compartment ensures adequate amounts of folates as polyglutamates necessary for optimizing folate-dependent biosynthetic activities during rapid cycling of these cells. Moreover, it is well known [4] that the proliferative fraction of this epithelial cell compartment is the site of limiting toxicity to antifolates in this rodent. Data obtained during the current studies should now allow a more valid estimate of the extent that membrane transport and folylpolyglutamyl synthetase play a role in the accumulation of specific 4-aminopterin polyglutamates in this proliferative tissue compartment. Finally, although there are a number of possible explanations for this variability in FPGS activity among the cellular fractions, it most likely reflects "down-regulation" of FPGS gene expression during maturation in this issue compartment. Further work will be required to confirm this notion.

In summary, folylpolyglutamyl synthetase activity in maturating luminal epithelial cells from mouse small intestine was measured by direct enzymologic assay in cell-free extracts and by monitoring *in vivo* [^{3}H]aminopterin polyglutamate synthesis following administration to mice. Our results revealed that FPGS activity was proliferation-dependent and decreased during maturation of this epithelial cell compartment.

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Interindividual variation in phase II detoxification enzymes in normal human colon mucosa*

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Human colon tissue is in continuous contact with the chemical environment defined by the diet as modified by endogenous gut mucosal metabolism. Some experimental data have given rise to speculation that various compounds or their metabolites play a role in carcinogenesis [1]. If this is true, colon tissue pathways that detoxify xenobiotics are of potential importance in tumor susceptibility. Indeed, an association has been suggested previously between cytochrome P-450-dependent phase I enzyme activity and inducibility and cancer incidence [2]. It has also been suggested that in the process of chemical carcinogenesis some detoxification pathways may be induced, resulting in anti-cancer drug-resistant tumors [3, 4]. A number of studies have reported colon cancer tissue levels of some enzymes to suggest a role in drug resistance [5, 6]. However, there is little data available on normal mucosal phase II detoxification components and, in particular, on the degree of interindividual variability. Such variability would be predicted based on the data on the phase I enzymes and the spectrum of inducers that can influence these pathways [7]. This information will be useful in evaluating levels described in tumors. This paper describes the activities and interindividual variabilities of six detoxification components measured in the normal appearing colon mucosa from fifteen human subjects with colon cancer.

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Materials and methods

Eight women and seven men, mean age 70.1 ± 7.0 years, were studied. All had locally advanced tumors, without any evidence of abdominal or systemic metastasis. Specimens

Table 1. Phase II detoxification components in normal mucosa

	GST (nmol/min/mg protein)	GPx	GSH (μmol/g tissue)	GGT (mUnits/mg protein)	GGCS (μmol/60 min/mg protein)	DTD (nmol/min/mg protein)
Mean ± SD	122.9 ± 49 (15)*	10.1 ± 5 (14)	3.0 ± 1.8 (15)	2.7 ± 2 (9)	0.38 ± 0.2 (15)	27.7 ± 18 (5)
Range	26–213	2–20.4	0.4–6.0	0.6–7.6	0.1–0.6	8.4–44.6
Variation (-fold)	8	10	16	14	5	5

Abbreviations: GST, glutathione-*S*-transferase; GPx, glutathione peroxidase; GSH, total glutathione; GGT, gamma-glutamyl transpeptidase; GGCS, gamma-glutamylcysteine synthetase; and DTD, D,T-diaphorase.
* Figures in parentheses represent the number of patient samples assayed.

removed at surgery were dissected free of fat and "snap" frozen within 1 hr. In most cases the tumors were readily identified grossly, and normal appearing adjacent mucosa was taken from at least seven inches beyond the tumor margin. Three were located in the ascending colon, five in the caecum, ane seven in the sigmoid colon. Where margins were less obvious, histologic confirmation was made. Tissues were homogenized using a Polytron homogenizer (Brinkmann Instruments, Toronto) in a buffer containing 0.25 M sucrose, 150 mM KCl, and 50 mM Tris, pH 7.4. Centrifugation at 10,000 *g* for 30 min resulted in a post-mitochondrial supernatant (PMS), and a second centrifugation at 100,000 *g* for 1 hr resulted in the cytosolic preparation.

Biochemical assays were done in batch runs of at least five samples, in triplicate. Protein was determined according to the method of Lowry *et al.* [8], using fatty acid free bovine serum albumin as the control. Enzymes were measured on the preparations described above, but total glutathione (GSH) was assayed according to the technique of Ellman, as described in Ref. 9, in tissue homogenized in 3% sulfosalicylic acid. Glutathione-*S*-transferase (GST) was assayed in cytosol using 2,4-chlorodinitrobenzene as substrate according to Habig *et al.* [10], glutathione peroxidase (GPx) was assayed in cytosol using hydrogen peroxide as substrate [11], gamma-glutamyl transpeptidase (GGT) was measured in homogenate using the method of Szaz [12], and gamma-glutamylcysteine synthetase (GGCS) was assayed as described by Richman and Meister [13]. D,T-Diaphorase (DTD) was measured in the cytosol using menadione as substrate as described by Ernster [14].

Results and discussion

Table 1 shows the mean results of each of the biochemical assays performed. The standard deviations and ranges demonstrate the interindividual variability of these data. Consistent with reports of other enzymes studied in this manner, the fold variation is provided. There was no influence of sex, age, tumor location, drug exposure or cigarette smoking among the enzymes assayed. The only significant correlation in activity was between GST and GPx ($r = 0.7$, $P = 0.007$).

As noted above, previous reports have shown that marked interindividual differences occur for the metabolism of chemicals in humans, and that these differences result from complex interactions between environment and genetic systems. These studies have focused on the cytochrome P-450-dependent enzymes [15]. Recently, attention has been turned to the phase II detoxification components of drug metabolism. Induction of some of these has resulted in protection from carcinogens in a number of models [16]. Although these generally appear to be separate systems under different regulatory control, there is some evidence of coordinate regulation. All components are inducible, and in the case of drug-resistant carcinogen-induced preneoplastic liver nodules in rats, simultaneous activation of all of the components studied here compared to the normal adjacent hepatocytes has been shown [4]. As a preliminary step in the examination of this phenotype in human colon cancer, it is important to establish the variability of these activities among different individuals. Metabolic pathways of mutagens and carcinogens have been comprehensively examined in human liver tissue, both normal and malignant, in view of the important role this organ plays in drug disposition. Similarly, drug metabolism enzymology was examined in human lungs because of their large contact surface with environmental toxins. Significant interindividual variation was found in normal lung tissue [17]. Although altered by enteric bacteria and digestive enzymes, the colonic contents represent an important point of contact with environmental agents, those in food. The interindividual variation in the normal appearing mucosa

of drug detoxification enzymes may represent the effects of subtle dietary variation among them.

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