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Biochemical Localization of Aryl Hydrocarbon Hydroxylase in the Intestinal Epithelium of the Rat[1]

C. W. Porter,[2] D. Dworacyzk,[3] and H. L. Gurtoo[4]

Department of Experimental Therapeutics, Grace Cancer Drug Center, Roswell Park Memorial Institute, New York State Department of Health, Buffalo, New York 14263

ABSTRACT

The distribution of the carcinogen-metabolizing enzyme system, aryl hydrocarbon hydroxylase (AHH), was biochemically determined in the intestinal epithelium of the rat. A method of epithelial cell isolation in which fractions of cells are sequentially collected as a villus tip-to-crypt gradient was used. AHH activity was highest in the midvillus region, 40% lower at the villus tip, and practically nonexistent in the crypt region where active cell proliferation takes place. This distribution differed from those of sucrase and alkaline phosphatase (used here as markers for cellular differentiation), which were characteristically lowest in activity at the crypts and increased continuously to the villus tips. Conceivably, the midvillus peak of AHH activity may serve to protect or enhance the susceptibility of cells undergoing cell division in the nearby crypt regions, depending on whether the predominant function of AHH in the intestinal epithelium involves detoxication or activation of polyaromatic carcinogens.

INTRODUCTION

It is now apparent that carcinogen-metabolizing systems are not restricted in their tissue distribution to the liver. Cytochrome P-450-dependent AHH,[4] also known as BP hydroxylase, is present in the tissues of all major portals of entry into the body (13) and in several other tissues as well (4). Significant enzyme activity is associated with the gastrointestinal tract (14) and the lung and skin (13). In the gastrointestinal tract, the greatest AHH activity is found in the proximal small intestine, while much less activity is located in the large intestine and portions of the stomach (14). Oral administration of certain inducers such as benzanthracene significantly increases AHH activity along the entire gastrointestinal tract. By histochemical reaction, AHH was found to be confined to the epithelial cells covering the villi (14). Cells in the crypts, stroma, and muscle layers of the intestine were essentially without enzyme activity. Since this particular histochemical methodology is semiquantitative at best, the precise distribution of enzyme activity along the epithelial cells of the villus was not determined.

In this study, the AHH activity was biochemically measured in intestinal epithelial cells isolated by sequential elution (16) as a villus tip-to-crypt gradient. The gradient corresponds to the location of the cells along the villus and to their stage of differentiation as they migrate towards the lumen. The data indicate that most AHH activity is associated with the mature cells of the villus walls, particularly in the midvillus region, while little is found in the crypts where cell proliferation is actively occurring.

MATERIALS AND METHODS

Isolation of intestinal epithelial cells was accomplished according to the method of Weiser (16) except that 10 rather than 9 cell fractions were collected following incubation times of 4, 2, 2, 3, 4, 5, 7, 10, 10, and 10 min with the eluting buffer. The technique relies on tissue dissociation with citrate and removal of cells in the presence of buffered 1.5 mM EDTA. The 10 cell fractions that were obtained form a villus tip-to-crypt gradient of epithelial cells, which are representative of the intestinal mucosa.

To confirm that the methodology for sequential elution of epithelial cells was functioning as expected in these experiments, the brush border marker enzymes, sucrase and alkaline phosphatase, were measured in the cell fractions eluted from the intestine of an untreated rat. Sucrase was assayed according to the method of Dahlqvist (3), and alkaline phosphatase was assayed according to the method of Weiser (16). Protein determinations were performed according to the method of Lowry *et al.* (8) on aliquots of cells having known cell densities.

Microsomes were prepared from intestinal cell fractions according to reported methods (12) and assayed for AHH activity by a highly sensitive fluorescent assay adapted from the procedure of Nebert and Gelboin (10). The buffer mixture (pH 7.4) containing 80 mM phosphate, 250 mM sucrose, 3.3 mM $MgCl_2$, an NADPH-generating system (0.81 mM NADPH:17 mM DL-isocitrate:200 mg protein of isocitrate dehydrogenase, Sigma type V), microsomes (1.5 to 2 mg protein), and 0.1 mM BP in a total volume of 1 ml was incubated for 30 min at 37° and extracted with 4 ml of acetone:hexane (1:3). The organic phase (3 ml) was then extracted with N NaOH and read on a spectrofluorometer using a wavelength of 396 nm for excitation and 522 nm for emission. AHH activity is expressed in terms of BP phenol formation quantitated by comparison with a standard curve based on the fluorescence of 3-hydroxy-BP.

