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

CONJUGASES

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Abstract—The potential of the epithelial cells of the villus-to-crypt surface of the small intestine of the rat to conjugate xenobiotics was studied. The cells were isolated sequentially in the villus-to-crypt gradient and were found to exhibit heterogeneous distribution patterns and inducer-sensitivities of the conjugating enzymes and their cofactors. UDP-glucuronosyltransferase (GT) activities towards 3-hydroxybenzo[*a*]pyrene (GT_1) and 4-hydroxybiphenyl (GT_2) were present in all the cells. The mature upper villus cells were rich in both GT_1 and GT_2 activities, which declined toward the highly replicating undifferentiated crypt cells. The specific enzyme activities were four times lower in crypt cells than in upper villus cells. The presence of GT_1 activity always predominated over GT_2 activity. 3-Methylcholanthrene (3-MC) given orally increased GT_1 activity by 2-fold in villus cells and about 6-fold in crypt cells, while phenobarbital sodium salt (PB) also markedly induced GT_1 of the crypt region. Unlike GT_1, GT_2 activity was distinctly induced only by PB in all the cells. Both GT_1 and GT_2 of crypt cells were highly sensitive to inducers, in comparison to the villus cells. The uridine-5-diphosphoglucuronic acid (UDPGA) content ranged from about 0.07 to 0.2 mM in cells from crypts to villus-tip respectively. 3-MC caused a 3-fold increase in UDPGA content in all the cells; PB, however, did not affect UDPGA. The highest glutathione-*S*-transferase (GST) activity, however, was towards the substrate 1-chloro-2,4-dinitrobenzene; the basal specific enzyme activity varied from about 0.05 to 0.2 μmol per min per mg protein in cells from crypt to upper villus. The enzyme was induced by both types of inducers, being about 2-fold in villus cells and 3- to 5-fold in crypt cells. In contrast, the GSH content was lower in cells with higher GST activity. The endogenous GSH content ranged from 0.8 mM in the upper-villus cells to 3 mM in the crypt cells. The GSH content, however, was not altered by 3-MC or PB treatment of rats. The results demonstrate that xenobiotic conjugation reactions in intestinal cells are much stronger than monooxygenase reactions. The differential and higher sensitivity of the intestinal cells to inducers appears to provide protection to the intestine against xenobiotics during intestinal "first pass".

In our preceding companion publication [1] it was observed that monooxygenase activities of epithelial cells of the crypt-villus surface of small intestine exhibit functional heterogeneity, and differential but strong sensitivity to xenobiotics. This was evidenced by the presence of significantly higher monooxygenase activity toward substrates such as benzo[*a*]pyrene, 7-ethoxycoumarin and ethylmorphine upon xenobiotic exposure. The higher sensitivity of the cells, particularly crypt cells, perhaps protects these cells by accelerating the oxidative metabolism of xenobiotics and, thus, the quick elimination of them by complimentary groups of conjugases. This, apparently, would mitigate the burden of xenobiotic load on the systemic circulation during an intestinal "first pass". During cytochrome P-450-mediated metabolism, however, some reactive metabolites of certain chemicals and drugs may be formed [2, 3] which may turn out to be toxic *per se*, especially to the highly replicating crypt cells in the proliferation zone, if otherwise not counteracted by opposing forces, e.g. conjugating enzymes and their cofactors [4, 5]. Rarely have these conjugation reactions been shown to be involved in the activation of xenobiotics to toxic forms [6–8]. Furthermore, the *in situ* rate of conjugation is limited not only by the forms and amount of enzymes present but also by the cofactor content [9–12]. Therefore, determination and characterization of these activities and quantitation of their cofactors in epithelial cells along the villus-crypt surface of small intestine would provide a deeper understanding of the drug-biotransforming competence of these cells and their vulnerability to xenobiotic insult.

In the present study, two groups of enzymes are described with respect to substrate specificity, i.e. UDP-glucuronosyltransferases (GTs) and glutathione-*S*-transferases (GST). Several UDP-glucuronosyltransferases (EC 2.4.1.17) have been described based upon their developmental pattern [13, 14], partial purification [15–17], substrate specificity and differential induction [18–21]. At least two major groups of GT have been distinguished in rat liver. GT_1 is inducible by 3-MC and requires mostly

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planar substrates, viz. 3-OH-BP, and GT_2 is induced by phenobarbital and requires mostly non-planar phenols, viz. 4-hydroxybiphenyl [22]. Glutathione-*S*-transferases (EC 2.5.1.18) also exhibit very broad and overlapping substrate specificities and differential induction patterns [23–26].

