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Biotransformation of Xenobiotics in Human Intestinal Mucosa

by H. P. Hoensch,* R. Hutt,* and F. Hartmann*

Drug-metabolizing enzymes, especially monooxygenases, play a major role in biotransformation and detoxification of many foreign compounds including environmental carcinogens. Although largely localized in the liver they are also found in the small intestine, which is the portal of entry of dietary toxins. Therefore cytochrome P-450 content as well as monooxygenase (7-ethoxycoumarin *O*-deethylase) and NADPH-cytochrome c reductase activities were determined in surgical specimens of the human small intestine and in jejunal biopsy material obtained from patients by use of a hydraulic biopsy instrument.

Microsomes were prepared from surgical material; these ranged in P-450 content from 30 to 120 pmole/mg protein and in monooxygenase activity from 60 to 110 pmole/min-mg protein.

In the 20,000g supernatant of the homogenized biopsy material, monooxygenase activity was undetectable in patients who had total villous atrophy, and low enzyme rates were found when the mucosa showed a partial villous atrophy. The mucosal monooxygenase activity of patients with normal jejunal histology and steatorrhea was significantly higher than in mucosa with villous atrophy but was only half of that observed in normal controls. These eight control patients had normal histology and no malassimilation.

Our results suggest that monooxygenase activity in the human small intestine is dependent on the morphological integrity of the mucosa and that in normal mucosa the enzyme rates are reduced when malassimilation is present.

There is increasing evidence linking environmental pollution and intestinal cancer (*1-3*). Colon cancer constitutes a major clinical problem, since its incidence is steadily rising in the countries of the western hemisphere (*4*). Although it was originally thought, that genetic factors determine the development of colon carcinomas, it is now recognized that environmental determinants are more important (*5-7*). Among other factors, a high dietary content of saturated fat and animal meat and a deficiency of undigestable bulk material seem to be related to colon neoplasms. However the causative carcinogen and the mechanism of action of these types of diet have not been identified yet. On the other hand several carcinogenic chemicals have been found in the human diet representing either naturally occurring compounds (*8*) or environmental pollutants (*2*).

Xenobiotics are chemical compounds foreign to the human organism without nutritional value. Various types of xenobiotics which are found in human food include drugs, nitrosamines, aflatoxin, aromatic hydrocarbons, biphenyls, halogenated hydrocarbons, and cycasin.

Xenobiotics ought to be considered potential toxins, because in many instances the body is deficient in specific biological mechanisms to deal efficiently with these compounds. The intestine, particularily the epithelial mucosal cell layer, is primarily exposed to dietary xenobiotics and thus might be affected by these chemicals. On the other hand, it has been shown that the intestinal mucosa possesses enzyme systems capable of various types of biotransformation of xenobiotics (*9*). Biotransformation can lead to bioactivation and/or bioinactivation of toxic substances. The biological result of the interaction of the foreign compound with the biotransformation enzyme system depends on a variety of factors and can not be predicted. Generally, biotransformation increases polarity and water solubility, resulting in facilitation of excretion of chemicals. Wattenberg (*10*) has suggested that microsomal enzyme systems can act as a first line of defense towards luminal harmful substances. Anyway, the toxicity of luminal xenobiotics might be decisively influenced by the activity of the intestinal biotransformation enzyme

*University of Tübingen, Department of Medicine, Gastroenterology Section, D-7400 Tübingen, West Germany.