RESULTS

The cells from the intestine were collected in 10 fractions, beginning with those from the villus tips (Fractions 1 to 3) and ending with those of the crypt region (Fractions 7 and 10). Cell viability by trypan blue exclusion was examined shortly after the isolation procedure, and approximately 90% cell viability was noted for all cell fractions. The effectiveness of the methodology for separating the epithelial cells as a villus tip-to-crypt gradient was confirmed by the distribution of sucrase and alkaline phosphatase along the 10 fractions (Chart 1). The greatest activity for both enzymes was located in Fractions 1 and 2 and declined progressively in subsequent fractions. This

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[2] To whom requests for reprints should be addressed.

[3] Present address: Medical Department, Norwich-Eaton Pharmaceutical, Norwich, N. Y. 13815.

[4] The abbreviations used are: AHH, aryl hydrocarbon hydroxylase; BP, benzo(*a*)pyrene.

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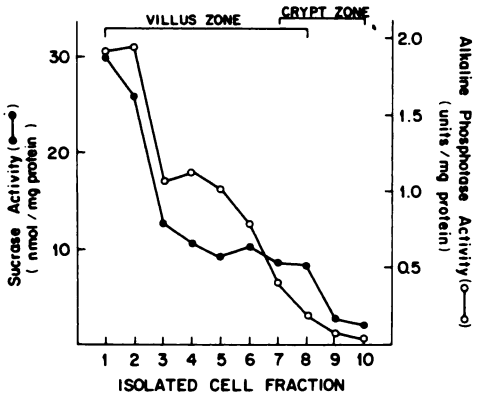

Chart 1. The distribution of sucrase and alkaline phosphatase activities along the villus tip-to-crypt gradient of the rat intestinal mucosa. Data were obtained from samples taken from 3 different rats. The unit for alkaline phosphatase activity is μM *p*-nitrophenol liberated per 15 min.

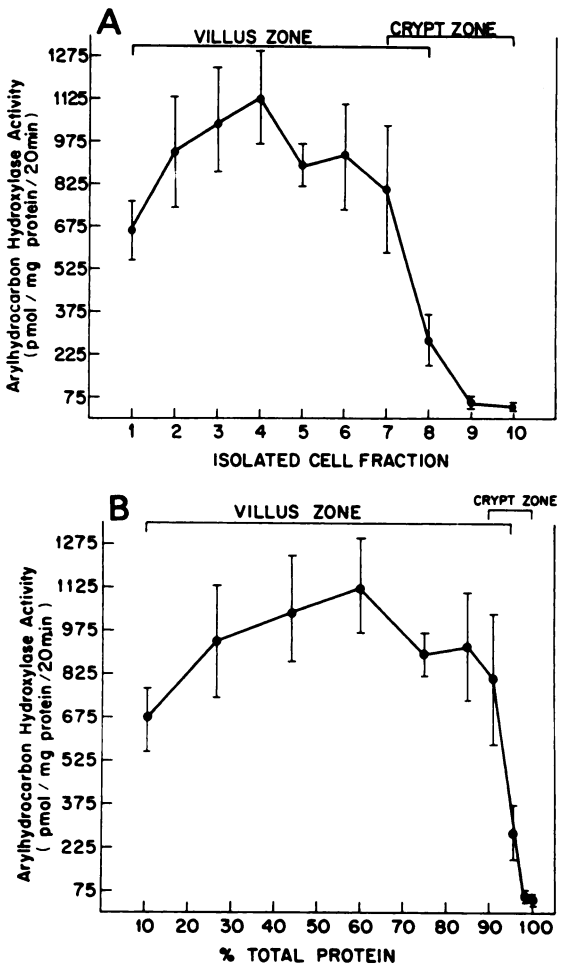

Chart 2. AHH activity along the villus tip-to-crypt gradient of the rat intestinal mucosa plotted according to fraction samplings (*A*) or percentage of total gradient protein represented in each fraction (*B*). *Points*, mean of data obtained from 6 different rats, 3 of which were represented in Chart 1. *Bars*, S.D.

is consistent with the findings of others (1, 11, 16) and with the concept that these enzymes, which are associated with brush border of columnar cells, are acquired as the cells differentiate and migrate towards the villus tip. The undifferentiated proliferating cells of the crypts are essentially lacking in these enzymes.

