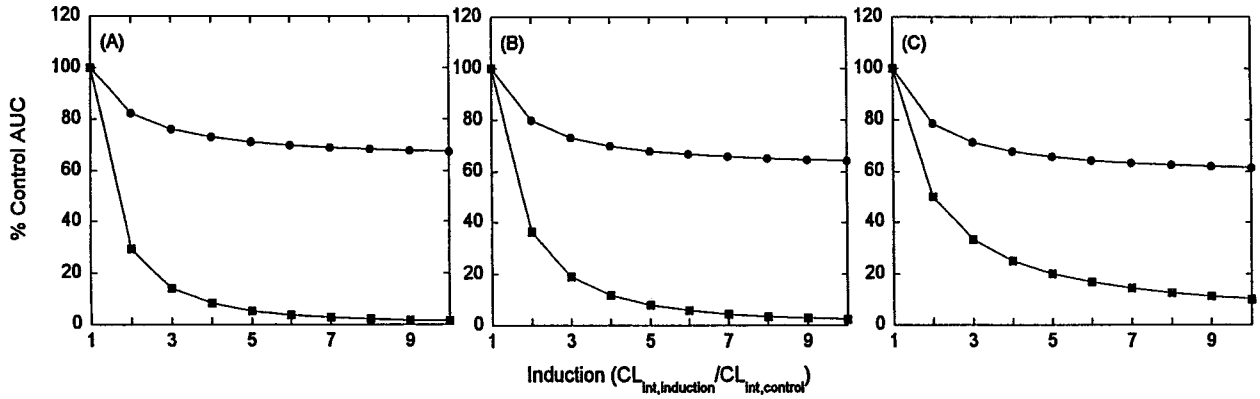

FIG. 4. Simulated effect of enzyme induction on the $AUC_{i.v.}$ (●) and $AUC_{p.o.}$ (■) of an intermediate-clearance drug. The changes in the AUCs are expressed as percentage of the AUC before induction. The value of $f_B \cdot CL_{int,h}$ (2000 ml/min) is constant for A–C, whereas the $f_B \cdot CL_{int,g}$ is 1000 ml/min for A, 200 ml/min for B, and 0 ml/min for C.

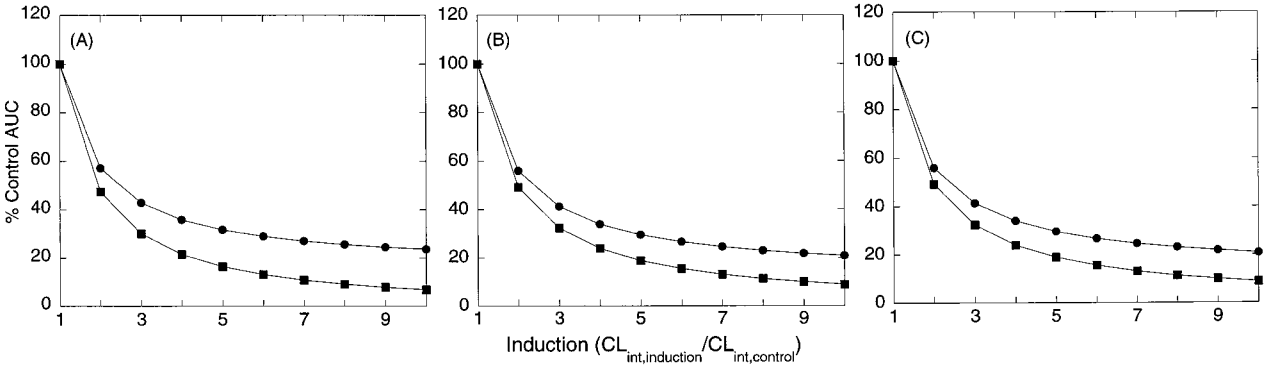

FIG. 5. Simulated effect of enzyme induction on the $AUC_{i.v.}$ (●) and $AUC_{p.o.}$ (■) of a low-clearance drug. The changes in the AUCs are expressed as percentage of the AUC before induction. The value of $f_B \cdot CL_{int,h}$ (200 ml/min) is constant for A–C, whereas the $f_B \cdot CL_{int,g}$ is 100 ml/min for A, 20 ml/min for B, and 0 ml/min for C.

should be performed with caution. In addition to issues of enzyme activity, there are many factors, such as blood flow, protein binding, and intracellular residence time, that influence both intestinal and hepatic metabolism. At the present time, quantitative aspects of the interrelationship between these factors in the intestine are not as well understood as those for the liver. Additional kinetic studies of intestinal metabolism are required to establish an appropriate model for describing intestinal metabolism in vivo.

Although many in vivo methods have been applied to assess directly intestinal first-pass metabolism in experimental animals, they have been used only rarely in humans because of ethical limitations. Alternatively, intestinal first-pass metabolism may be determined indirectly by comparing plasma AUCs after i.v. and oral administration. Often, the conclusions from the indirect AUC approaches are based on assumptions that may not be valid. Although the intestinal first-pass metabolism of some drugs is probably substantial, the role of intestinal metabolism may have been exaggerated as a result of problems inherent in the methodology currently available. We believe that the true importance of intestinal metabolism to the overall first-pass effect can be assessed only by a judicious combination of in vitro and in vivo studies, and that the results are interpreted in the context of a good understanding of intestinal physiology.

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