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Rat Small Intestinal Cytochromes P450 Probed by Warfarin Metabolism

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SUMMARY

Small intestinal cytochromes P450 (P450s) provide potential first-pass metabolism of ingested xenobiotics. To investigate this system, this study addresses the procedure for elution of enterocytes from male rat small intestine, histological evaluation of the elution procedure, and assessment of the functional microsomal P450s in small intestine of untreated and induced rats, using warfarin metabolism as a probe. Histologically it was demonstrated that villous enterocytes are initially detached in sheets and are subsequently eluted without clear resolution into villous tip, midvillous, and lower villous cells, contrary to previous reports. Crypt cells are eluted after cells from the villus. The following functional P450s were identified, using stereo- and regioselectivity of warfarin metabolism, in small intestinal microsomes: P4502B1 in untreated rats, P4501A1 in β-naphthoflavone-induced rats, P4502B1 in phenobarbital-induced rats, and P4503A1/2 in pregnenolone-16α-carbonitrile-induced rats. In contrast to hepatic microsomes from untreated or induced rats, P4502C11 and -2C6 were not present or inducible by these inducing agents in rat intestine. Western immunoblots, warfarin assays, and P450 assays all indicated that β-naphthoflavone induced P4501A1 in small intestinal villous and crypt cells, but in contrast to the liver neither apo-P4501A2 nor functional P4501A2 was induced.

P450s are ubiquitous in mammalian species, where they exhibit extensive heterogeneity. Although their preponderance in the liver has resulted in a focus of research efforts on the hepatic P450s, observations of organ-specific toxicity of xenobiotics have produced recent growth in research efforts associated with the bioactivation of xenobiotics by extrahepatic P450s (1).

Small intestinal P450s have the potential to play significant roles in environmental toxicology and in pharmacology but have received relatively little research attention (2). Because the small intestine is a major route of exposure to xenobiotics, P450-catalyzed small intestinal metabolism may detoxify these generally hydrophobic xenobiotics by facilitating their excretion to the intestinal lumen. However, such P450-mediated metabolism can activate some xenobiotics, with toxic consequences; for example, intestinal metabolism of 2,2,2,-trifluoroethanol to trifluoroacetic acid produces intestinal lesions in rats, with resultant lethality (3).

Immunoblotting techniques have demonstrated the presence of a few P450 apoproteins in small intestine of untreated rats, i.e., P4501A1 (4), -2B1 (4), and -3A1/2 (4, 5), whereas P4504A (6), -2C11 (4), and -2E1 (4, 7) have been probed for but not detected. Immunoblotting has also confirmed the inducibility of rat intestinal P450s. P4501A1 is induced by BNF (8, 9), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (10), 3-methylcholanthrene (11, 12), isosafrole (11), and indole-3-carbinol (6); P4502B1 is induced by PB (9, 13), indole-3-carbinol (6), and Brussels sprouts (6); P4502E1 is induced by ethanol (7); and P4503A1/2 is induced by DEX (5). In addition, several P450 activities have been determined in rat, mouse, rabbit, guinea pig, and human small intestinal microsomal preparations (for review, see Ref. 2), but no systematic evaluation of functional rat small intestinal P450s has been undertaken.

Many of these available data on small intestinal P450s contain inconsistencies (2). A probable basis for these inconsistencies is variation in the procedures for preparation of intestinal epithelial cell microsomes. A useful method for isolation of enterocytes for investigations of P450 should reproducibly provide separation of villous and crypt epithelial cells. Two principal methods for isolation of enterocytes for microsomal preparation are scraping and elution of the intestinal mucosa. The former procedure is based on the scraping of the mucosa with a hand-held spatula, with differential pressures for isolation of villous and crypt cells (14, 15); however, this technique is difficult to apply reproducibly. The most favored elution methods have been based on the procedures of Weiser (16), which use an eluting buffer containing EDTA and dithiothreitol. Modifications incorporating elution at 4° and tapping (13)

ABBREVIATIONS: P450, cytochrome P450; PB, phenobarbital; BNF, β-naphthoflavone; PCN, pregnenolone-16α-carbonitrile; DEX, dexamethasone; HPLC, high performance liquid chromatography; SDS, sodium dodecyl sulfate.

or shaking (5) of the intestinal preparation in the presence of the eluting buffer have yielded reproducible cellular preparations. However, the products of these modified procedures have not been well characterized.

Metabolism of the anticoagulant drug warfarin has been shown, in an extensive series of studies, to be useful as a probe of individual functional P450s in a mixture of microsomal P450s, particularly for rats (17–19). Warfarin is metabolized regio- and stereoselectively by a number of P450s, to produce metabolite profiles that are characteristic of particular forms of P450 (Fig. 1). These properties, allied with a well developed assay for simultaneous assessments of the rates of formation of all the metabolites, provide a potentially useful probe to assess the functional intestinal P450s present in untreated and induced animals.

In this paper the method for eluting enterocytes from male rat small intestine is further refined and evaluated. Warfarin metabolism is applied as a probe of functional forms of intestinal P450s and of the effects of inducing agents in enhancing levels of functional P450s in rat small intestine.

Experimental Procedures

Materials. Resolution of racemic warfarin (Calbiochem, La Jolla, CA) into the optically pure *R* and *S* sodium salts (20) and synthesis of the warfarin metabolite standards (17) have been described previously. The *R,R/S,S* and *R,S/S,R* warfarin alcohol diastereoisomeric mixtures were prepared by a previously described method (21).

