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THE GLUTATHIONE S-TRANSFERASES AS A POSSIBLE DETOXIFICATION SYSTEM OF RAT INTESTINAL EPITHELIUM

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Abstract— Because the glutathione *S*-transferases perform a detoxification function in liver and kidney, evidence for them was sought in the intestine, another major site of contact with xenobiotics. The range of activity with several different substrates was similar to that of liver; highest activity was observed with 1-chloro-2,4-dinitrobenzene. Antibodies prepared against homogeneous rat liver transferases A, B, and E gave lines of identity with cytosol obtained from intestinal epithelial cells. With 1-chloro-2,4-dinitrobenzene as substrate a shallow gradient of increasing activity was observed from crypt to tip cells of jejunal epithelium. In cells at all stages of maturation, activity was increased in response to phenobarbital whereas alkaline phosphatase, thymidine kinase and γ-glutamyltranspeptidase were not induced. Activity was greatest in the duodenum and jejunum, and lowest in the colon and stomach. The data are consistent with a detoxification role for the glutathione *S*-transferases in intestine.

The glutathione *S*-transferases (EC 2.5.1.18) are a family of enzymatically active binding proteins that catalyze the initial step in mercapturic acid formation [1], among other reactions [2, 3], and may play a significant role in the transport [4] and detoxification [3] function of vertebrates. At least seven distinct glutathione *S*-transferases, characterized by the letters AA, A, B, C, D, E, and M, have been isolated from rat liver [2] of which all but transferases D and M have been purified to homogeneity [5–8]. These enzymes catalyze the conjugation of glutathione (GSH) with an enormous variety of compounds that bear a sufficiently electrophilic carbon and have a hydrophobic topography [2, 3]. Substrates of this sort include benzo[a]pyrene 4,5-oxide, bromosulfophthalein, ethacrynic acid and numerous halo-and nitrobenzene derivatives [2, 9]; the product in each case, is the corresponding thioether with GSH. The same enzymes catalyze the glutathione-dependent formation of cyanide from thiocyanates [10]; the formation of nitrite from such organic nitrate esters as trinitroglycerol [10]; the isomerization of Δ^5 to Δ^4-3-ketosteroids [11]; and a number of disulfide interchange reactions [12]. Thus, this group of enzymes is active in the detoxification of a wide range of compounds that have in common only a reactive group subject to nucleophilic attack by GSH and the ability to bind to the protein.

Glutathione *S*-transferase activity of the rat has been observed in all tissues examined and has been calculated to comprise approximately 10 per cent of the soluble protein of rat liver [2]. Transferase B alone constitutes about 5 per cent of rat liver extracts [13] and about 3 per cent of kidney extracts [13, 14]; it has also been observed in the intestine by immunofluorescence techniques [14]. Glutathione *S*-transferase activity with bromosulfophthalein has been reported in the gastrointestinal tract of several vertebrate species [15].

It is possible that those organs which have primary contact with the large number of xenobiotics to which animals are exposed, would contain the glutathione *S*-transferases as one system of detoxification. We have therefore investigated the gastrointestinal tract of the rat with a view to gathering qualitative data for the possible function of the transferases in this tissue. The data indicate that the intestinal transferases from rat are present in villus and crypt cells of intestinal epithelium, are immunologically related to the liver transferases, and are increased in concentration by treatment of the animals with phenobarbital.

MATERIALS AND METHODS

Adult, male Osborn–Mendel rats (200–300 g), fed NIH 07 open-formula stock ration, were used in all studies.

Tissue preparations. To obtain mucosa from the rat gastro-intestinal tract, particulate matter was freed by rinsing with phosphate buffered saline at pH 7.4. The adhering mesentery was trimmed, segments were opened, and additional mucus was removed by blotting with moist tissue paper. Mucosa was freed from the underlying muscular layer by scraping with a glass slide on an ice-cold surface. Enzyme extracts were obtained from mucosal scrapings by disruption in a Waring blender at between 0° and 6° for 45 sec in ten volumes of a solution containing 75 mM potassium phosphate at pH 7.6, 330 mM sucrose and 2 mM dithiothreitol. The presence of a mercaptan disrupts

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mucus which can interfere with subsequent centrifugation and pipetting procedures [16]. Extracts were centrifuged for 20 min at 15,000 *g* and the resulting supernatant liquid was used for determination of enzyme activity.

