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LOCALIZATION AND CHARACTERIZATION OF DRUG-METABOLIZING ENZYMES ALONG THE VILLUS-CRYPT SURFACE OF THE RAT SMALL INTESTINE—I.

MONOOXYGENASES

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Abstract—To investigate the drug-metabolizing potential of different sub-populations of cells along the villus-crypt surface of the small intestine, the major monooxygenase activities directed towards the substrates benzo[*a*]pyrene (BP), 7-ethoxycoumarin and ethylmorphine were studied. The cells were isolated in sequential fractions corresponding to the villus tip-to-crypt gradient in the small intestinal epithelium of the rat. Cells from the upper- and mid-villus regions were rich in aryl hydrocarbon (BP)hydroxylase (AHH) and 7-ethoxycoumarin deethylase (7-ECDE) activities whereas in crypt cells the activities of these enzymes were at the level of detectability. Ethylmorphine demethylase (EMD) was not detectable in the entire villus-crypt surface. The intestinal epithelial cells responded strongly to inducers. 3-Methylcholanthrene (3-MC), given to rats 24 hr previously, induced increases in AHH activity of 4- to 7-fold in the villus and of 19- to 26-fold in the crypt cells. 7-ECDE had a similar pattern. The induced level of monooxygenase activity in crypt cells was sustained for a longer time, followed in order by consecutively higher cells of the villus. Phenobarbital caused maximal expression of EMD activity in the mid-villus region whereas the activity in crypt cells was half the maximal activity. PB also significantly induced AHH and 7-ECDE in the intestinal epithelium. 7,8-Benzoflavone inhibited AHH activity to the same degree in all the cell fractions. The apparent K_m for AHH was 5 μM (BP). Treatment of rats with 3-MC, after 24 hr, enhanced the K_m and V_{max} differently in the cells along the villus-crypt surface. The K_m value in the villus region increased, whereas it did not change in the crypt cells; V_{max} increased 6-fold in the villus and about 12-fold in the crypt cells, above their basal levels.

The results suggest that the intestinal cells are capable of biotransforming various xenobiotics. The higher sensitivity of their monooxygenases, particularly of the crypt cells, might protect them directly or render the cells capable of generating metabolites that aid and abet toxicity toward target tissue *in vivo*.

The small intestine is one of the major avenues for the entry of large numbers of chemicals and drugs into the body. The cells responsible for their absorption and transport are the epithelial cells lining the villus-crypt surface of the intestinal lumen. The cells in the crypt actively divide, differentiate and migrate upward to the villus-tip so that the cells in the upper third of the villus are fully mature and differentiated. Various studies suggest that the drug-metabolizing activity of the small intestine could be modulated to actively metabolize xenobiotics, modifying their fate and bioavailability [1] although basal drug-metabolizing activity is considerably less than in the liver [2]. A number of reports describe the high sensitivity of intestinal monooxygenases to induction by polycyclic aromatic hydrocarbons and to compounds present in cigarette smoke or certain foods [2–5]. The activity of the monooxygenases is highest in the proximal part of the small intestine [2, 6], declining towards the caudal end. The activity on the villus-crypt surface has been found to be mainly localized in the villus cells and to be almost at the level of detection in the crypts [7–9] where cell proliferation is actively occurring.

The various cell types along the villus-crypt surface represent different stages of differentiation and proliferation. There is a possibility that these cell types and their monooxygenases may express differential sensitivities towards xenobiotics or their modulators. This would affect the steady-state levels of drugs and environmental carcinogens *in situ* and may simultaneously influence the functions of these cells. Further, the consequences of damaging the highly proliferating crypt cells would be most serious if these cells were preferred for cytotoxic and genotoxic actions of xenobiotics. Thus, it is important to know the capacity of the cells along the villus-crypt surface for activation and inactivation of chemicals mediated by drug-metabolizing enzymes. This would involve the isolation of the epithelial cells corresponding to the villus tip-to-crypt surface of the small intestine so that the drug-biotransforming competence of these cells could be quantitated, localized and characterized.