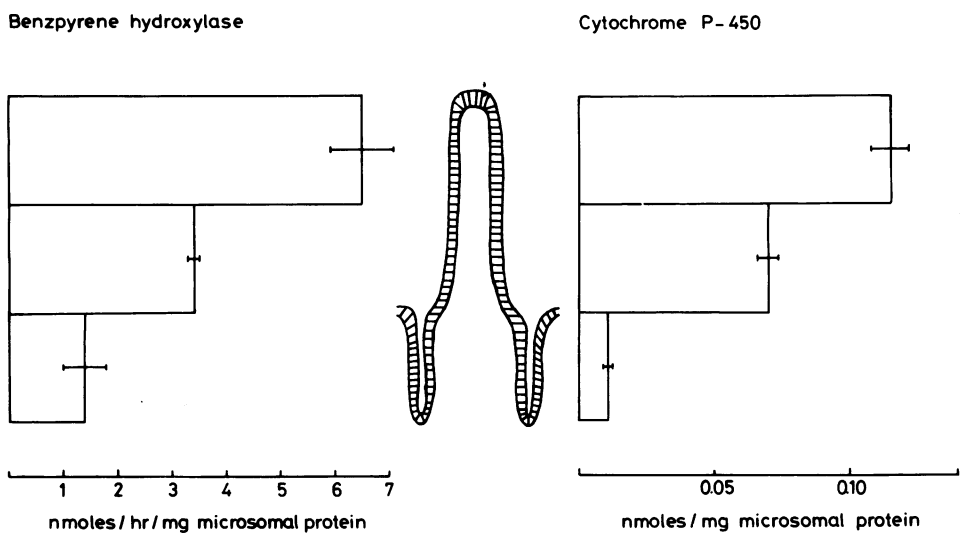

FIGURE 1. Localization of specific activity of benzo(a)pyrene hydroxylase and content of cytochrome P-450 in three cell populations of duodenal mucosa of rats: (upper bar) villous tip cells; (middle bar) lower villous cells; (lower bar) crypt cells. Values represent means ± standard error of mean of three individual experiments using mucosal cell fractions pooled from four rats on commercial rat chow diet (*13*).

system. The epithelial cells of the intestinal mucosa are equipped with enzymes which are structurally bound to the smooth endoplasmic reticulum (SER) and which catalyze three types of metabolic reactions. UDP-glucuronyltransferases (*11, 12*), monooxygenases (*13-15*) including NADPH-cytochrome c reductase and cytochrome P-450, and epoxide hydratase (*16*). Lipophilic xenobiotics of various molecular structures are initially oxidized by monooxygenase enzymes which require cytochrome P-450 as oxygen activator and NADPH-cytochrome c reductase to supply electrons (*17*). Usually the products of monooxygenation are further metabolized by epoxide hydratase to dihydrodiols and UDP-glucuronyltransferases to conjugates (*18-20*).

Localization of Intestinal Monooxygenases

In previous experiments (*13*) with rats we have shown that uninduced microsomes of the small intestinal mucosa contain stable cytochrome P-450 and cytochrome b_5 with high concentrations (50-120 pmole/mg microsomal protein) of both microsomal hemoproteins. These microsomes oxidatively metabolize benzo(a)pyrene and p-nitroanisole at rates which are approximately 10-20% of those with rat liver microsomes. Recently we found that intestinal microsomes of rats catalyze the metabolism of 7-ethoxycoumarin to umbelliferon (*21*) at rates between 25 and 45 pmole/min-mg protein (liver microsomes: 300-400 pmole/min-mg). The enzymes of the oxidative metabolism of foreign compounds are not evenly distributed within the mucosa of the gut. Figure 1 illustrates the distribution of cytochrome P-450 and benzpyrene hydroxylase in the different cell populations of the duodenum of rats. The poorly differentiated, actively dividing crypt cells contain only minute activity, whereas the highly specialized, mature villous tip cells exhibit much greater activities. In the lower villous cells an intermediate enzyme activity could be demonstrated. Moreover *p*-nitroanisol *O*-demethylase followed the same pattern, whereas the reductase and cytochrome b_5 had a less pronounced gradient. These findings suggest that metabolism of xenobiotics seems to be a property of the developed enterocytes of the tip cell region which are also most active in the absorption of nutrients.