PB was purchased from J. T. Baker (Phillipsburg, NJ), BNF and DEX were from Sigma Chemical Co. (St. Louis, MO), and PCN was a gift from Upjohn (Kalamazoo, MI). NADPH, NADH, and phenylmethylsulfonyl fluoride were from Sigma. Immobilon P and the semi-dry transfer apparatus used in Western immunoblotting were purchased from Integrated Separation Systems (Natick, MA). Alkaline phosphatase-conjugated goat anti-rabbit IgG, 5-bromo-4-chloro-3-indolyl phosphate (*p*-toluidine salt), and nitro-blue tetrazolium chloride were from Pierce Chemical Co. (Rockford, IL).

Treatment of animals. Male Wistar rats (232 ± 14 g) were obtained from a colony maintained by this Center. Animals were acclimated for at least 5 days at 22° with a 12-hr on/12-hr off light cycle and had free access to feed (autoclaved Agway RMH 3500 rodent chow, with 5.5% fat and 22.5% protein) and water. All procedures involving animals were approved by this Center's Institutional Animal Care and Use Committee.

For induction studies rats were treated by intraperitonal administration of inducing agent for 3 days and were killed by CO_2 overdose on day 4. Inducing agents and doses used were PB (100 mg/kg) in saline, BNF (80 mg/kg) in corn oil, PCN (100 mg/kg) in corn oil, and DEX (100 mg/kg) in corn oil. Controls received only corn oil.

Preparation of microsomes. In general, four male Wistar rats were used for each intestinal preparation. Intestinal cells were isolated by modifications of the methods of Weiser (16) and Watkins *et al.* (5).

A small intestinal segment, starting at the pyloric valve and ending at approximately 60% of the total length of the small intestine, was removed and immediately placed into freshly prepared 0.154 M NaCl solution containing 0.5 mM dithiothreitol, at 4°. Each segment was then flushed with 50 ml of solution A (1.5 mM KCl, 96 mM NaCl, 27 mM sodium citrate dihydrate, 8 mM KH_2PO_4, 5.6 mM Na_2HPO_4, pH 7.4), as described by Weiser (16). The proximal end of the intestinal segment was then clamped and the intestine was filled with solution A, clamped at the distal end, and incubated at 4° for 30 min. The solution was then drained from the distal end and replaced with EDTA-containing solution B (phosphate-buffered saline, pH 7.2, containing 1.5 mM EDTA disodium salt, 3 units/ml heparin sodium salt, and 0.5 mM dithiothreitol). The intestine was then resealed, immersed in a solution of phosphate-buffered saline, pH 7.2, containing 20% glycerol, in a plastic dish, and shaken vigorously in a shaking stand at 4° for 5 min. The solution and suspended cells were then drained into a centrifuge tube from the distal end. Each segment was refilled with fresh solution B and shaken for an additional 15 min. This process was repeated seven or eight times to collect separate and sequential samples of eluted cells, identified as elution fractions 1 through 8. The cells were pelleted by spinning at 2000 × *g* for 10 min at 4° and were washed twice with a solution containing 5 mM histidine, 0.25 M sucrose, 0.5 mM EDTA, and 230 μM phenylmethylsulfonyl fluoride (added in dimethylsulfoxide just before use), to remove mucus (13). The cells were resuspended in this solution, homogenized using a Potter-Elvehjem homogenizer, and then sonicated for 15 sec. The homogenized and sonicated suspensions were centrifuged at 12,000 × *g* for 30 min, and the supernatant was decanted, filtered through two layers of cheesecloth, and centrifuged at 100,000 × *g* for 1 hr. The microsomal pellets were resuspended in 0.1 M Tris·HCl buffer, pH 7.4, containing 15% glycerol and 0.15 M NaCl, and were used immediately or frozen at −85°.

Histology. Histological evaluations were conducted to establish the source of eluted intestinal epithelial cells and the influence of position along the small intestine on cell release, by taking 1-cm sections of small intestine from untreated and PB-, BNF-, or PCN-treated rats at distances of 5, 25, 45, and 65 cm from the pyloric valve. These sections were collected after 0, 15, 30 45, 60, 75, and 90 min of shaking in elution buffer B at 4°. In a separate study the same experimental conditions were maintained except that the intestinal preparations were not shaken. All sections were fixed in neutral-buffered formalin, stained with hematoxylin and eosin, and examined microscopically.

Analytical methods. Intestinal P450 concentrations were determined from reduced CO versus reduced difference spectra (22). Protein concentrations were determined by the Pierce bicinchoninic protein assay reagent (Pierce). Rates of formation of warfarin metabolites in intestinal microsome preparations were determined by HPLC by using

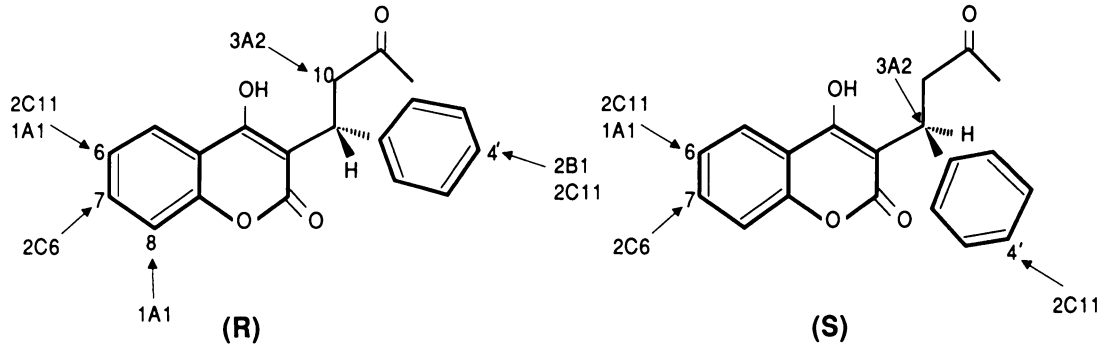

Fig. 1. Regioselectivities of purified rat liver P4501A1, -2B1, -2C6, -2C11, and -3A2 for (*R*)- and (*S*)-warfarin metabolism in reconstituted systems (19).

previously described methods (17). Alkaline phosphatase activity of 10,000 × *g* supernatant fractions from homogenized intestinal cell preparations was determined with *p*-nitrophenyl phosphate as substrate, by a previously described spectrophotometric method (16). Erythromycin *N*-demethylase activity was determined by analysis of the released formaldehyde (23).