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A few attempts have been made in the past to localize AHH* activity along the villus-crypt surface either histochemically [6] or biochemically [9]. These studies, however, did not examine the abilities of the various cell types of the villus-crypt surface to oxidize other model drug substrates, i.e. they did not examine the presence and activity of other cytochrome P-450 isozymes. Cytochromes P-450 exhibit a wide variety of substrate specificities, differential induction patterns and tissue distributions [10]. Based on various lines of evidence, the cytochromes P-450 are broadly classified into two major groups: (1) the cytochrome P-450 forms that are present predominantly in liver and are inducible by compounds typified by phenobarbital (PB) and that express preferential specificities towards substrates, e.g. aldrin, ethylmorphine, nitrosamine or aflatoxin B_1 [11–15], and (2) the cytochrome P-448 forms that are ubiquitous in nature and inducible by polycyclic aromatic hydrocarbons [11, 12, 14]. Benzo[*a*]pyrene is metabolized by cytochrome P-448 forms whereas 7-ethoxycoumarin is metabolized by both types of monooxygenases [11].

In the present study we have tried to systematically quantitate and characterize the major monooxygenase activities (directed towards the substrates, benzo[*a*]pyrene, 7-ethoxycoumarin and ethylmorphine) in cells isolated in sequential fractions [16] that correspond to the villus tip-to-crypt gradient in the small intestinal epithelium of the rat. This and the accompanying paper [17] attempt to provide some understanding of the drug-metabolizing potential of the rat small intestinal cells.

MATERIALS AND METHODS

Chemicals. Benzo[*a*]pyrene (BP), 7-ethoxycoumarin (EC), umbelliferone, 7,8-benzoflavone (7,8-BF), 3-methylcholanthrene (3-MC), bovine serum albumin, dexamethasone and γ-glutamyl-*p*-nitroanilide were procured from the Sigma Chemical Co., St. Louis, MO, U.S.A. NADPH and NADH were obtained from Boehringer Mannheim GmbH, West Germany; phenobarbital-sodium salt (PB) was from Merck & Company, Inc., Rahway, NJ, U.S.A., ethylmorphine-HCl and 3-hydroxybenzo[*a*]pyrene were gifts from Drs. T. Wolff and F. J. Wiebel of the Department of Toxicology, GSF, D-8042 Neuherberg, Munchen, West Germany, Radioactive [*methyl* ^{3}H]thymidine (sp. act. 18,800 mCi/mmol) was obtained from the Bhaba Atomic Research Centre, Bombay. All other chemicals used were of analytical grade.

Animals and treatment. Adult male albino Charles Foster rats (200–220 g body wt) maintained on standard pelleted food (Hindustan Lever, Bombay) and water *ad lib.* were used. The animals received a single oral dose of 1.0 ml coconut oil or 3-MC (40 mg/kg body wt) in 1.0 ml coconut oil. PB (80 mg/kg body wt) in 1.0 ml normal saline was administered i.p. to the rats. The animals were kept for another 5 days on drinking water containing 0.1% of PB, and 12 hr before sacrifice the PB water was replaced by tap water. Wherever required, dexamethasone (55 μg/kg body wt) or theophylline (20 mg/kg body wt) was administered orally in 0.1 ml coconut oil daily for 5 days. One day before sacrifice the rats from PB, PB and dexamethasone, or PB and theophylline groups were given an oral dose of PB (120 mg/kg body wt) in 1 ml of normal saline.

Isolation of small intestinal epithelial cells. Cells from small intestinal epithelium were isolated by the method of Weiser [16]. The method involves loosening and dissociating cells through chelation and, probably, –S–S– reduction at the baso-lateral attachments. By a series of incubations and washings of gut loops, sequential fractions of isolated epithelial cells are obtained. Briefly, the animals were killed by cervical dislocation, and a segment (about 50 cm) of the small intestine 10 cm beyond the pylorus end of the stomach was excised. The lumen was flushed with saline containing 1 mM dithiothreitol at 37°. Gradient elution of the small intestinal epithelial cells from the villus-tip to the crypt region was accomplished in nine fractions of cells isolated by incubation of the intestine with cell eluting buffer for different specified time intervals of 4, 2, 2, 3, 4, 5, 7, 10 and 15 min. The isolated cell fractions were washed with phosphate-buffered saline (PBS) at 0–4°. The nine isolated cell fractions were pooled sequentially into three categories for certain studies: fractions 1, 2 and 3 were pooled to represent upper villus; fractions 4, 5, 6 and 7 as mid and lower villus; and fractions 8 and 9 as crypt cells. The cells in small batches were frozen in liquid nitrogen and stored at −70° without any loss of activity for at least 7 days. The cells were thawed, stirred on a vortex, and used to assay monooxygenase and other enzyme activities. The frozen-thawed cell preparations exhibited enzyme activities almost the same as those of freshly isolated cells homogenized in PBS using a Potter–Elvehjem type glass homogenizer.