Epithelial cell fractions were obtained by the Weiser procedure [17] from intestine excised between the ligament of Trietz and the cecum. The lumen was rinsed with phosphate buffered saline at 37°, clamped at one end, filled with approximately 18 ml of Weiser buffer A, clamped at the other end, and incubated at 37° with gentle shaking in a beaker containing phosphate buffered saline supplemented with 1 mM dithiothreitol. After 15 min, the lumen was drained, refilled with Weiser buffer B at 37°, and the incubation continued. This procedure was repeated at intervals of between 2 and 15 min for a total of 45 min, i.e. until few additional cells were released into the buffer. Cells were collected separately at each step, chilled in an ice bath, centrifuged at 500 *g* for 3 min and washed twice with cold phosphate buffered saline.

Cells were homogenized with 30 strokes of a Dounce B homogenizer in 8 volumes of 125 mM potassium phosphate at pH 7.6 containing 330 mM sucrose. After centrifugation for 20 min at 15,000 *g*, the resulting supernatant fractions were used for determination of enzyme activity.

Enzyme assays. Glutathione *S*-transferase activity was determined spectrophotometrically with 1-chloro-2,4-dinitrobenzene and GSH as substrates. The procedures for this assay as well as those for each of the other transferase substrates used, have been described [6]; fresh, unfrozen extracts were necessary for reproducible determination of enzyme activity. Thymidine kinase was assayed in a solution containing 50 mM Tris HCl, pH 8.0 [18], 5 mM ATP, 5 mM $MgCl_2$, and 4 μM [^{3}H]thymidine at 37°; final volume was 0.1 ml. Aliquots were withdrawn at intervals for 15 min and the phosphorylated products were collected on Whatman DE-81 filters as described by Breitman [19]. γ-Glutamyl-transpeptidase was assayed with L-γ-glutamyl-*p*-nitroanilide as described by Orlowski and Meister [20] with the addition of glycylglycine as acceptor [21]. Alkaline phosphatase was assayed with *p*-nitrophenyl phosphate as described by Weister [17]. Protein was determined colorimetrically [22].

Immunodiffusion studies. Antibody to transferases B and E were raised in separate sheep; antibody was specific for the respective glutathione *S*-transferase [3]. Antibody to transferase A was raised in New Zealand rabbits; this antibody was equally active with either transferase A or C but not with any of the other transferases [6]. Immunoglobulin was prepared from each serum by standard methods [23] and used for double diffusion studies by the procedure of Ouchterlony [24].

RESULTS

Glutathione S-transferases in mucosal epithelium. Preliminary determination of glutathione *S*-transferase activity was carried out with a spectrum of substrates using extracts derived from scrapings of jejunal mucosa. The highest activity was found with 1-chloro-2,4-dinitrobenzene (Table 1). Indeed, the activity with the other substrates was sufficiently low that, in the interest of accuracy, subsequent determinations of enzyme activity were limited to the standard assay with GSH and 1-chloro-2,4-dinitrobenzene. The range of activity with the several different substrates was similar to that observed in extracts of rat liver [2, 6].

Table 1. Glutathione *S*-transferase activity of extracts of jejunal epithelium as determined with GSH and each of a number of substrates by standard [9] assay procedures

Substrate	Specific activity*
1-Chloro-2,4-dinitrobenzene	0.35
1,2-Dichloro-4-nitrobenzene	0.03
Ethacrynic acid	0.02
1,2-Epoxy-3-(p-nitrophenoxy)propane	0.01
p-Nitrobenzyl chloride	0.02

* Specific activity is defined as the number of μmoles of product formed per min per mg of protein.

More than 96 per cent of the total activity present in mucosal homogenates was recovered in the supernatant fluid following centrifugation at 100,000 *g* for 1 hr. No activity was detected in purified [25] brush border membranes.

Localization of activity. When transferase activity was measured in different regions of the gastrointestinal tract (Table 2) the highest activity appeared in the duodenal and jejunal mucosa. Over 90 per cent of the total activity in scrapings was found in the small intestine whereas lower specific activity and total activity were observed in the stomach, cecum, and colon.