Small intestine is very rich in conjugases and many workers have determined various conjugation reactions in the intestine using microsomal preparations [13, 27], scraped-off mucosa [28, 29] or total enterocytes [30–32]. From these observations it is not possible to understand the prevalence and inducibility of various forms of GT or GST and their cofactors in the epithelial cell population of the villus-crypt surface of small intestine where these cells are specifically known to express different degrees of differentiation and proliferation. This is considered important in estimating the differential sensitivities of these cells in modulating xenobiotic conjugations during their transcellular transport. The present studies are, therefore, an effort in this direction.

MATERIALS AND METHODS

Materials. Uridine-5-diphosphoglucuronic acid (UDPGA), 3-methylcholanthrene (3-MC), Brij-58, dexamethasone (DEX), 1-chloro-2,4-dinitrobenzene (CDNB), 3,4-dichloronitrobenzene (DCNB), 5,5-dithiobis (2-nitrobenzoic acid) (DTNB), and 1,2-epoxy-3 propane (epoxy prop) were procured from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). Reduced nicotinamide adenine dinucleotide (NADH), β-nicotinamide adenine dinucleotide phosphate reduced (NADPH), reduced glutathione (GSH) and glutathione reductase-type III were purchased from Boehringer, Mannheim GmbH (West Germany). Phenobarbital sodium salt (PB) was purchased from Merck & Company Inc. (Rahway, NJ, U.S.A.). 3-Hydroxybenzo[*a*]pyrene (3-OH-BP) was a gift from Dr. F. J. Wiebel, Department of Toxicology, GSF, D-8042 Neuherberg, Munchen, West Germany. All other chemicals used were of analytical grade available locally.

Animals and treatment. Adult male albino Charles Foster rats weighing 200–225 g, maintained on pelleted food (Hindustan Lever, Bombay) and water *ad lib.*, were used. 3-MC, PB and/or dexamethasone treatment was given as described in the preceding paper by Dubey and Singh [1].

Isolation of small intestinal epithelial cells. Sequential cell isolation corresponding to the villus-crypt surface of the small intestinal epithelium was accomplished according to Weiser [33]. The isolated cell fractions were finally washed with phosphate-buffered saline (PBS) at 0–4° and stored in small batches at −70° after freezing in liquid nitrogen for assaying the activity of conjugases. Enzyme activity was stable for at least 7 days. The cells were frozen-thawed, stirred on a vortex [1] and used for enzyme assays.

Preparation of hepatic microsomes. Adult male guinea pigs weighing 300–350 g were used for the isolation of liver microsomes. The animals were starved overnight, and microsomes were prepared by the calcium aggregation method of Kamath and Rubin [34]. The fraction from male guinea pig liver contains negligible amounts of pyrophosphatase activity directed toward UDPGA [35], and the preparation was used as a source of GT for the enzymatic determination of UDPGA.

Enzyme assays. UDP-glucuronosyltransferase activity toward 3-OH-BP(GT_1) as substrate was determined by a fluorometric and highly sensitive method of Singh and Wiebel [36]. The reaction mixture in a total volume of 0.2 ml contained 100 mM Tris–HCl, pH 7.6, 0.025% Brij-58, 5 mM $MgCl_2$, 3 mM UDPGA, and 25–75 μg cellular protein. The reaction was started with 50 μM 3-OH-BP in 10 μl methanol at 37° for 3 min. The reaction was terminated with 6 ml chloroform-methanol (2:1) and 0.8 ml water. The tubes were shaken vigorously on a vortex. The BP-3-glucuronide formed was measured fluorometrically in 1 ml aqueous phase [36] at 378 nm excitation and 425 nm emission. UDP-glucuronosyltransferase activity toward 4-OH-biphenyl (GT_2) was assayed according to Bock *et al.* [37]. The assay system in a total volume of 0.5 ml contained 0.1 M Tris–HCl, pH 7.5, 0.05% Brij.58, 5 mM $MgCl_2$, 5 mM UDPGA, and 0.5 to 1 mg cellular protein. The reaction was started with 0.5 mM 4-OH-biphenyl in 10 μl methanol at 37° for 30 min. After termination and extraction, the glucuronide in the aqueous phase was measured in the glycine buffer, pH 10.3, fluorometrically at an excitation setting of 290 nm and 325 nm emission. Glutathione-*S*-transferase activity toward the major three substrates (CDNB, DCNB and epoxy prop) was determined according to Habig *et al.* [38]. GST activity was determined at 25° in the supernatant fraction of cells sonicated (Soniprep-150 MSE, 5–10 sec) in PBS and centrifuged at 20,000 *g* at 0–4°. The reaction was started with enzyme, and initial linear change in optical density per unit time was recorded in a Pye-Unicam spectrophotometer.