To establish that the sequentially isolated cells were specifically from the villus tip-to-crypt region [16], a systematic study of the pattern of distribution of brush border marker enzymes, showing contrasting areas of activities, was done. Enzymatic activities of sucrase [18], alkaline phosphatase [16] and γ-glutamyltranspeptidase [19] were determined. Radiolabelled thymidine incorporation along the intestinal mucosa was accomplished by injecting (i.p.) 100 μCi of [*methyl*-^{3}H]thymidine. Three hours after injection, the animals were killed, and the cells were isolated. The cell protein was precipitated with chilled (10%, w/v) trichloroacetic acid; the precipitate was washed with 5% TCA followed by subsequent washing with 95% alcohol containing 5% potassium-acetate, alcohol:ether (3:1) and ether. The pellet was dissolved in 1 N NaOH, and the radioactivity was measured in Bray's scintillation fluid using a Beckman LS-3150P scintillation counter.

Assay of monooxygenase activities. Ethylmorphine demethylase (EMD) was assayed as described by Mazel [20] based on the method of Anders and Man-

* Abbreviations: AHH, arylhydrocarbon hydroxylase; 7-ECDE, 7-ethoxycoumarin deethylase; EMD, ethylmorphine demethylase; BP, benzo[*a*]pyrene; EC, 7-ethoxycoumarin; 7,8-BF, 7,8-benzoflavone; 3-MC, 3-methylcholanthrene; and PB, phenobarbital sodium salt.

nering [21]. Aryl hydrocarbon hydroxylase (AHH) using BP as substrate was measured according to Wiebel *et al.* [22]. The phenolic products formed were quantitated using 3-hydroxybenzo[*a*]pyrene as a standard. Fluorescence was determined with an excitation setting of 396 nm and an emission wavelength of 520 nm in a Kontron spectrofluorometer (Kontron Instruments Ltd., St. Albans). 7-Ethoxycoumarin-*O*-deethylase (7-ECDE) activity was determined by the fluorometric method of Greenlee and Poland [23].

Protein was determined by the method of Lowry *et al.* [24] with bovine serum albumin as standard.

RESULTS

Isolation of epithelial cells from villus tip-to-crypt gradient. The enzymatic activities of γ-glutamyltranspeptidase, sucrase and alkaline phosphatase were highest in the differentiated cells of the villus region, and their activities declined progressively towards the crypt region where these activities were at the level of detectability (Fig. 1). In contrast, the incorporation of [^{3}H]thymidine was highest in the crypt cells and declined steadily towards the villus tip. These data are consistent with the observations of others [9, 16, 25] which confirm that the procedures of isolating cells correspond to the villus tip-to-crypt distance.

Effect of monooxygenase inducers on the activities of AHH and 7-ECDE along the villus-crypt surface of intestinal epithelium. The biochemical localization and distribution of AHH activity are shown in Fig. 2. The enzyme activity was predominant in the upper and mid-villus region and at the level of detection in the crypt region. However, a single oral dose of 3-MC induced AHH from 4 to 7-fold in the villus cells and 19- to 26-fold in the crypt cells; the extent of AHH induction tended to increase towards the proliferating zone. A significant induction of AHH activity by PB occurred in almost all the cell fractions, though these were relatively weaker than those obtained by 3-MC. The enzyme activity was induced 3- to 6-fold in crypt cells and 50–100% in cell fractions from the villus, above their corresponding untreated controls. The extent of induction by 3-MC was at least 4- to 5-fold higher than that obtained by PB.

The biochemical localization and the distribution of 7-ECDE along the villus-crypt surface followed a pattern similar to that of AHH (Fig. 3). The enzyme activity was highest in the upper-villus region and decreased steadily to the crypt cells where it was one-fifth of the upper villus. 3-MC induced 7-ECDE increasingly in all the cell fractions beginning from villus tip-to-crypt. The extent of increase was from villus tip (2-fold) to the crypt cells (7-fold) compared to the cells from control rats. The extent of induction was lower than that of AHH. PB also induced 7-ECDE significantly from 50 to 70% in villus cells and 2- to 3-fold in crypt cells. The capacity of PB to induce 7-ECDE activity was lower than that of 3-MC by a factor of almost two in all the fractions.