The intestinal muscularis remaining after scraping of mucosa contained 19 per cent of the total activity, probably the result of residual mucosal cells contaminating the preparation. The muscularis remaining after selective elution of epithelial cells (see below) and subsequent scraping, was virtually devoid of activity.

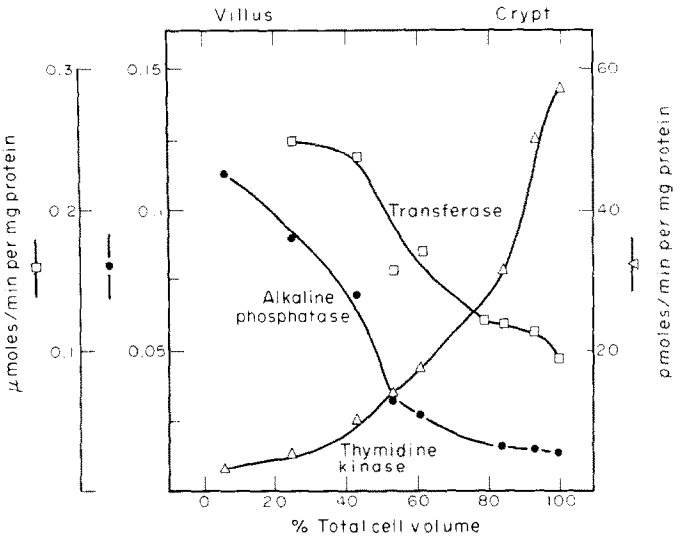
Distribution among villus and crypt cells. Mucosal epithelial cells are produced in the crypts of Lieberkuhn and undergo enzyme and morphological changes as they migrate up the villi and mature into

Table 2. Distribution of glutathione *S*-transferases activity, in mucosal scrapings of sections of the gastrointestinal tract with GSH and 1-chloro-2,4-dinitrobenzene as substrates*

Tissue	Specific activity†	% of Total units
Stomach	0.012	2
Duodenum	0.41	20
Jejunum (proximal)	0.40	23
Jejunum (distal)	0.35	15
Ileum (proximal)	0.26	10
Ileum (distal)	0.10	3
Cecum	0.14	5
Colon	0.13	3

* The intestine was divided into four equal segments between the ligament of Trietz and the ileocecal valve. Difficulty in completely separating epithelium and muscularis accounts for recovery of only 81 per cent of the total activity of the scrapings (see text).

† Specific activity is defined as the number of μmoles of product formed per min per mg of protein.



1. Distribution of glutathione S-transferase activity with glutathione and 1-chloro-2,4-dinitrobenzene as substrates in villus and crypt cells of rat intestinal mucosa obtained by the general method of Weiser [17] described in the text. The total packed volume of cells obtained was considered as 100 per cent of the cell volume.