Extraction and determination of UDP-glucuronic acid from isolated intestinal cells. UDPGA was extracted from freshly isolated cells and determined enzymatically [39]. Briefly, 500-μl aliquots of freshly isolated cells containing approximately 5–10 mg cellular protein were taken and heated for 3 min in a boiling water-bath and the samples were suitably diluted and centrifuged at 3000 *g* at 0–4° to sediment the denatured protein. The supernatant fraction served as a source of UDPGA which was kept in ice and used within 1–2 hr. The UDPGA content was determined by a simple and high sensitive fluorometric microassay developed on the basis of formation of BP-3 glucuronide catalyzed by GT of male guinea pig liver microsomes according to Singh *et al.* [39].

Extraction and determination of glutathione content of isolated intestinal cells. GSH was extracted from isolated intestinal cells according to Tietz [40]. Aliquots (250 μl) of the fresh cell suspension (2–4 mg of cellular protein) were taken in plastic centrifuge tubes, and 100 μl of chilled 35% perchloric acid containing 10 mM EDTA was added to it; the tubes were stirred immediately and placed in ice. The samples were sonicated (Soniprep-150 MSE) for 5 sec, and the precipitate was spun down in a Beckman microfuge. The clear supernatant (300 μl) was

transferred into another plastic vial, and 50 μl of chilled 1 M triethanolamine containing 1.65 M K_2CO_3 and 30 mM EDTA was added three times to it under chilled conditions. The tubes were subsequently centrifuged at 10,000 *g* for 5 min, and the supernatant fraction was transferred into another vial. The extract was stored at −70° to be used within 7 days.

GSH content was measured spectrophotometrically at 405 nm in 1-ml glass cuvettes at 25°. Briefly, the reaction mixture in a total volume of 1 ml contained 0.08 M phosphate buffer, 0.8 mM EDTA, 0.025% NADPH, 1 μg glutathione reductase and 20–100 μl of the biological extract. The reaction was initiated by the addition of 50 μg DTNB. The linear reaction rate was monitored by measuring the increase in absorbance as a linear function of time.

Protein was assayed by the method of Lowry *et al.* [41].

RESULTS

Glucuronidation potential of the epithelial cells along the villus-crypt surface of small intesine. The epithelial cells isolated in gradient fractions 1–9 corresponded to the villus-crypt surface of rat small intestine observed earlier [1]. The observations presented in Fig. 1 revealed that glucuronic acid conjugation was expressed at remarkably high levels in intestinal cells. 3-OH-BP and 4-OH-biphenyl are the prototype substrates for two forms of GT representing GT_1 and GT_2 activities respectively. GT_1 activity was present in all the cells; it was highest in the highly differentiated mature cells of the villus and tended to decline gradually toward crypts in parallel with the decline in cellular differentiation. This also is in agreement with the cytochemical observations of others [42]. The crypt cells expressed activity that was relatively four times less than that observed in villus cells, although GT_1 activity present in crypt cells was found to be markedly above the level of detection. The conjugase was inducible by 3-MC in all the cell fractions isolated. Crypt cells were found to be highly sensitive to 3-MC induction of GT_1 activity, being about 6-fold more sensitive in contrast to the 2-fold sensitivity of the villus cells, when compared to their respective untreated control cells. 3-MC-induced GT_1 activity was also induced by PB in the crypt cells, as measured by the rate of glucuronidation of 3-OH-BP (Fig. 1).