Using villous tip cells as the source of enzyme activity we also obtained a gradient along the consecutive segments of the small intestine with highest activity in the upper region and a progressive decrease to the terminal ileum (*13*). The colon is low in benzpyrene hydroxylase (BPH) activity (*22*) in comparison to the small intestine. Recently several monooxygenase enzymes were demonstrated in rat colon (*23*) with activities of BPH less than 10% of rat duodenum. It appears, that monooxygenase activity is concentrated in the villous cells of the upper intestine, which is also the region primarily and most intensively exposed to xenobiotics in the diet.

Human Intestinal Monooxygenase Activity

Human intestinal monooxygenase activity has not been investigated so far. Since these enzymes might influence the toxicity of xenobiotics in food, we have developed a method to determine enzyme activity in intestinal surgical specimens.

Immediately after resection the gut segment was rinsed in ice-cold saline solution and frozen at −20°C for 24 hr. Subsequently the tissue was kept at −70°C for at least 24 hr. Thawing was done as a stepwise timed procedure (12 hr at −20°C, then 5 min at 4°C), resulting in thawing just of the villous cell region of the mucosa. This layer was removed from the rest of the tissue by gentle scraping with a metal spatula. In Figure 2 the histology of the mucosa before and after scraping is shown. Without scraping the histological structure of the mucosa is preserved and appears normal. After the procedure the villi are denuded of epithelial cells, leaving the lamina propria and the crypt cell area intact. From 10 coded specimens the pathologist identified correctly the five scraped samples as described earlier (loss of epithelial villous cells). In addition stereomicroscopy demonstrated lack of villous structure in the scraped material, although due to Kerckring's folds not all villi were removed completely by scraping. The scraped material was homogenized, sonicated, centrifuged at 20,000*g* for 10 min and the supernatant sedimented at 104,000*g* for 1 hr. The microsomal pellet was washed once in 1.15% KCl solution and suspended in 0.1*M* potassium phosphate buffer (*p*H = 7.4, K_2HPO_4-KH_2PO_4) to yield a concentration of 2-3 mg microsomal protein/ml, equivalent to 200 mg wet weight of mucosa/ml.

To validate the microsomal preparation, the 104,000*g* pellet was subjected to transmission electron microscopy. As shown in Figure 3, the typical vesicular structures of the smooth endoplasmic reticulum (SER) could be demonstrated. Moreover, there was no contamination by mitochondrial fragments observed. The mitochondrial marker enzyme glutamate dehydrogenase was only present in trace activity in the microsomal suspension, while high rates of this enzyme were measured in the 20,000 *g* pellet and the homogenate. Small amounts of amorphous granular material were seen which could not be identified but presumably constitute nuclear breakdown products. Some of the vesicles might represent microvillous remnants although no attempts were made to differentiate these particles from SER. With 7-ethoxycoumarin as a model substrate (*21*) we were able to demonstrate monooxygenase activity in microsomes of human small intestinal mucosa. This was possible by using thin-layer chromatography to separate the substrate and the product of the enzyme reaction (*24*). In the microsomal suspension the specific activity of the microsomal marker enzyme, NADPH-cytochrome c reductase, was three times higher than in the homogenate. Additionally specific microsomal monooxygenase activity (EOD, BPH) was raised threefold compared to the homogenate.

Intestinal human microsomes contained cytochrome P-450 of high concentrations (Fig. 4 and Table I). The difference absorption spectrum of the CO versus reduced microsomal suspension (*25*) is shown in Figure 4 for microsomes from jejunum and ileum. The shape of the peak is similar to the liver P-450 spectrum, although we consistently noted a shift of the peak to wavelengths between 451 and 453 nm. This suggests that the intestinal P-450 differs from the type found in the liver.