Western immunoblotting studies on intestinal microsomal preparations used as primary antibodies rabbit anti-rat P4501A1 and P4501A2, which were purified by immunoabsorption chromatography (24) and kindly provided by Dr. Andrew Parkinson, University of Kansas Medical Center. SDS-polyacrylamide gel electrophoresis was performed by the method of the Laemmli (25); the separating gel was 7.5% total monomer concentration/2.67% crosslinking monomer concentration and the stacking gel was 4% total monomer concentration/2.67% crosslinking monomer concentration. The running buffer was 0.05 M Tris·HCl, 0.384 M glycine, 0.2% SDS. All procedures and incubations were performed at room temperature. Small intestine and liver microsomal proteins were solubilized with 1% SDS immediately after preparation and were extracted with methanol/chloroform to remove excess lipid, as described by Wessel and Flugge (26). The solubilized pellets (5% SDS) were diluted with an equal volume of 12% glycerol, 10% β-mercaptoethanol, 4% SDS, 0.05 M Tris·HCl, pH 6.8, heated to 95° for 2 min, and electrophoresed at a constant current of 25 mA/gel. The proteins were transferred to Immobilon P with a semi-dry apparatus, using buffers supplied by the manufacturer and a current of 1.5 mA/cm^2 of gel, for 1 hr. The membrane was blocked overnight in 0.2 M Tris·HCl, 0.15 M NaCl, pH 7.4, containing 0.05% Tween 20 (Tris-Tween buffer) and 3% (w/v) nonfat dry milk, and was exposed to the primary antibody in the same solution for 1 hr, with gentle shaking for both steps. The membrane was washed extensively with the Tris-Tween buffer and gently shaken for 1 hr in the same solution containing the alkaline phosphatase-conjugated second antibody. The membrane was again washed extensively with the Tris-Tween buffer, followed by brief sequential washes in 0.2 M Tris·HCl, 0.15 M NaCl, pH 7.4, and 0.1 M Tris·HCl, 0.1 M NaCl, 0.05 M $MgCl_2$, pH 9.5. Antibody-reactive proteins were visualized by reaction with the 5-bromo-4-chloro-3-indolyl phosphate/nitro-blue tetrazolium chloride substrate in the latter buffer.

Results

The progression of elution of epithelial cells from the small intestine of untreated or variously induced rats, as a consequence of the preparative technique described previously, is shown for untreated rats in a series of photomicrographs (Fig. 2). These particular intestinal sections were collected 5 cm from the pylorus and represent the intestine after 0, 15, 45, and 60 min of shaking in elution buffer. In general, villous epithelial cells (Fig. 2A) initially separated from the lamina propria (Fig. 2B). This was followed by detachment of villous epithelial sheets (Fig. 2C) before the detachment of the epithelial cells of the crypts (Fig. 2D). This process of cell detachment and elution was not homogeneous throughout the tissue, because some villi that were denuded of cells were near villi where epithelium was still partially attached. Although there was no strong indication that cells situated at different distances along the intestine were differentially susceptible to cell detachment by this process, duodenal epithelial cells appeared to detach slightly earlier from the lamina propria than did those more distally located. No microscopic difference was detected in the epithelial cell detachment process between the shaking and static incubation procedures.

Alkaline phosphatase assays of cell lysates of the various eluted epithelial cell fractions showed marked variability. However, the overall trends were for a decreasing alkaline phosphatase activity gradient from fraction 1 to fraction 8, as exemplified by data from cells from PCN-treated rats (Fig. 3).

The wet weights of isolated and washed small intestinal epithelial cells from untreated and BNF-, PB-, or PCN-induced rats as a function of the elution fraction are shown in Fig. 4. In all cases the initial two and final two fractions contained relatively small quantities of cells. The largest quantities of cells were collected in fractions 4 or 5. None of the inducing agents significantly altered the quantities of intestinal cells eluted.

The total spectrally determined P450 concentrations in small intestinal microsomal preparations from untreated and BNF-, PB-, or PCN-induced rats as a function of the elution fraction are presented in Fig. 5. All data are from microsomes frozen at −85° for at least 96 hr before thawing and analysis. Frozen and thawed microsomal preparations exhibited an apparent increase of spectrally determined P450 of 55%, compared with freshly prepared microsomes. P450 concentrations in untreated rats were constant across the various fractions. Both BNF and PB significantly increased P450 concentrations, relative to controls, but PCN did not produce significant induction of P450.

Intestinal microsomes from untreated rats yielded 4′-hydroxywarfarin as the only hydroxylated warfarin metabolite, with a marked stereoselectivity for (*R*)-warfarin. The average rate of (*R*)-4′-hydroxywarfarin formation was 12 pmol/min/mg of microsomal protein, whereas only trace quantities of (*S*)-4′-hydroxywarfarin were formed.