The glucuronidation potential of the epithelial cells toward 4-OH-biphenyl (GT_2 activity) was found to be almost four to seven times lower than observed with 3-OH-BP, indicating clearly that GT_1 activity predominated over the GT_2 activity in the intestine. The cells from the mid-villus and villus tip regions exhibited maximum GT_2 activity; the activity gradually decreased toward the crypt region (Fig. 2). GT_2 activity was induced markedly by PB and was unaffected by exposure of rats to 3-MC. In contrast, GT_1 activity in the immature crypt cells was induced markedly by both types of classical inducers, PB and 3-MC. A maximum induction of about 4-fold of GT_2 activity by PB was observed in the crypt cells, whereas in the villus-tip cells the maximum induction was only 2-fold. The pattern of PB-induced GT_2 activity was similar to that of 3-MC-induced GT_1 activity along the villus-crypt surface of the intestinal epithelium. The combination of PB and dexamethasone enhanced the GT_2activity marginally, similar to that of PB alone (Fig. 2). Thus, the GT_2 activity induced by PB was expressed uniquely in all the cell populations along the villus-crypt surface; the activity in the untreated crypt cells was, however, low, near the level of detection.

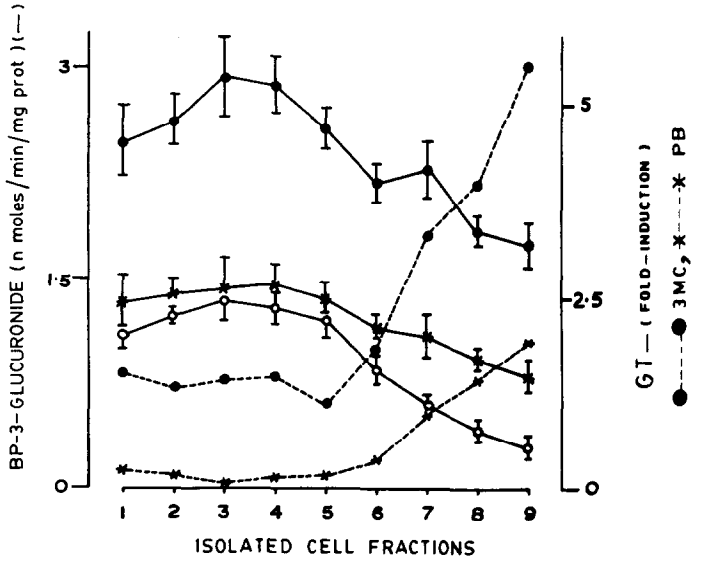

Fig. 1. Effects of 3-MC and PB on the biochemical localization and distribution of GT_1 activities toward 3-OH-BP in rat small intestinal epithelial cells from villus tip-to-crypt gradient. Determination of GT activity and treatment of animals with inducers are described in "Materials and Methods". GT_1 activity: (○——○) basal, (●——●) 3-MC-treated, and (★—★) PB-treated. The data are means ± SE of four separate experiments. The fraction numbers 1 to 9 correspond to villus tip-to-crypt gradient as described in Table 1 and this is followed in subsequent figures.

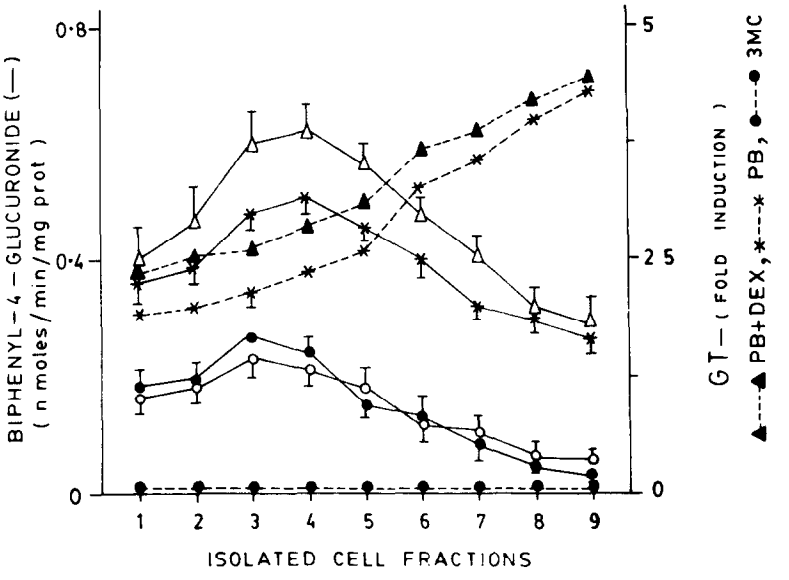

Fig. 2. Influence of 3-MC and PB on the biochemical localization of GT_2 activity toward 4-OH-biphenyl in rat small intestinal epithelial cells from villus tip-to-crypt gradient. Conditions for enzyme assay and procedures for treatment with inducers are described in "Materials and Methods". GT_2 activity: (○——○) basal, (●——●) 3-MC, (★——★) PB, and (△——△) PB + DEX. The data are means ± SE of four separate experiments.