Expression of ethylmorphine-N-demethylase and its modulation. EMD is a preferred substrate for PB-inducible cytochrome P-450. Previous attempts by others [2] to demonstrate EMD activity in the intestinal mucosa from untreated rats have been unsuccessful. We also observed that EMD activity was below the level of detectability in cells isolated from untreated rats (Fig. 4). However, treatment with PB markedly increased the enzyme activity in all cell fractions. The maximum activity of 80 pmol per min

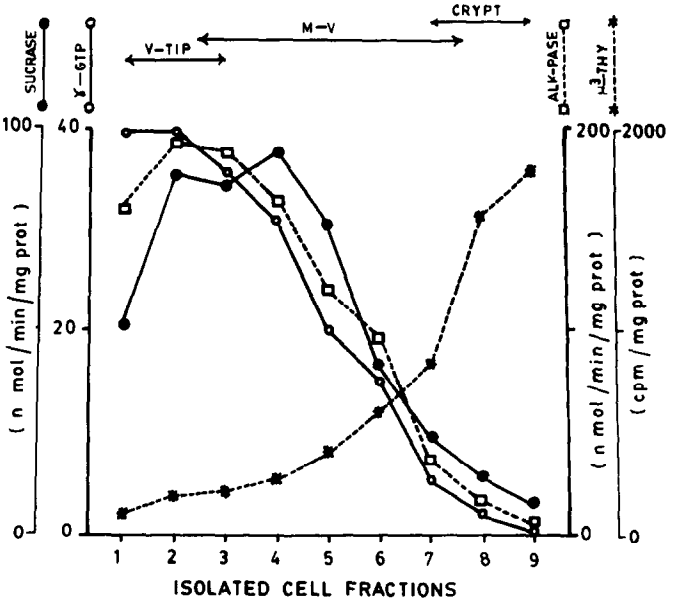

Fig. 1. Gradient elution of rat small intestinal epithelial cells from villus-tip to the crypt. The isolation of cells, determination of marker enzymes and incorporation of [^{3}H]thymidine into the cells are described in Materials and Methods. The data are the means of at least four separate experiments. The coefficient of variation was less than 10%. The villus-crypt surface is marked into three regions: V-tip, villus tip or upper villus (fractions 1–3); M-V, mid and low villus (fractions 4–7); and crypt (fractions 8 and 9).

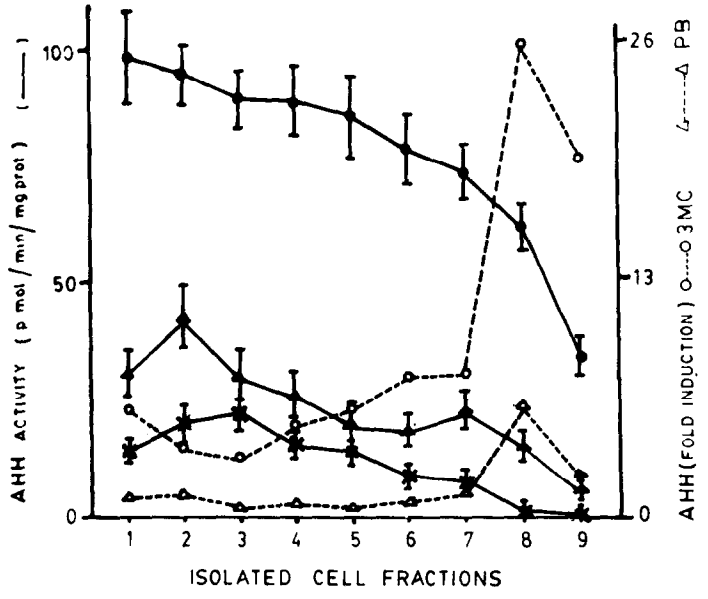

Fig. 2. Effects of 3-MC and PB on the biochemical localization and distribution of AHH in rat small intestinal epithelial cells from villus tip-to-crypt gradient. Determination of AHH activity and treatment of animals with inducers are described in Materials and Methods. AHH activity: (★——★) basal; (▲——▲) PB-treated; and (●——●) 3-MC-treated. The data are means ± SE of four animals. The significance of changes, compared to respective control fractions, were: for all fractions of the 3-MC-treated group, $P < 0.001$. For the PB group: $P < 0.02$ (fr. 1 and 2), $P < 0.05$ (fr. 3–6), $P < 0.01$ (fr. 7–9).

per mg protein was observed in cell fractions from the upper mid-villus region which was double the activity of crypt cells. Thus, the differentiated cells expressed the highest demethylase activity upon exposure to PB. Coadministration of dexamethasone or theophylline with PB did not modulate significantly the enzyme activity.