The rates of formation of (*R*)- and (*S*)-warfarin metabolites catalyzed by intestinal microsomal preparations from the various eluted epithelial cell fractions from BNF-induced rats are shown in Fig. 6. Warfarin was regioselectively metabolized to 6- and 8-hydroxywarfarin, with stereoselectivity for the *R*-enantiomer in both cases. Markedly smaller quantities of 4′- and 7-hydroxywarfarin were also produced. The warfarin metabolite profiles indicated that the P450 composition did not change between the various eluted fractions.

The rates of formation of (*R*)- and (*S*)-warfarin metabolites catalyzed by intestinal microsomal preparations from the various eluted epithelial cell fractions from PB-induced rats are shown in Fig. 7. Warfarin was regioselectively metabolized to 4′-hydroxywarfarin, with stereoselectivity for (*R*)-warfarin, and (*R*)-6-hydroxywarfarin was also formed, stereospecifically. Slow rates of 10- and 8-hydroxywarfarin formation were also detected, but 7-hydroxywarfarin was not detected in any of the eluted fractions.

Intestinal microsomes from PCN-treated rats were similar to microsomes from untreated rats with respect to their metabolism of warfarin regioselectively for 4′-hydroxywarfarin (19 nmol/min/mg of microsomal protein) and stereoselectively for (*R*)-warfarin. These microsomal preparations yielded trace quantities of 10-hydroxywarfarin regioselectively for (*R*)-warfarin. Similar results were obtained when DEX, either by gavage or by intraperitonal administration, was used as the inducing agent. Erythromycin *N*-demethylase activity of microsomal preparations from combined small intestinal eluted fractions 3 through 5 from PCN-treated rats was 1.8 nmol/min/mg of microsomal protein, compared with 0.3 nmol/min/mg of microsomal protein for the corresponding preparation from untreated rats.

Western immunoblots of small intestinal microsomal frac-

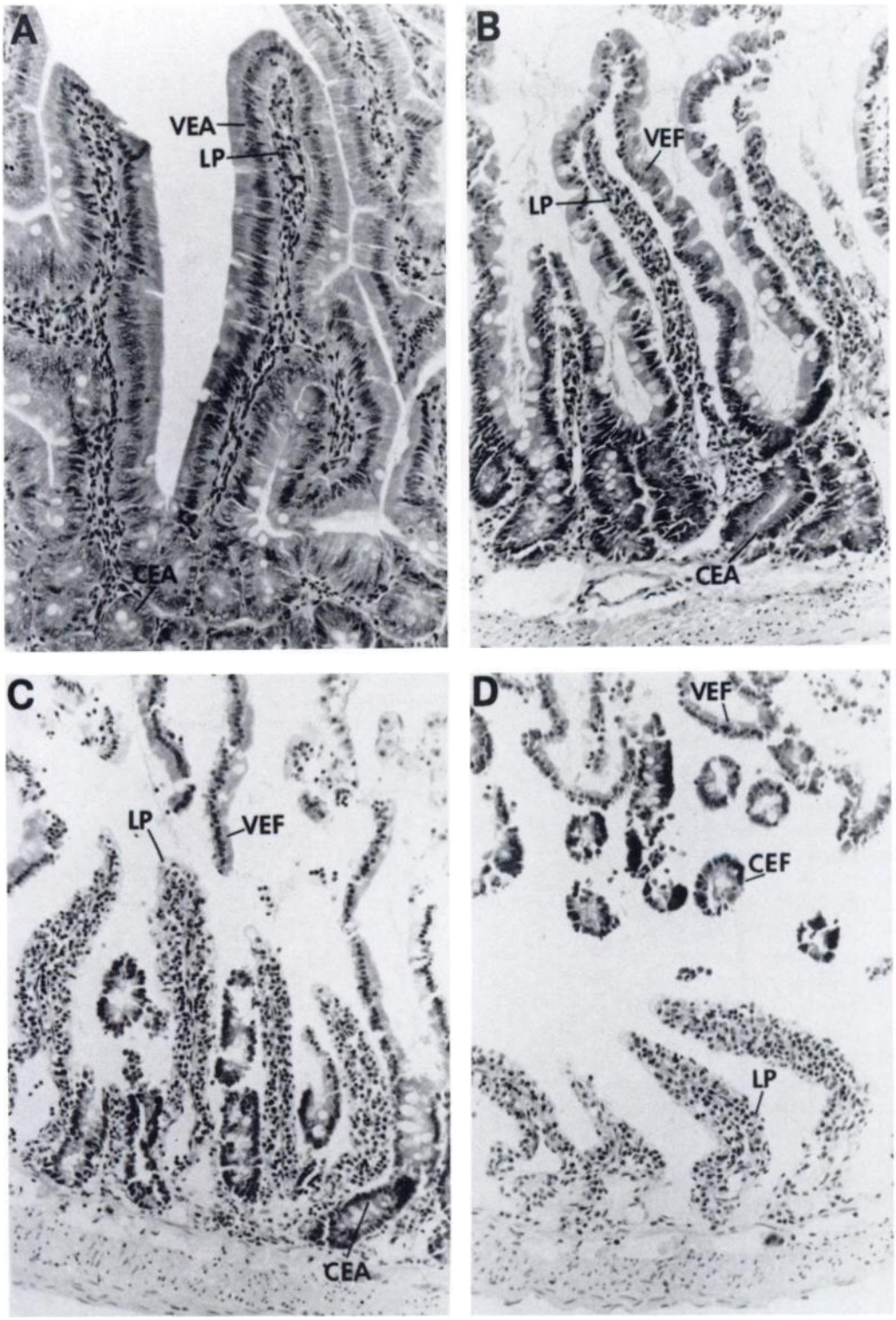

Fig. 2. Photomicrographs of untreated rat small intestinal tissue (5 cm from the pyloric valve). A, Before incubation in elution buffer, showing columnar epithelium of villi and of the crypts of Lieberkuhn attached to lamina propria. B, After 15 min in elution buffer, showing separation of intact villous epithelial sheets from lamina propria. C, After 45 min in elution buffer, showing detachment of sheets of villous epithelial cells from the lamina propria into the intestinal lumen and cells retained in the crypt. D, After 60 min in elution buffer, showing lamina propria completely devoid of villous and crypt epithelial cells. *VEA*, villous epithelium, attached; *VEF*, villous epithelium, free; *LP*, lamina propria; *CEA*, crypts of Lieberkuhn epithelium, attached; *CEF*, crypt epithelium, free. Stain, hematoxylin and eosin; magnification, 300×.