Effect of inducers on UDP-glucuronic acid content of the rat intestinal epithelial cells along the villus-crypt surface. Rat small intestinal cells were found to contain remarkably high amounts of UDPGA (Fig. 3). The UDPGA content was in the range of 0.3 to 0.8 nmol/mg of cellular protein in the intestinal cells from crypt to villus (0.07 to 0.2 mM) surface; the highest content was found in cells of villus-tip, and this decreased in a gradation toward the crypt cells where the amount of UDPGA was less than half the amount present in the villus tip cells. The higher UDPGA content appeared to be associated with higher conjugase activities in these cells. 3-MC modulated the UDPGA content in the cells, increasing the cofactor by about 3-fold in all the epithelial cells. Relatively, the modulation by PB of UDPGA content was not significant (Fig. 3).

Ratio of hydroxylation to glucuronidation of xenobiotics by intestinal cells. In Table 1 the ratios of GT_1/AHH and GT_1/UDPGA are presented. The

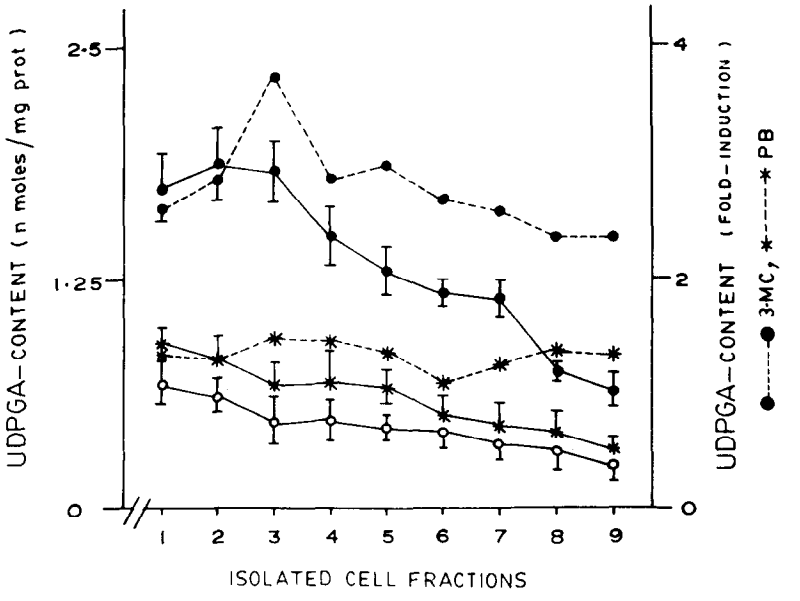

Fig. 3. Amount and modulation of UDPGA content in rat intestinal epithelial cells isolated as villus tip-to-crypt gradient. Determination of UDPGA is described in "Materials and Methods". Other conditions were the same as described in the legend of Fig. 1. UDPGA content: (○——○) basal, (●——●) 3-MC, and (★——★) PB. Data are means ± SE of four different experiments.

Table 1. Effect of oral administration of 3-MC on the proportions of GT_1 to B[*a*]P hydroxylation (AHH) and to glucuronidation (UDPGA) activities in the rat small intestinal epithelial cells isolated in fractions of villus to crypt gradient

Cell fractions (villus to crypt) Fractions eluted	GT_1*/AHH†		GT_1*/UDPGA**	
	Untreated	3-MC-treated	Untreated	3-MC-treated
1	77	25	1.7	1.4
2	59	28	2.0	1.4
3	60	33	2.7	1.6
4	82	33	2.6	1.9
5	87	30	2.7	2.0
6	95	28	2.0	1.9
7	71	31	1.6	2.1
8	246	30	1.3	2.5
9	369	51	1.2	2.6

* Values, as nmole × min^{-1} × $(mg\ protein)^{-1}$, are taken from Fig. 1, and ** nmole × $(mg\ protein)^{-1}$ are taken from Fig. 3.