Time course of AHH and 7-ECDE induction. Two types of effects were discernible with two different sub-populations of cells from rats pretreated with a single oral dose of 3-MC (Figs. 5 and 6). In cell fractions of the villus region, the maximal AHH activity of 4- to 8-fold was observed at 24 hr and declined to 2- to 3-fold at 72 hr after a single oral dose

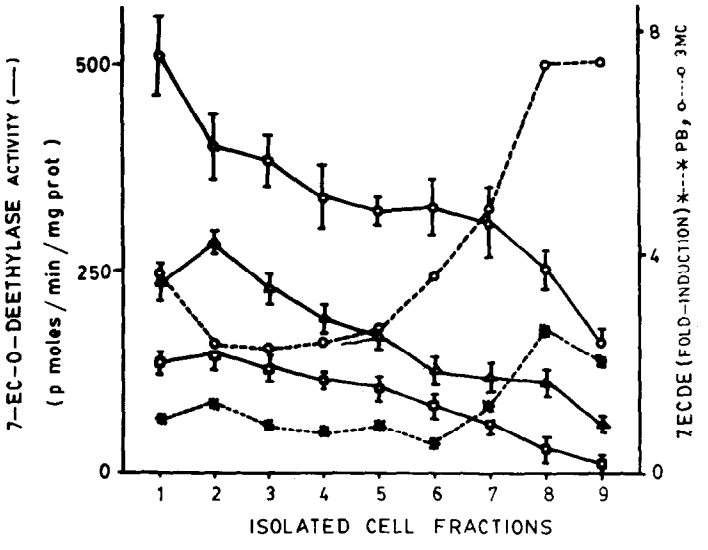

Fig. 3. Influence of 3-MC and PB on the biochemical localization of 7-ECDE in rat small intestinal epithelial cells from villus tip-to-crypt gradient. The method for the assay of enzyme activity is given in Materials and Methods. Other conditions were the same as in Fig. 2. Key: (□——□) basal; (△——△) PB-treated; and (○——○) 3-MC-treated. The data are means ± SE of four animals. Significance of changes: for all fractions of the 3-MC-treated group, $P < 0.001$. For the PB group, $P < 0.01$ (fr. 1–6) and $P < 0.001$ (fr. 7–9).

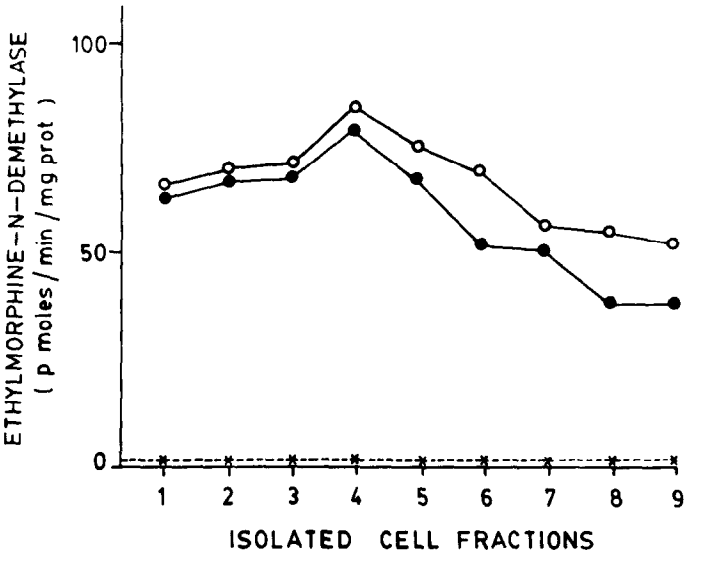

Fig. 4. Expression and modulation of EMD in rat intestinal epithelial cells. The data are the means of four animals. The coefficient of variation was less than 12%. Other conditions are the same as described in Materials and Methods. EMD activity: (*----*) basal; (O—O) PB-treated; and (●—●) PB plus dexamethasone.