tions from untreated, PB-treated, or BNF-treated rats were used to probe the status of P4501A1 and -1A2 (Fig. 8). With monospecific anti-P4501A1 or with anti-P4501A1/2, there was no detectable P4501A1 or -1A2 in small intestinal microsomes from untreated or PB-induced rats. P4501A1 was clearly detected in all eight eluted intestinal fractions from BNF-induced rats (Fig. 8) but P4501A2 was not, under conditions where this form was clearly detected in hepatic microsomes from identically treated rats (Fig. 8).

Reduction of the warfarin ketone moiety to a secondary alcohol generates an asymmetric center. Thus, (*R*)-warfarin can be converted to the *R*,*R* and *R*,*S* diastereoisomeric alcohols and (*S*)-warfarin to the *S*,*R* and *S*,*S* diastereoisomeric alcohols. The warfarin alcohols generated from rat intestinal microsomal preparations were identified by HPLC retention time, UV

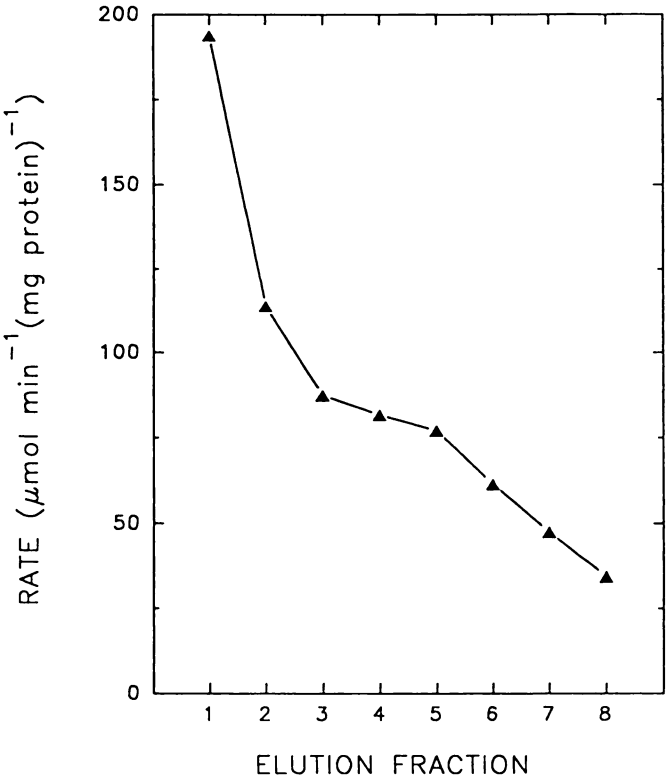

Fig. 3. Rates of alkaline phosphatase activity in whole-cell lysates from small intestinal epithelial cells of PCN-treated rats as a function of the elution fraction. The elution fractions are intestinal cell fractions eluted by sequential treatments of intestinal tissue. There are eight such elutions.

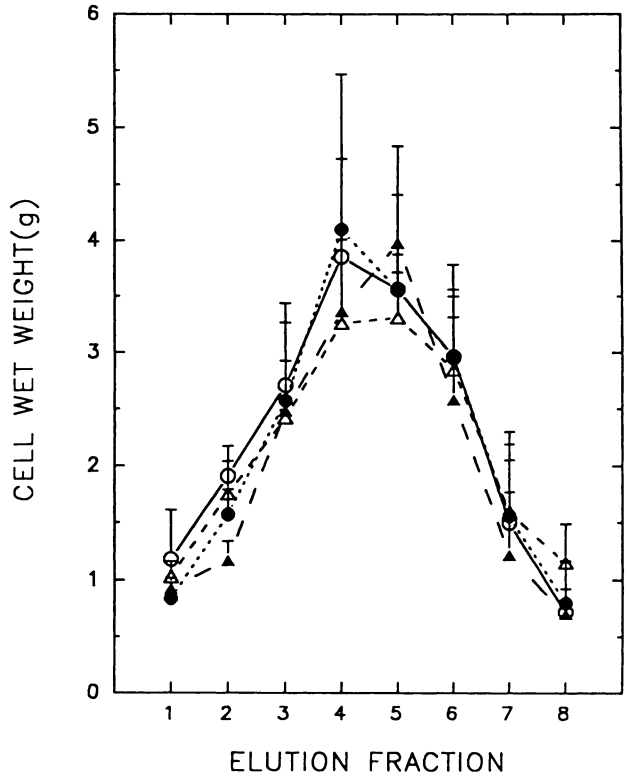

Fig. 4. Wet weight of small intestinal cells from untreated (△) and BNF- (○), PB- (●), or PCN-induced (▲) rats as a function of the elution fraction. Values are mean ± standard deviation (three experiments).