† Values for arylhydrocarbon hydroxylase, in nmol × min^{-1} × $(mg\ protein)^{-1}$, are taken from Fig. 2 of the preceding companion paper [1]. Other conditions are the same as described in "Materials and Methods". Fractions 1–9 correspond to villus tip-to-crypt gradient [1]: fractions 1–3, upper villus; fractions 4–7, mid and lower villus; and fractions 7–9, crypt.

ratios of these enzymic entities provide a rough approximation of the balance between activation and inactivation of intestinal cells upon exposure to the procarcinogen, 3-MC. The cell fractions from the villus region of the untreated rat intestine were found to have almost the same ratios of GT_1/AHH, whereas in the crypt cells the ratios were three times higher. A single oral dose of 3-MC to rats, 24 hr before sacrifice, reduced this ratio to less than half the value found in untreated rats; this ratio, however, was about the same in all the cell fractions. The GT_1 was about thirty times higher than the corresponding AHH activity. The ratio GT_1/UDPGA, however, was found to be about 70% higher in villus cells than in crypt cells, and this was reversed by 3-MC exposure.

Localization and induction of glutathione-S-transferases along the villus-crypt surface of rat small intestinal epithelium and status of glutathione content. GST activity towards three substrates, viz. CDNB, DCNB and epoxy prop, was determined. The most significant activity of the transferase was observed toward CDNB, whereas the basal activities toward the other two substrates were too small to be measured. In the following, the transferase activity towards CDNB is described (Fig. 4). A significant amount of basal GST activity was found in the crypt-cells; the relative amount found in the cells increased successively as they differentiated and migrated towards the upper villus region. Consequently, the highest GST activity was found in cells of the upper mid-villus region, whereas cells in the proliferation

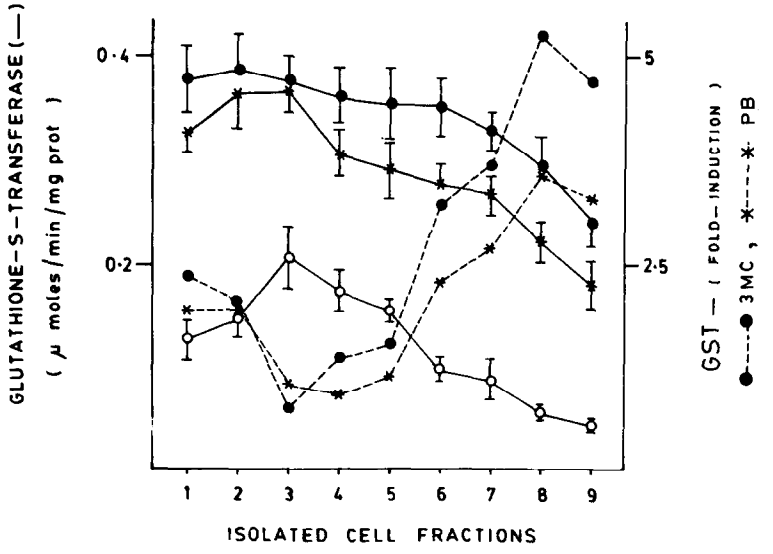

Fig. 4. Expression and modulation of GST activity toward CDNB in rat intestinal epithelial cells along the villus-crypt surface. Conditions were the same as described in "Materials and Methods". GST activity: (○——○) basal, (●——●) 3-MC, and (★——★) PB. The data are means ± SE of four separate experiments.

zone (crypts) had less than half the activity found in mature villus cells. Thus, GST was one of the constitutive enzymes present in the intestinal cells, the level of which varied by a factor of 2–3 depending upon the degree of maturation of cells. Both PB and 3-MC modulated the GST activity in a somewhat similar manner in all the cells of the villus-crypt surface except that in the crypt cells where 3-MC appeared to be more potent than PB in increasing the GST activity. A 2-fold increase of GST was observed in upper villus cells whereas a 3- to 5-fold increase was seen in cells of the lower villus and crypts. The induction of intestinal GST activity was therefore sensitive to both types of inducers used in the present study. Pinkus *et al.* [43] also made similar observations with PB inducibility of intestinal GST.