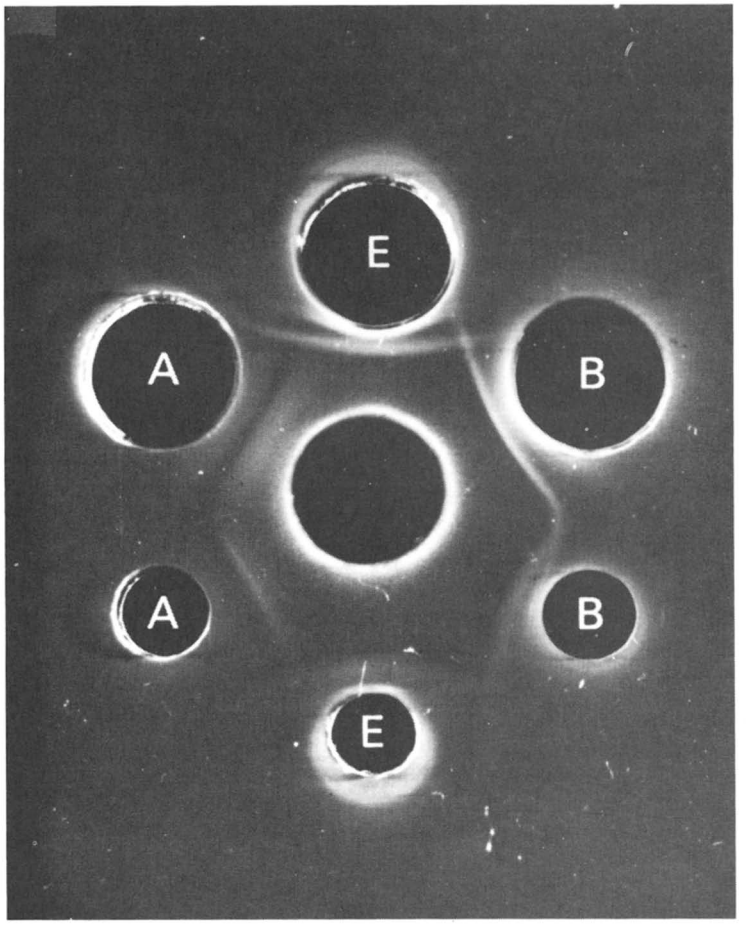


Fig. 2. Ouchterlony double-diffusion study of the glutathione S-transferases present in rat intestinal epithelium. A soluble extract (6 mg/ml) from epithelial scrapings was placed in the center well and the IgG against transferases A, B and E were placed in the surrounding wells.

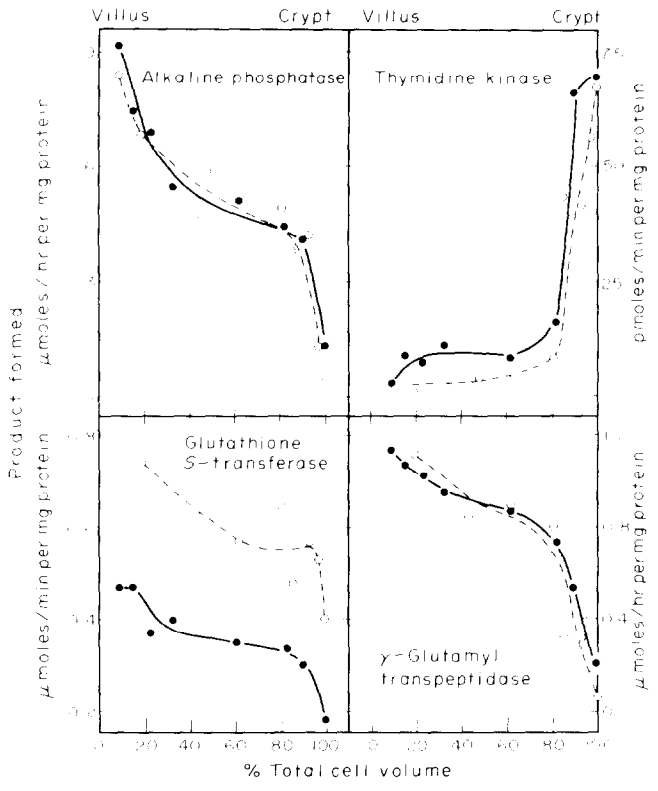


Fig. 3. Response of glutathione *S*-transferase activity and that of several other enzymes to phenobarbital. Villus and crypt cells were derived from jejunal segments of intestine from male rats treated with 80 mg per kg of phenobarbital per day for 3 days. Rats were sacrificed 24 hr after the third intraperitoneal injection of phenobarbital. With phenobarbital (O—O); without phenobarbital (● ●).

the primary absorptive cells [26–28]. Cells at different stages of maturation were obtained from a whole rat intestine by the method of Weiser [17]. Villus cells were identified by their alkaline phosphatase activity whereas crypt cells were identified by their high thymidine kinase activity (Fig. 1). The gradient in activity of the marker enzymes attests to the success of the separation. Thus, a shallow gradient of decreasing transferase activity was observed from villus to crypt cells (Fig. 1). The results indicate that significant transferase activity is present in the crypt cells but that activity increases by a factor of about two as the epithelial cells mature. A similar gradient with a two-fold increase in specific activity in all fractions was obtained with segments of jejunum; lower specific activities were observed with fractions derived from ileum. These observations are consistent with the data on the differential distribution of transferase activity in intestinal mucosa (Table 2).