In Table 1 the results of the cytochrome and enzyme determinations in surgical material of the small

Table 1. Cytochrome and protein content and specific enzyme activity of microsomes from villous cells of intestinal mucosa of patients.[a]

Diagnosis	Localization	Cytochrome P-450, nmole/mg	Cytochrome b_5, nmole/mg	7-Ethoxycoumarin O-deethylase, pmole/mg-min	NADPH-cytochrome c reductase, nmole/mg-min	Protein, mg/g mucosa
Gastrectomy of BI stomach with gastric carcinoma	Duodenum	0.092	0.245	64.8	20.7	15.2
Gastrectomy of BII stomach with gastric carcinoma	Afferent loop	0.039	0.233	ND	ND	10.3
	Efferent loop	0.090	0.274	108.9	18.4	10.0
Gastrectomy of BII stomach with ulcer	Afferent loop	0.036	0.339	64.4	19.7	6.5
	Efferent loop	0.072	0.348	66.0	20.2	9.7
Gastrectomy of BII stomach with gastric carcinoma	Efferent loop	0.119	0.181	101.9	21.6	13.5
Traumatic intestinal injury	Ileum	0.031	0.146	61.5	13.6	8.4

[a]ND = not determined.

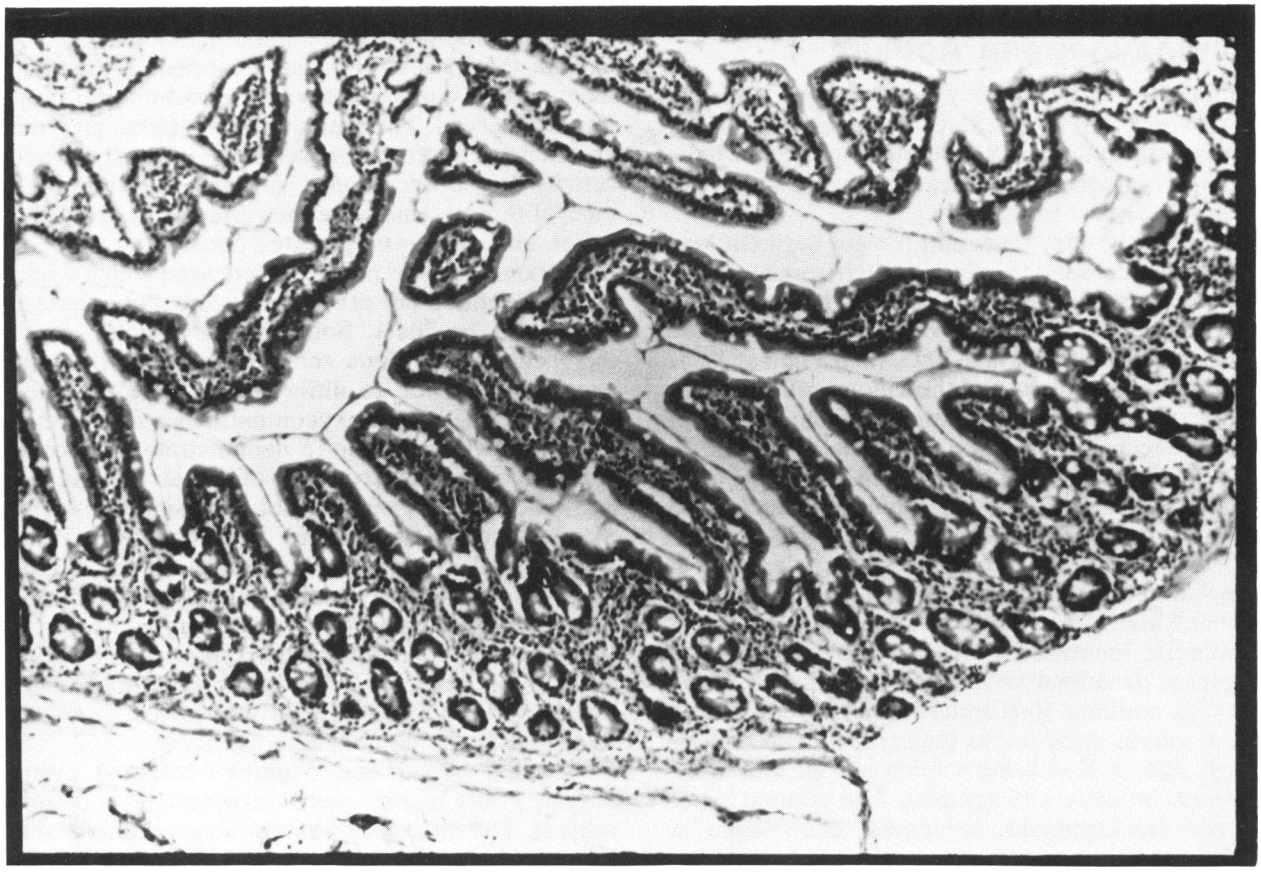

FIGURE 2*a*. Histology of human small intestinal mucosa before the scraping procedure. Hematoxylin-eosin stain. 16×.

intestine are presented. We were able to obtain intestinal tissue of sufficient quantity from patients who developed gastric cancer (n = 3) and gastric ulcer (n = 1) after gastric surgery for benign ulcer disease. One patient had a resection of the ileum after traumatic damage of the small intestine due to a traffic accident. Cytochrome P-450 content ranged from 30 to 120 pmole/mg microsomal protein, with higher values in the duodenum and efferent loop than in ileum and afferent loop.