GSH content was also simultaneously determined in the same groups in the sequentially isolated cells of the villus to crypt surface of small intestine (Fig. 5). The cells of the upper and mid-villus (fractions 2–6), which perform the major absorption of nutrients and xenobiotics, had the lowest GSH content. A steep increase in GSH content was thus observed in epithelial cells from villus tip-to-crypt gradient, so that the GSH level was about 4- to 6-fold higher in the rapidly dividing crypt cells when compared to upper villus cells. Considering an intracellular volume of an average of 4.2 ± 1.1 μl/mg of cellular protein of intestinal cells [44], the endogenous concentration of GSH would thus range from about 0.8 mM in upper villus cells to about 3 mM in the crypt cells. Unlike GST activity, treatment of animals with 3-MC or PB did not modulate the *in situ* level of GSH content in the rat small intestinal epithelium (Fig. 5). If one observes the ratio of GST specific activity to the content of GSH per mg protein after inducer treatment, it would appear that the endogenous GSH content might be a limiting factor consequent to GST induction, because the intestinal GSH content was not altered significantly by test inducers.

DISCUSSION

The present studies show that both GT and GST followed distribution patterns similar to that observed for monooxygenases [1] along the villus-crypt surface of the small intestine. This indicates that mature villus cells are well equipped with a conjugation system to detoxify various types of aglycones. The planar molecules are likely to be excreted faster because of the predominance of GT_1 activity. The GT activity of the intestinal cells was different for two groups of substrates, i.e. GT_1 activity was 6-fold higher than GT_2 activity in almost all the cell fractions eluted. Nevertheless, the ratio of GT_1/GT_2 activities remained almost constant in the villus-crypt surface of the entire mucosa of small intestine. Further, 3-MC and PB pretreatment was followed by a marked and selective induction of the GTs activity toward 3-OH-BP and 4-OH-biphenyl respectively. It may be mentioned here that the *in vivo* rate of glucuronidation is also related to the intracellular content of UDPGA [9]. It has been observed recently that intestinal cells are quite active in the synthesis of UDPGA [10], and this *in vivo* concentration is increased markedly by 3-MC as observed in the present study. Though the intracellular UDPGA in the intestinal cells (0.07 to 0.2 mM) may be present at less than saturating concentrations for maximal GT activity [45], yet its active synthesis and modulation by inducers may continuously fulfill the requirement of GT activity *in situ*. Moreover, the rate of elimination of an aglycone from the intestinal cells to the serosal or mucosal side would also depend upon its concentration and biological half-life within the cell. In this context it

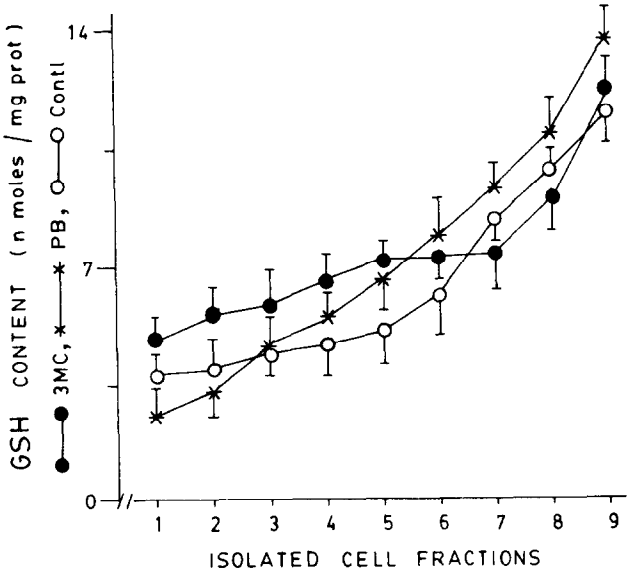

Fig. 5. Influence of 3-MC and PB on the amount and modulation of GSH content in rat intestinal epithelial cells along the villus-crypt gradient. The assay procedure is described in "Materials and Methods". Other conditions were the same as described in the legend of Fig. 1. The data are means ± SE of four different experiments.

may be mentioned that there has always been difficulty in finding the apparent K_m of UDPGA, because the dependency of *in vitro* GT activity on UDPGA followed Michaelis–Menten kinetics only in the presence of detergent, with an apparent K_m of 0.2 mM whereas in the absence of detergent the kinetics are non-linear and the increasing UDPGA content appears to behave like a chaotropic agent [45].