spectral comparison with a synthetic mixture of *R,R/S,S* and *R,S/S,R* diastereoisomeric pairs (21), and the enantiomeric identity of the substrate. The mean rates of formation of the alcohols and the stereoselectivity of these reactions for substrate for the intestinal microsomal fractions 3 through 6 are presented in Table 1. Microsomes from untreated and BNF-, PB-, or PCN-induced rats were all stereoselective for (*R*)-warfarin, with the BNF-induced sample being more markedly so. In all cases formation of the warfarin alcohols was NADPH dependent and essentially stereospecific for formation of *S*-alcohol product. Microsomal preparations from intestinal cell fractions 7 and 8, which showed a greater stereoselectivity for (*R*)-warfarin (data not shown) than did microsomes from fractions 3 through 6 (Table 1), yielded alcohol products in the presence of NADPH. Microsomal metabolism of warfarin to the alcohols was not inhibited when the reaction mixture was bubbled with N_2 gas or with CO/O_2 at a ratio of 2:1. Thus, the reduction of the warfarin ketone moiety is NADPH dependent and O_2 independent and is not catalyzed by a P450. Cytosol from intestinal cell fractions 3 through 6 yielded NADPH- and not NADH-dependent formation of (*RS*)-warfarin alcohol at a rate of 50 pmol/min/mg of cytosolic protein from (*R*)-warfarin and of (*SS*)-warfarin alcohol at a rate of 7 pmol/min/mg of cytosolic protein from (*S*)-warfarin. These low rates, relative to rates with microsomal preparations, indicate that the microsomal rates cannot be due to cytosolic contamination.

Discussion

The procedure for elution of intestinal epithelial cells at 37° as originally published by Weiser (16) produced inactivation of

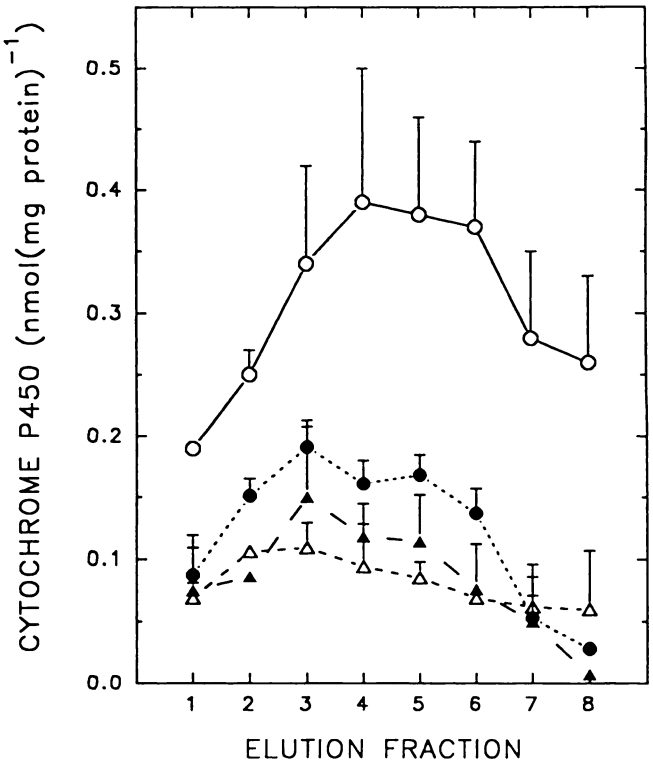

Fig. 5. Spectrally determined P450 concentrations of small intestinal microsomal preparations from untreated (△) and BNF- (○), PB- (●), or PCN-induced (▲) rats as a function of elution fraction. Values are mean ± standard deviation (three experiments).

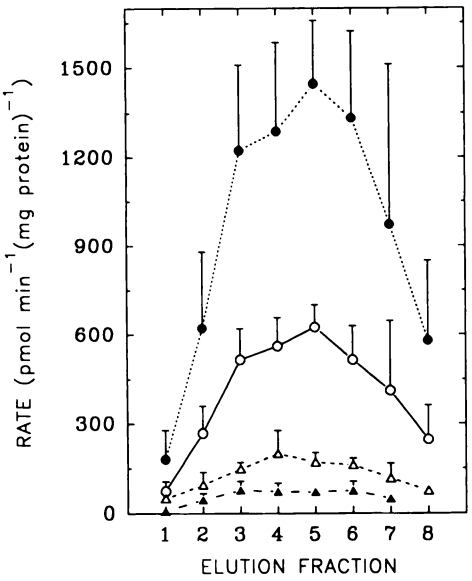

Fig. 6. Rates of formation of (*R*)-6- (○), (*R*)-8- (●), (*S*)-6- (△), and (*S*)-8-hydroxywarfarin (▲) from (*R*)- and (*S*)-warfarin catalyzed by intestinal microsomes from BNF-induced rats as a function of the elution fraction (three experiments).