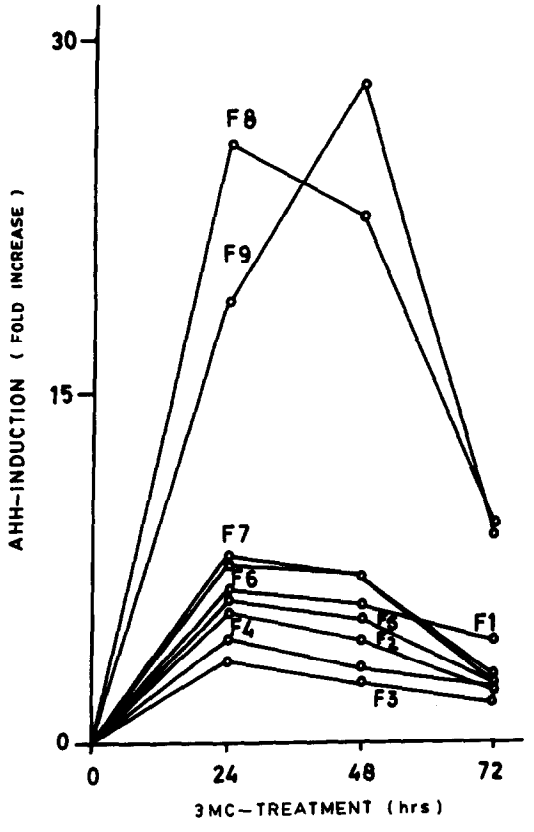

Fig. 5. Time course of AHH induction by 3-MC along the villus-crypt surface of small intestine. Rats were killed at the indicated time period after a single oral dose of 3-MC. Data are means of two separate experiments (10% variation) of two rats each. "F" denotes sequence of fraction number. Other conditions were the same as in Materials and Methods.

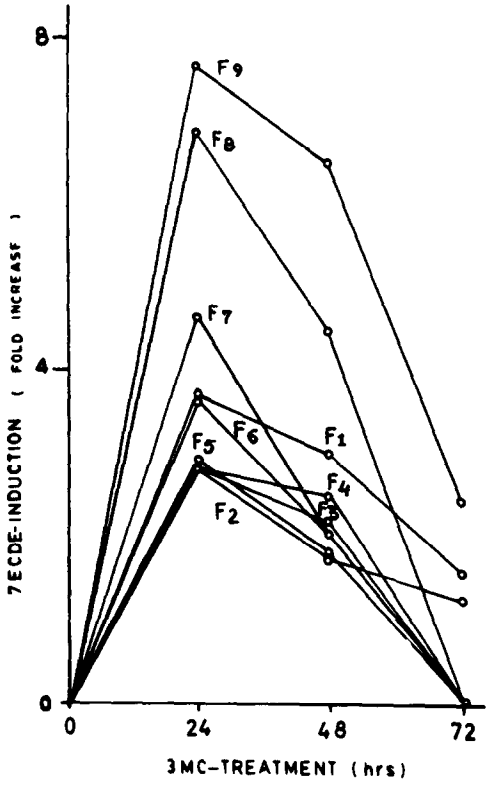

Fig. 6. Time course of 7-ECDE induction by 3-MC along the villus-crypt surface of the small intestine. Conditions were the same as described in the legend of Fig. 5.

of 3-MC. The time-related *in vivo* stability of the induced AHH level appeared similar in fractions 1–7 of the villus. In the crypt cells (fractions 8 and 9), the AHH activity was 26-fold at 24 hr for fraction 8 while in fraction 9 the enzyme activity was 19-fold and continued to increase to about 28-fold at 48 hr of 3-MC exposure; thereafter, it declined to about one-third of its maximal induced level at 72 hr (Fig. 5). The time course of 7-ECDE induction followed almost the same pattern as that of AHH (Fig. 6). The crypt cells appear to have responded more strongly to inducers of the monooxygenases, and the maintenance of the induced level followed a similar trend in all the cell types. The studies demonstrate that the crypt cells expressed relatively greater sensitivity of monooxygenase induction to inducers. Because the magnitude of enzyme induction still remained predominantly higher in crypt cells than in mature villus cells, the enzyme activity had levelled to the control value at 72 hr of 3-MC treatment.

Kinetic constants of AHH in cells from villus tip, mid villus and crypt regions of intestinal villi. The apparent K_m and V_{max} values of AHH from control and 3-MC-treated rats are shown in Table 1. The apparent K_m values for AHH in all fractions from untreated cells obtained was 5.5 μM. The V_{max} was highest in the mid-villus region followed by upper villus and crypt. 3-MC treatment enhanced the K_m and V_{max} values disproportionately. The K_m value was increased by 3-fold in cells corresponding to the villus region and marginally in the crypt cells. However, the V_{max} increased by about 6-fold in the villus region and about 12-fold in the crypt cells. PB had no significant effect on K_m from controls, while V_{max} increased by 3-fold in crypt cells and 50–70% in the villus cells.

Effect of 7,8-BF on AHH activity. 7,8-BF is a potent inhibitor of 3-MC-inducible hepatic monooxygenase form [26] and differentiates this from the PB-inducible monooxygenases. 7,8-BF causes a dose-dependent inhibition of both constitutive and 3-MC-induced AHH of intestinal cells of upper and mid-villus and crypt. The magnitudes of inhibition were similar in pooled fractions representing three zones of villus-crypt surface (Fig. 7). Both the constitutive as well as the induced monooxygenase activities from intestinal cells were inhibited to the same extent by 7,8-BF, indicating that the AHH form in all the cells belonged to the 3-MC-inducible cytochrome P-448 dependent monooxygenases.