An interesting feature of the study was the induction of GT_1 activity by PB in the crypt cells, because GT_1 activity is specifically inducible by 3-MC and not by PB [22] as observed in the differentiated villus cells. This, possibly, may have been due to proliferation of endoplasmic reticulum of the highly dividing crypt cells by PB as reported for liver [46]. This is in contrast to the effect of 3-MC on GT_2 activity toward 4-OH-biphenyl, which was not induced by 3-MC in any of the cell fractions. Further, it may be observed that the induction of GT_1 by 3-MC was similar to that of AHH activity in the whole villus-crypt surface of the small intestine and that the maximal induction of AHH and GT_1 by 3-MC was found to be localized in the crypt cells. This suggests a regulatory link between 3-MC-inducible cytochrome P-450-dependent monooxygenases and GT_1 activity within the same cell. It is possible that the genes responsible for the synthesis of these enzymes may be located on the same chromosome or closely linked, which would be advantageous for an efficient and co-ordinated induction of 3-MC-inducible cytochrome P-450 and GT_1 in order to eliminate a variety of xenobiotics from the intestine. In mice, the inducibility of this cytochrome P-450 and GT_1 appears to be genetically regulated by the same Ah locus [47]. We believe that a similar link may also exist between the 3-MC-inducible GT_1 and UDPGA synthesis in intestinal cells as evidenced by 3-MC-induced modulation of UDPGA in these studies.

A significant amount of glutathione-*S*-transferase activity toward CDNB as substrate was observed in the cytosol of the intestinal cells and was largely localized in the upper and mid-villus cells. The GST and γ-glutamyltranspeptidase activities were highest in the upper and mid-villus cells and, correspondingly, the GSH content was found to be much lower in these cells as compared to the crypt cells. This may have been due to high GSH turnover in these cells, which are associated with the transport of amino acids, smaller peptides and biotransformation of xenobiotics. GST has been shown to catalyze the conjugation of GSH with a variety of compounds, viz. epoxides and numerous halo- and nitrobenzene derivatives [48]. In small intestine GST may be the principal route of epoxide biodegradation, since the levels of epoxide hydrase are extremely low in this organ [49, 50]. High GSH and GST activities and higher sensitivity of GST to inducers along the villus-crypt surface would suggest that these enzymes may play an important protective role against many intestinal xenobiotics that are metabolized through the microsomal system. Higher sensitivity of colonic epithelium to chemical induction of neoplastic growth could be due to low GST activity [51]. Thus, the intestinal cells and particularly the crypt cells may adapt rapidly to environmental exposure so as to enable them to eliminate quickly various metabolites through glucuronidation. The crypt cells were found to be capable of acquiring a conjugation potential equivalent to that of mid-villus and villus-tip cells and, therefore, may function successfully in terminating the biological activity of the foreign compounds or their metabolities. This would offer greater protection to these highly dividing cells against harmful drugs and chemicals.

From the above discussion it may be concluded that the phase-II reactions that occur parallel to the oxidative reactions or subsequent to them are much more dominant in the intestinal mucosa than reported in hepatic and other extrahepatic tissues [32]. The activities become more pronounced with inducers especially, in the highly dividing crypt cells, indicating thereby that cells can develop an armory to offer resistance to the onslaught of toxic xenobiotics. Obviously, this bears greater significance with crypt cells that have high mitotic indices and are likely to be prone to the genotoxic and cytotoxic action of chemicals. A higher prevalance of conjugases thus appears to offer protection to the small intestinal mucosa against xenobiotics and their metabolites. In this way the intestine is called upon to modulate the load of xenobiotics to the systemic circulation. There are, however, instances in which the sulfhydryl (GSH) contents were found to activate xenobiotics such as *N*-methyl-*N*-nitrosoguanidine, 1,2-dichloroethane or 1,2-dibromomethane [52, 53]. Under such circumstances the higher GSH content of the crypt cells might be a determining factor in contributing to the development of intestinal malignancy due to such procarcinogens or toward their tissue specific toxicity. It is, thus, the delicate balance of activating and inactivating systems that determine the harmful effect of xenobiotics on the intestine. Nevertheless, the xenobiotic conjugating capability of the small intestine appears to be of much benefit to the tissue. The present studies indicate that the intestinal cells along the villus-crypt surface are equipped with systems to inactivate the xenobiotics so as to protect the intestine from exposure to xenobiotics as well as to reduce their load on the systemic circulation.

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