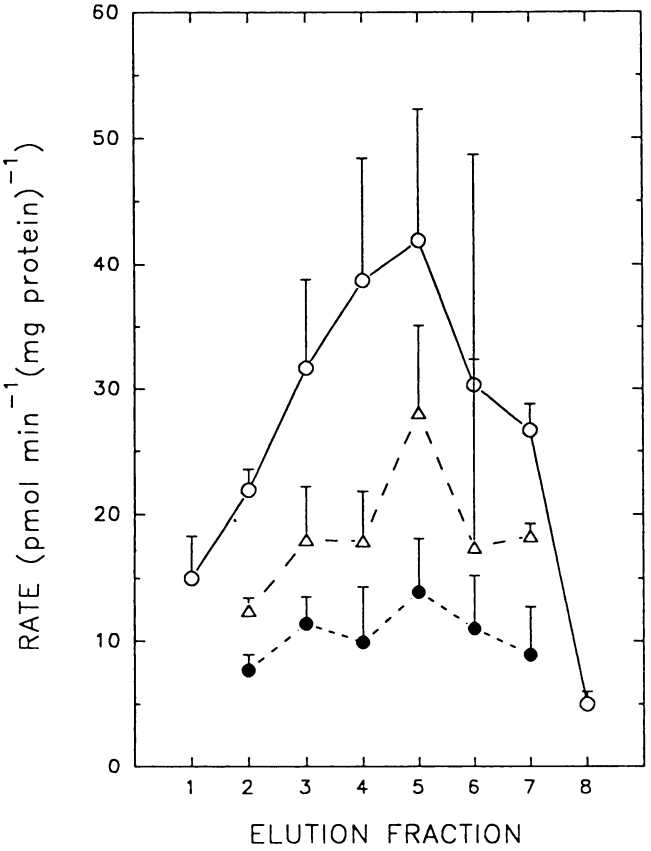

Fig. 7. Rates of formation of (*R*)-4′- (○), (*S*)-4′- (●), and (*R*)-6-hydroxywarfarin (△) from (*R*)- and (*S*)-warfarin catalyzed by intestinal microsomes from PB-induced rats as a function of the elution fraction.

the constituent P450s (5). A subsequent modification of the procedure incorporated incubations at 4°, which stabilized P450 activities in the isolated intestinal cells (5). In these studies (5, 16) the elution method for intestinal epithelial cell isolation was presumed to provide separate cell preparations, first from

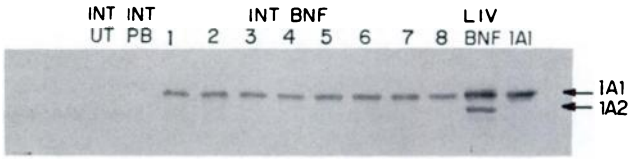

Fig. 8. Immunoblot depicting the reaction of affinity-purified antibodies to P4501A1/2 with microsomes (3 μg of protein) from small intestine (*INT*) elution fraction 4 from untreated (*UT*) and PB-treated (*PB*) rats and small intestine elution fractions 1 through 8 from BNF-treated rats (*BNF*). Purified P4501A1 (*1A1*) (1.0 pmol) and liver (*LIV*) microsomes (0.3 μg of protein) from BNF-treated rats are included for comparison. Experimental conditions were as described in Experimental Procedures.

TABLE 1

Rates and stereoselectivity of rat intestinal microsomal reduction of warfarin to the diasterioisomeric alcohols

Rates were determined by HPLC, and alcohols were identified by comparison with synthesized standards. The rates are means of rates obtained from intestinal fractions 3 through 6.

Induction	Substrate	Rate of alcohol formation				Substrate stereoselectivity, R/S
		R,R	R,S	S,S	S,R	
		nmol/min/mg of microsomal protein				
Untreated	(*R*)-Warfarin	Trace[a]	0.19			1.7
	(*S*)-Warfarin			0.11	ND[b]	
BNF	(*R*)-Warfarin	Trace	0.26			2.6
	(*S*)-Warfarin			0.10	ND	
PB	(*R*)-Warfarin	Trace	0.22			1.2
	(*S*)-Warfarin			0.18	ND	
PCN	(*R*)-Warfarin	Trace	0.25			1.3
	(*S*)-Warfarin			0.19	ND	

[a] Trace, <0.02 nmol/min/mg of microsomal protein.
[b] ND, not detected.

the villus tip and then from the midvillus, the lower villus, and finally the crypt. Evidence for this order was based on several parameters, including a decreasing gradient of alkaline phosphatase activity. Although decreasing gradients of alkaline phosphatase activity were obtained in the present studies, the histological data do not support the conclusion that villous tip, central villus, and lower villus epithelial cells are clearly resolved into separate fractions. Initial incubation of the isolated small intestine appears to loosen all of the villous intestinal cells in sheets (Fig. 2). This would appear to preclude the possibility of initial elution of villous tip cells. The histological data presented here are representative of many such studies and are very clear cut. This suggests that the interpretation of the alkaline phosphatase activity as indicating a gradient of elution from the tip of the villus down is not correct. The histological data do strongly support the differential elution of villous and crypt epithelial cells, with the latter being eluted in only the last two or three fractions.

Of the three inducing agents used in this study, BNF was the most effective in enhancing total small intestinal P450 concentrations (maximally 5.5-fold), PB was less effective (maximally 2-fold), and PCN produced no significant induction. All of the induced values are relative to values from untreated, standard diet-fed rats. Some studies have indicated that any intestinal P450s in rats fed such a diet are induced by components of the diet (27, 28), and thus these reported extents of induction are probably representative of changes not from constitutive levels but, rather, from diet-induced levels.

The warfarin hydroxylated metabolite profiles for a number of rat hepatic P450s are shown in Fig. 1. The relative formation rates of the metabolites produced by the P450 forms in Fig. 1

have been reported previously (19). We have previously made assessments of the functional P450 composition of hepatic microsome preparations from untreated and variously induced rats (29). These assessments are based on the regio- and stereoselectivities of warfarin metabolism by purified and reconstituted rat liver P450s (18, 19) and on studies that have demonstrated that these regio- and stereoselectivities determined in reconstituted systems reflect those of P450s in microsomal systems (29). A similar approach has been used in the current study to make assessments of the composition of functional P450s in intestinal microsomal preparations from untreated and induced rats.