Immunological comparison of rat liver and intestinal transferases. Upon examination by Ouchterlony double-diffusion an extract of rat intestinal mucosa, placed in the center well, reacted with the immunoglobulin prepared against homogeneous preparations of rat liver transferases A, B, and E (Fig. 2). The pattern shows that several transferase species are present in gut epithelium. This observation is consistent with the spectrum of catalytic activity found in Table 1, a spectrum which requires the presence of more than one transferase [2].

Response to phenobarbital. Phenobarbital is known to induce microsomal drug metabolizing systems in rat liver [29] and intestine [30]. Glutathione *S*-transferase activity in liver is also increased to between 130 per cent and 180 per cent of normal over a period of several days [4, 31]; the kidney transferases are not affected by phenobarbital [4]. To ascertain if the activity of the intestinal transferases is affected by phenobarbital, the inducer was administered intraperitoneally for 3 days (80 mg/kg daily), a period sufficient to increase activity in liver [4]. Treatment for this period seemed sufficient since intestinal epithelial cells of the rat turn over every 2 to 3 days [28]. The results, shown in Fig. 3, indicate that transferase activity with 1-chloro-2,3-dinitrobenzene increases in both villus and crypt cells after exposure to the inducer. An approximately 2-fold increase was measured whereas alkaline phosphatase, thymidine kinase, and γ-glutamyl transpeptidase remained the same. Specific activity of the transferases in extracts of the livers from the same rats was 1.2 μmoles min^{-1} mg^{-1} and 2.3 μmoles min^{-1} mg^{-1}, respectively, for control and phenobarbital treated animals.

The time course of the increase in activity due to phenobarbital was also followed in jejunal mucosal scrapings. Virtually no increase was observed after 1 day (110 per cent). However, activity increased to 132, 160 and 155 per cent of control at days 2, 3, and 4, respectively. In two other cell-separation experiments, transferase activity in villus cells was increased to 155 and 190 per cent of the control value after injection of phenobarbital.

DISCUSSION

The intestinal and colonic epithelium is repeatedly exposed to metabolites and xenobiotics derived from dietary constituents and bacterial metabolism. Products of digestion or drugs taken orally can be removed from the lumen by the intestinal villus cells and both villus and crypt cells are potential sites for absorption of metabolites present in arterial blood. Several detoxication systems have been detected in the gut including the enzymes of glucuronide formation [32], sulfate transferases [33] and the mixed function oxidases [33–35]. For the latter, highest activity was observed in the villus cells of the duodenum and jejunum [36] with specific activities similar to that of the glutathione S-transferases.

The data indicate that significant amounts of the transferase activity are located in the cytosol of intestinal villus and crypt cells. These enzyme species from intestinal epithelium cross-react with antibody to glutathione S-transferases A, B and E that had been isolated previously from rat liver. As with liver, the highest specific activity is observed with 1-chloro-2,4-dinitrobenzene and this value is increased in both tissues by treatment of the animals with phenobarbital. Based on the assumption that the maximum specific activity of intestinal and liver transferases is the same, it may be calculated that the transferases comprise 3 per cent of the extractable protein from duodenal and jejunal mucosa. Immunofluorescence studies with an antibody directed specifically to transferase B, led to an estimate of transferase B concentration in this tissue as 2 per cent [14].

Although the potential of the intestinal transferases in detoxification has not been evaluated *in vivo*, there are several factors that are in accord with this role. Glutathione S-transferase activity is greatest in those cells at which considerable absorption of dietary metabolites and xenobiotics occurs, i.e. the villus cells of duodenum and jejunum. Additionally, the concentration of one of the substrates, GSH, is sufficiently high to be compatible with this function. Thus, the K_m for GSH of liver transferases AA, A, B, and C is 0.2 mM [2] whereas the concentration of GSH is about 4 mM in crypt and 0.3 mM in villus cells [37]. Products generated by transferase action may be metabolized further in the intestine or liver. γ-Glutamyl-transpeptidase, the enzyme believed to catalyze the second step in mercapturic acid formation, is present in intestinal epithelium where its activity approaches 0.1 μmoles/mg/min in the villus cells [37, 38].

On a more speculative level, the low glutathione transferase activity found in colonic epithelium may be significant if a correlation can be established between this tissue's sensitivity to chemical induction of neoplastic growth [39, 40] and a diminished ability to detoxify absorbed substances.

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