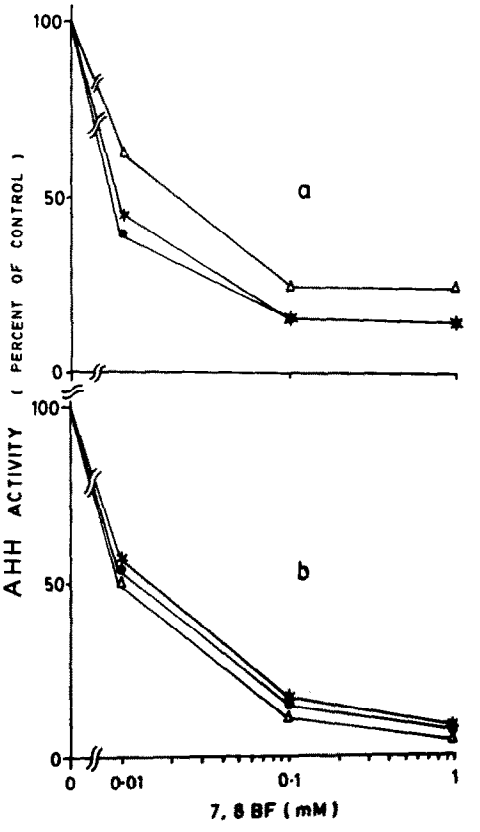

Fig. 7. Inhibitory effect of 7,8-BF on basal and 3-MC-induced AHH activity of rat small intestinal cells. AHH activity was measured in pooled fractions in duplicate from two separate experiments yielding similar results. Villus tip (●——●) fr. 1–3; mid-villus (★——★) fr. 4–7; and crypt (△——△) fr. 8 and 9. Rats were given 3-MC p.o., and after 24 hr the cells were isolated. AHH activity was measured *in vitro* with or without 7,8-BF. 7,8-BF was added in 10 μl of DMSO before the reaction was started with BP. Controls received the vehicle only. Other conditions were the same as described in Materials and Methods. Panel A: basal; and panel B: 3-MC-treated.

Table 1. Kinetic constants of aryl hydrocarbon hydroxylase in isolated epithelial cells corresponding to villus-tip, mid-villus and crypt regions of the small intestine of the rat

Villus-crypt cells	Control		3-MC treated		PB-treated	
	K_m*	V_{max}†	K_m*	V_{max}†	K_m*	V_{max}†
Upper-villus	5.5	14.6	15.4	104	6.2	23.0
Mid-villus	5.5	21.2	15.4	120	6.2	37.5
Crypt	5.5	4.8	8.0	66.6	6.2	16.0

The nine cell fractions isolated [16] were finally pooled into three groups. Fractions 1–3 were pooled as upper villus or villus-tip cells, fractions 4–7 as lower and mid-villus, and fractions 8 and 9 as crypt cells. The V_{max} and K_m values were obtained using Lineweaver–Burk plots. The data are the mean values from two different experiments which varied by less than 10%. Rats were treated with 3-MC or PB as described in Materials and Methods.

* Expressed in μM.

† Expressed as pmol/mg protein/min.

DISCUSSION

The methodology adopted offered a simple system of choice for quantitation, characterization and distribution of the monooxygenases along the villus-crypt surface of the intestinal epithelium. The three substrates used, i.e. benzo[*a*]pyrene, 7-ethoxycoumarin and ethylmorphine [11], reflect the general metabolic competence of the cells in biotransforming xenobiotics along the villus-crypt surface. For the present discussion, the broad classification of monooxygenases into cytochrome P-450 and cytochrome P-448 forms [10, 13, 14, 27] has been used, though multiple forms of cytochrome P-450 have been isolated to homogeneity [28, 29].

Our studies provide evidence that the metabolic competence of crypt cells in the oxidation of BP, hitherto considered insignificant [6, 9], can be raised significantly. This is quite apparent from the 3-MC inducibility of AHH and other monooxygenase activities in the crypt cells. The intestinal cells were found to express higher sensitivity to inducers than the liver [4, 30], observed over time-dependent induction of AHH. The induction would thereby reduce the difference between intestinal and liver monooxygenase activities when the intestinal cells show maximum induction after a single oral pretreatment with 3-MC as also observed by others [31].