The distribution of AHH along the gradient (Chart 2*A*) differed from those of sucrase and alkaline phosphatase. Microsomal enzyme activity was greatest in the midvillus region (Fractions 2 to 6), lower in the villus tip (Fraction 1), and lowest in the crypt region (Fractions 8 to 10). When the data were plotted according to the total gradient protein present in each fraction (Chart 2*B*), the point of greatest activity was even more obviously in the midvillus region. The latter representation gives a better correlation of the location of enzyme to the actual morphology of the intestinal mucosa. This is because each gradient fraction shown in Chart 2*A* contains a different number of cells and hence a different amount of protein (*i.e.*, approximately 75% of the total protein is removed in the first 5 of the 10 gradient fractions). Chart 2*B* therefore shows the enzyme data according to their real distribution along the villus and crypts, whereas Chart 2*A* presents a somewhat artificial representation.

DISCUSSION

The epithelial cells of the intestinal mucosa undergo a morphological segregation as they migrate from the crypt region to the villus tip. The cells located at the bottom of the crypts are mitotically active but poorly differentiated. These multipotential cells give rise to villus columnar mucous, enteroendocrine, and Paneth cells which, excepting for Paneth cells, become differentiated as they move towards the villus tip (2). The most common of these, the columnar cells, reach full maturity at the upper third of the villus and are eventually shed into the lumen of the intestine. In the rat, this migration and maturation process requires 36 to 72 hr (6). Weiser (16) has developed a nonenzymatic means for isolating these various cells in a gradient representative of their location along the villus or crypts of the intestinal mucosa. That this sequential elution methodology does in fact reflect in *in situ* villus tip-to-crypt cell gradient has already been established (16) and further confirmed here by sucrase and alkaline phosphatase distribution.

In the present study, this methodology was used to determine the distribution of AHH activity in the epithelial cells along the villus tip-to-crypt gradient of the rat intestinal mucosa. Overall, the data are similar to those reported by Wattenberg *et al.* (14) on the basis of histochemical studies. Namely, AHH activity is associated with the epithelial cells along the walls of the villus but not with the cells of the crypts. The present study, however, indicates that the greatest AHH activity is localized in the midvillus region. This differs from the distribution of enzymes associated with the differentiation process (*i.e.*, sucrase and alkaline phosphatase) which, as demonstrated here and elsewhere (11, 16), tend to be the lowest in the crypt region and increase consistently in activity as the cells migrate to the villus tip. Thus, the distribution of AHH would suggest that it is not exclusively related to differentiation.

Various lines of evidence have established the existence of multiple forms of cytochrome P-450 in animal tissues (5, 9). It is now widely believed that more than one cytochrome P-450 are involved in the metabolism of BP to epoxides, quinones, and phenols (4, 12). It is also conceivable that different forms of this cytochrome could convert BP to the same metabolite at different rates. The cytochrome P-450-dependent monooxy-

genase system and the metabolically coupled enzyme epoxide hydrolase convert BP to diols, quinones, and phenols (4). The diols are derived by the epoxide hydrolase-mediated hydration of the intermediate epoxides formed by the cytochrome P-450-dependent reactions. Diols can be recycled by the monooxygenase system to form the ultimately carcinogenic and mutagenic diol oxides, especially BP:7,8-diol 8,10-oxide. Phenols and diols can be further detoxified via conversion by metabolically related transferases to glutathione, glucuronide, and sulfate conjugates.

The data presented here do not identify the association between one or more specific forms of the phenols measured in the fluorometric AHH assay but merely indicate the extent of formation of BP phenols (10) in certain regions of the intestinal epithelium. Since the induction of bowel AHH (7) is associated with a decreased incidence of bowel cancer by polyaromatic hydrocarbons (15), it is possible that, in this tissue, the balance between activation and detoxication of BP is favorable to the host, possibly indicative of predominant reactions catalyzed by the cytochrome P-450 forms leading more to the formation of phenol than to the ultimately carcinogenic diol epoxides. Alternatively, since epoxide hydrolase is not induced by 3-methylcholanthrene under the experimental conditions (12), it is probable that more of the initially formed BP epoxides are converted to phenols, which are subsequently detoxified via the various conjugation reactions. If the AHH of the intestinal epithelium is more active in detoxication rather than in activation of polyaromatic hydrocarbons, the midvillus peak in activity could serve to protect the nearly mitotically active crypt cells from exposure to active carcinogens.

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