Although cytochrome P-450 content was comparable to that in rat small intestinal mucosa, cytochrome b_5 was 2-5 times higher in human gut mucosa. Monooxygenase activity paralled cytochrome P-450 content and varied between 60 and 110 pmole/min-mg protein. NADPH-cytochrome c reductase activity was not dependent on the localisation of the gut segment. Protein and time dependencies as well as Michaelis-Menten kinetics of the enzyme reactions were established and indicated optimal enzyme conditions.

The limited supply of surgical specimens and the selected disease spectrum of these patients do not allow studies of intestinal monooxygenase activity in relation to various gastrointestinal disorders prevalent in internal medicine. Therefore we developed a technique to investigate enzyme activity in biopsy material of the small intestine taken with the hydraulic biopsy instrument (7 mm, Quinton Instruments, Seattle, Washington, USA) (*26*).

Specimens of 6-8 particles per patient were obtained from the upper jejunum, subjected to stereomicroscopy, and one or two samples were subjected to histological investigation. The remainder was homogenized, sonicated, and enzyme assays (EOD, reductase) were performed on the 20,000 g supernatant which contained the equivalent of 50 mg wet weight/ml of biopsy material.

As expected, in four patients with total villous atrophy due to gluten-sensitive enteropathy (n = 3) and malignant lymphoma (n = 1) no detectable EOD activity was found (Table 2). Partial restoration of mucosal villous structure by treatment with gluten-free diet (n = 3) was associated with appearance of low EOD activity, which was also observed in three cases with partial villous atrophy associated with