The absolute activities of the cytochrome P-448 monooxygenases, AHH and 7-ECDE, were not found uniformly distributed along the villus-crypt surface, though the apparent K_m for BP-hydroxylase was similar in cells from control animals. The higher apparent K_m for 3-MC-induced hydroxylase of the cell fractions from mid-villus and upper-villus than that of the crypts may not necessarily mean that the exposure of the intestinal epithelium to 3-MC results in different forms or population of cytochrome P-448 isozymes along the villus-crypt surface, because the enzyme is a part of a multicomponent system which has not been purified for the present study of Michaelis constants. Inasmuch as the velocity of enzyme reaction depends upon the concentration of free substrate available at the active site, the high K_m of AHH in villus cells from 3-MC-treated rats may presumably be due to the higher unspecific binding of BP in these cells. The intestinal wall is known to strongly accumulate carcinogens such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) [5] or 3-MC [31, 32], and slow rates of BP hydroxylation [31] and of 7-ethoxycoumarin deethylation [3] are reported in villus cells of rats treated daily with a single oral dose of 3-MC for 2 consecutive days. This may partly be related to enhanced K_m of AHH in villus cells from 3-MC-treated rats.

Our studies also indicate that 7-ECDE and AHH activities were closely related in the intestinal epithelium, based upon their induction and distribution pattern. It has also been observed in rabbit liver [28] that BP is metabolized by cytochrome P-450 form-6, and EC by forms-4 and -6, and that both cytochrome P-450 isozymes are inducible by polycyclic aromatic hydrocarbons. This would suggest that monooxygenase forms predominant in the rat intestinal epithelium are of the types inducible by polycyclic aromatic hydrocarbons. This is evidenced by 7,8-BF inhibition of intestinal monooxygenase activity in this study demonstrating the predominance of cytochrome P-448 in the intestinal mucosa of both 3-MC-treated and untreated rats.

The EMD in the small intestine of the rat was below the level of detection unlike that in the intestinal epithelium of other rodents [2]. However, PB caused significant expression of EMD in epithelial cells along the entire villus-crypt surface. Some workers considered PB to be a poor inducer of intestinal drug metabolism, whereas others noted induction of N- and O-dealkylation [33]. Sharf and Ullrich [34] demonstrated higher *in vitro* inducibility of ECDE with parallel increase in NADPH-cytochrome *c* reductase by PB in the small intestine of mice. In fact, the extrahepatic induction of monooxygenases by PB seems to be unusual for it is normally confined to adult hepatic tissue. However, from our studies, that of the aforementioned workers, and those of Wiebel *et al.* [13, 14] in differentiated and dedifferentiated cell lines derived from Reuber H-35 rat hepatoma, it may be viewed that PB can modulate the monooxygenase activities in tissues other than the adult liver. PB was also observed to cause increase in AHH and 7-ECDE activities, the relative increases being higher in crypt cells than in villus cells. Similarly, the induction of cytochrome P-448 dependent monoxygenases by PB has been observed in fetal rats at term [35]. It remains to be determined whether PB-induced cytochrome P-448 isozymes in the crypt cells are similar to 3-MC-induced cytochrome P-448 isozymes.

The present results indicate that the intestinal epithelium of the rat possesses at least two forms of monooxygenases which are specifically induced by different inducers. The induction of monooxygenases would, thus, mean either an increase in activation or inactivation pathways, resulting in formation of their reactive metabolites or detoxified products. It is therefore quite possible that the mitotically active crypt cells, which possess higher DNA/protein than the villus cells [36], might become susceptible to the toxic action of xenobiotics. The rapid induction of monooxygenases and maintenance of an induced level for a long time may provide protection to this compartment. But the steady-state level of reactive metabolites *in vivo* is also known to depend on other enzymes, such as conjugases. Currently we observed that the villus-crypt surface of the small intestine is also rich in conjugases and their cofactors, UDPGA and GSH [17]. Because of these properties the intestinal cells may protect themselves against the noxious effect of xenobiotics. The studies also indicate that exposure of intestinal epithelial cells to xenobiotics and the higher sensitivity and inducibility of their monooxygenases would render the cells capable of inactivating a considerable amount of the potentially toxic compounds and thereby reduce harmful action on other "target" tissues. This would reduce the systemic burden of xenobiotics during intestinal first-pass and affect their bioavailability *in vivo*. However, prolonged exposure to potentially toxic compounds and certain intestinal diseases may affect the delicate balance of activation and inactivation and consequently alter

the steady-state kinetics of the cells in the proliferation compartment. These and further studies may help in assessing and predicting the vulnerability of small intestinal epithelium to genotoxic and cytotoxic effects of xenobiotics.

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