The major hepatic P450s in untreated rats are P4502C11 [(*R*)- and (*S*)-warfarin 4′- and 6-hydroxylase], P4502C6 [(*R*)- and (*S*)-warfarin 7-hydroxylase], and P4503A1/2 [(*R*)-warfarin 10-hydroxylase] (19). In contrast, the failure of intestinal microsomes from untreated rats to produce detectable 6-, 7-, 8-, and 10-hydroxywarfarin metabolites indicates that P4502C11, -2C6, -1A1, and -3A1/2 are not detectable functionally in these intestinal preparations. However, the very low levels of erythromycin *N*-demethylase activity detected in the intestinal microsomes from untreated rats indicate that P4503A1/2 is probably present but at very low levels.

The absence of detectable P4502C11 is of particular interest because this is the major form present in liver, comprising 54% of the total hepatic P450 in untreated rats (19). The warfarin metabolite profile, which precludes the presence of functional P4501A1 in these microsomal preparations, is consistent with one immunoblot study of apo-P4501A1 (11) but not with another (4). The warfarin metabolite profile is most consistent with the presence of P4502B1 in the intestinal microsomes from untreated rats, a conclusion supported by immunoblot studies with a monoclonal antibody to P4502B1 (4).

The regioselectivity for 6- and 8-hydroxywarfarin, with stereoselectivity for (*R*)-warfarin, of intestinal microsomal preparations from BNF-induced rats resembles that of liver microsomes from these rats and is indicative of the induction of functional P4501A1. This is consistent with immunoblots of BNF-induced rat intestinal microsomes using a polyclonal antibody to P4501A1 (8, 9), which indicated the induction of P4501A1 protein. Northern blots of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced mouse intestinal RNA also demonstrated that P4501A1 mRNA was induced by this compound (30). The capacity of all of the intestinal cell fractions, including those isolated from the crypts, to catalyze warfarin metabolism to 6- and 8-hydroxywarfarin suggests that crypt epithelial cells are also inducible for P4501A1. This is supported by our Western immunoblotting studies, which demonstrate that P4501A1 protein is induced in all the small intestinal cells eluted, including those from the crypt. Although this observation is consistent with immunocytochemical evidence for the induction of P4502B1 in rat intestinal crypt cells (30), it is not consistent with the reported failure of BNF to induce P4501A1 in crypt cells (31). The Western immunoblots also confirm that P4501A2 protein is not induced in rat small intestine by BNF.

Intestinal epithelial cells from PB-induced rats yielded microsomal preparations that metabolized warfarin regioselectively to 4′- and 6-hydroxywarfarin, with stereoselectivity for (*R*)-warfarin, indicating the induction of functional P4502B1. Immunoblots of rat intestinal microsomes with monoclonal (13) and polyclonal (9) antibodies to P4502B1 have demonstrated that P4502B1 protein is induced by PB, whereas Northern blots have demonstrated that P4502B1 mRNA is induced in rat intestine by PB (32, 33). The low levels of 10-hydroxywarfarin metabolite, and associated stereoselectivities, suggest that PB induces correspondingly low levels of P4503A1/2 in rat intestinal microsomes. PB does similarly induce P4503A1/2 in rat hepatic microsomes (19). The Western immunoblots indicate that PB does not induce P4501A1 in rat small intestine, in contrast to suggestions based on activity measurements (see Ref. 2). In rat liver P4502C6 apoprotein is induced approximately 2-fold by PB, contributing 15% of the total hepatic microsomal apo-P450 and catalyzing the 7-hydroxylation of warfarin (19). The absence of any detectable 7-hydroxywarfarin formation in the intestinal microsomes from PB-induced rats demonstrates that PB did not induce functional P4502C6 in rat intestinal microsomal preparations. This result highlights an additional important difference between the regulation of rat hepatic and small intestinal P450s.

We have previously reported that PCN induces hepatic P4503A1/2 3.4-fold and hepatic microsomal 10-hydroxywarfarin hydroxylase activity 10.8-fold in rats (19). The regioselectivity of P4503A1/2 for 10-hydroxywarfarin has been clearly established in microsomal preparations and with purified and reconstituted P4503A1/2 (19). The extent of hepatic induction of P4503A1/2 is comparable to the relative extent of induction in the intestine of 6-fold, based on our erythromycin *N*-demethylase activities, although Watkins *et al.* (5) reported only a 2.2-fold increase in erythromycin demethylase activity after DEX induction. In the current study intraperitonal administration of PCN or DEX (data not shown) to rats induced trace quantities of 10-hydroxywarfarin hydroxylase activity with (*R*)-warfarin but undetectable activity with (*S*)-warfarin in intestinal epithelial cell microsomes. The corresponding preparations from untreated rats did not exhibit any detectable 10-hydroxylase activity with either (*R*)- or (*S*)-warfarin. Oral gavage of DEX, with or without fasting, did not induce any larger quantity of 10-hydroxylase activity than did intraperitonal administration. Thus, PCN and DEX induce functional P4503A1/2 in rat small intestine, based on the metabolism of warfarin and erythromycin; however, the induced concentrations of P4503A1/2 in the intestine are very low, compared with levels induced in the liver.

All of the intestinal microsomal preparations from untreated and BNF-, PB-, or PCN-induced rats exhibited a capacity to reduce the ketone moiety of warfarin in a NADPH-dependent, oxygen-independent reaction, which was not inhibitable by CO. This reduction, which is thus not P450 catalyzed, was stereoselective for (*R*)-warfarin but essentially stereospecific for *S*-alcohol formation. This activity contrasts with the corresponding rat liver activity, which is stereospecific for (*S*)-warfarin and stereospecific for *S*-alcohol formation (34).

In conclusion, warfarin metabolite profiles and Western immunoblots have been used to demonstrate that intestinal epithelial cells from untreated and induced rats express several functional P450s, some of which are inducible.

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