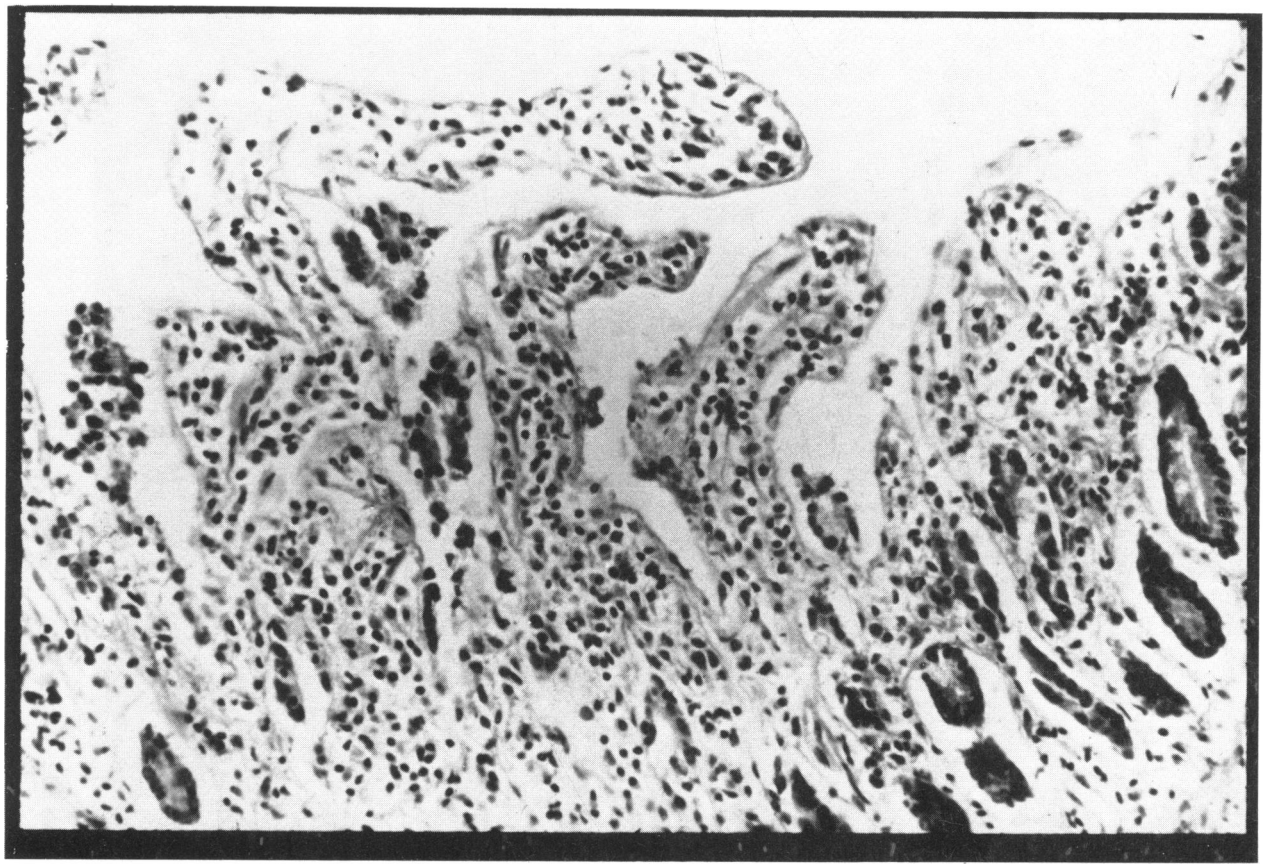

FIGURE 2*b*. Histology of human small intestinal mucosa after the scraping procedure. Hematoxylin-eosin stain. 16×.

Crohn's disease, hypogammaglobulinemia, and excretory pancreatic insufficiency (n = 1, each).

When a normal histology was demonstrated by light microscopy but signs of malassimilation were present, a further increase in enzyme activity compared to the first two groups of patients was found (Table 2). These patients (n = 9) had steatorrhea of various etiology (Crohn, with and without ileum resection, n = 5; exudative enteropathy, chronic pancreatitis, anorexia nervosa and idiopathic steatorrhea, n = 1 each). Previously we have reported, that in exocrine pancreatic insufficiency with steatorrhea

Table 2. Specific enzyme activities and protein content of small bowel biopsy specimens from patients with various intestinal diseases[a]

Group	Diagnosis	7-Ethoxycoumarin *O*-deethylase, pmole/min-mg	NADPH-cytochrome c reductase, nmole/mg protein-min	Protein, mg/10 mg of wet weight
A	Total villous atrophy	0 (4)	4.62 ± 0.72 (4)	0.58 ± 0.18 (4)
B	Partial villous atrophy	2.32 ± 2.36 (6)[b]	8.59 ± 5.46 (5)	0.85 ± 0.19 (6)
C	Normal jejunal histology with malassimalation	4.74 ± 2.11 (g)[c]	11.89 ± 2.57 (6)[d]	0.76 ± 0.15 (g)
D	Normal jejunal histology without malassimilation	9.94 ± 2.94 (8)	13.38 ± 3.18 (7)[d]	0.79 ± 0.07 (8)

[a]Values represent mean ± standard deviation of individual results obtained with 20,000*g* supernatant of homogenates from 3-6 biopsy particles pooled per patient. The number of patients per group is given in parentheses. For calculation of significance the Wilcoxon test of unpaired samples was used.

[b]Significantly lower than D at $p < 0.01$.

[c]Significantly lower than D at $p < 0.05$.

[d]Significantly higher than A at $p < 0.01$.

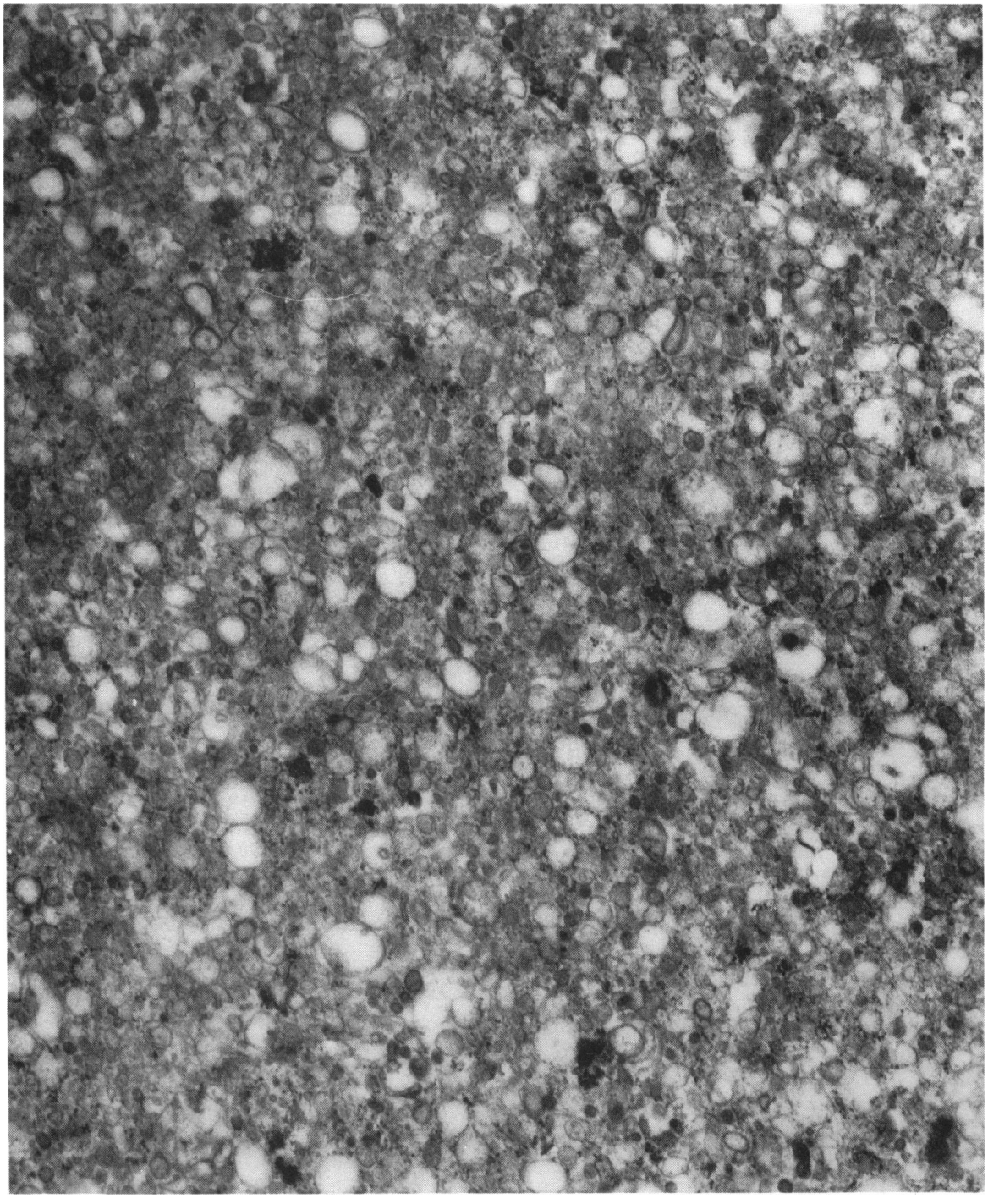

FIGURE 3. Transmission electron microscopy of microsomal pellet from human small intestine, 25,000 X.

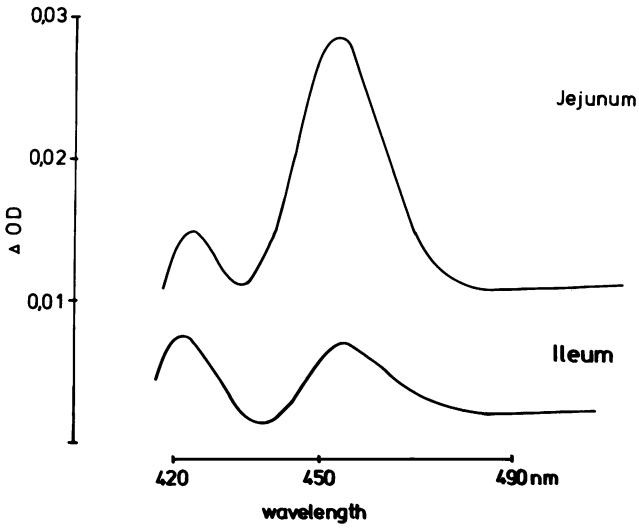

FIGURE 4. Original recording of difference absorption spectrum of cytochrome P-450: microsomes of (top) human jejunum and (bottom) human ileum. The microsomal suspensions (1.7-2.0 mg protein/ml = 200 mg mucosa wet weight/ml) were reduced with $Na_2S_2O_4$ and the sample cuvette was gassed with CO.

a reduction of EOD was observed (*27*). Eight patients with normal intestinal histology and no abnormal intestinal function tests were shown to have the highest monooxygenase activity [irritable bowel syndrome (n = 2), inactive pancreatitis (n = 2); lambliasis, intestinal pseudo-obstruction, diabetes mellitus, alcoholic hepatitis (n = 1) each]. The NADPH-cytochrome c reductase activity of the two groups with normal histology was significantly higher than that of those with total villous atrophy (Table 2). The protein content of the 20,000g supernatant did not differ significantly between the groups. These results indicate that loss of or damage to the villous cells is associated with a lack or severe reduction of intestinal monooxygenase activity. Moreover pathological alteration of the intraluminal gut content as seen in steatorrhea depresses the enzyme activity of the upper gut mucosa by about 50% in spite of normal findings by light microscopy. While the decrease in the number of mature villous cells in villous atrophy easily explains the depression of monooxygenase activity, the reason for the enzyme reduction in normal mucosa with steatorrhea is less obvious. In rats, a diet high in saturated fatty acid has been shown to decrease intestinal BPH activity (*28*). Possibly changes in the lipid environmental of the SER might be involved. On the other hand, subtle structural and functional alteration of the mucosa associated with steatorrhea appear to be conceivable (*29, 30*).

The biological consequences of the reduced activity of enzymes involved in biotransformation of foreign compounds are not known yet. It is conceivable however, that the altered metabolism of xenobiotics in the intestine might change the fate of these compounds in the body. This could lead to modified rates of activation, detoxification, or accumulation of dietary toxins with as yet undetermined biological effect not only to the gut mucosa but also to other organs beyond the intestinal barrier. Furthermore, the effect of carcinogenic compounds in the diet might be influenced by different enzymatic rates of intestinal monooxygenases possibly leading to changes in cancer incidence.

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