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OXIDANT DEFENSE MECHANISMS IN THE HUMAN COLON[1]

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Abstract—Reactive oxygen metabolites have been implicated as important mediators of inflammation-induced intestinal injury associated with ischemia (and reperfusion), radiation, and inflammatory bowel disease. Because the colonic mucosa may be subjected to significant oxidant stress during times of acute and chronic inflammation, knowledge of the oxidant defense mechanisms in the colon is of biologic and potential clinical importance. Therefore, the objective of this study was to quantify the specific activities of superoxide dismutase (SOD), catalase, and GSH peroxidase in the normal human colon. We found low, but significant, amounts of all three enzymes in the mucosa, submucosa, and muscularis/serosa of the human colon. However, the mucosal levels of SOD (3.6 ± 0.3 units/mg protein), catalase (11 ± 3 units/mg), and GSH peroxidase (15.2 ± 0.8 mU/mg) represented only 8%, 4%, and 40%, respectively, of those values determined for human liver. Colonic epithelial cells derived from mucosal biopsies exhibited significantly higher specific activities for SOD (12 ± 0.5 units/mg) and catalase (26 ± 6 units/mg) when compared to whole mucosa, suggesting most of the mucosal activity was associated with the epithelial cells and not the lamina propria. In a comparative study, we found that a human colonic carcinoma cell line (CaCo-2) contained significantly lower SOD (6 ± 0.5 units/mg) and catalase (6 ± 0.6 units/mg) activities when compared to colonic epithelial cells. Taken together, our data suggest that: (1) the colonic mucosa is relatively deficient in antioxidant enzymes when compared to liver, and (2) most of the protective enzyme activity is localized within the epithelium and not the mucosal interstitium.

INTRODUCTION

The colonic mucosa plays an important role in maintaining proper fluid and solute exchange as well as providing a restrictive barrier to the immigration of

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potentially noxious bacteria, bacterial products (lipopolysaccharide, *N*-formylated peptides), and luminal antigens. The mucosal epithelium in known to be injured in a variety of pathophysiological conditions such as ischemic colitis, radiation enteritis, necrotizing enterocolitis, and inflammatory bowel disease (IBD). The enhanced mucosal permeability that results from injury to the epithelium may facilitate the entry of luminal components such as bacteria and bacterial products into the mucosal interstitium and ultimately the systemic circulation. Recent work suggests that the gut ischemia induced by either hemorrhage or endotoxin results in mucosal injury that coincides with translocation of luminal bacteria into the mucosal interstitium (1, 2). These data have prompted investigators to suggest that a breech in the mucosal barrier may play an important role in the initiation and exacerbation of multiorgan failure (MOF) (3).

There is a growing body of experimental evidence to suggest that inflammation-induced intestinal injury associated with ischemia (and reperfusion), radiation, and IBD is mediated in part by reactive oxygen metabolites (ROM) generated by the parenchyma and/or inflammatory phagocytes (4, 5). Most tissues are protected from the injurious effects of these cytotoxic species by the action of certain antioxidant enzymes such as superoxide dismutase (SOD), catalase, and GSH peroxidase. The levels of these enzymes vary greatly from tissue to tissue, with the liver and spleen containing high concentrations and other tissues such as heart and skeletal muscle containing very low levels of these antioxidant enzymes. Virtually nothing is known regarding the levels of antioxidants in the human colon. Indeed, the relative paucity of antioxidant enzymes in certain tissues has prompted some investigators to suggest that these tissues may be easily overwhelmed by enhanced ROM production and thus more sensitive to oxidant injury (6). Since the colonic mucosa may be subjected to significant oxidative stress during times of hypoperfusion or inflammation and thus susceptible to mucosal injury, knowledge of the oxidant defense systems of the gut is of biological and potential clinical importance. Therefore, as a first step in clarifying the intrinsic oxidant defenses in the human colon, we measured the levels of SOD, catalase, and GSH peroxidase in segments of the human colon, in mucosal biopsies, and from a human colonic carcinoma cell line.

MATERIALS AND METHODS

Collection of Tissue and Preparation of Colonic Epithelial Cells (7–9). Colonic specimens were collected from a total of six patients undergoing elective colon resections for adenocarcinoma of the distal bowel including the descending and sigmoid colon as well as the rectum. For one set

of experiments, full-thickness portions of the uninvolved (normal) colon at the resection margin were excised and rinsed with ice-cold saline. The specimens then were transported on ice directly to the laboratory where they were processed as follows: The mucosa was separated from the submucosa/muscularis by scraping with the sharp edge of a glass slide. The submucosa was removed carefully from the muscularis/serosa by dissection with a small spatula. All colon samples were rinsed in ice-cold saline, blotted dry, and frozen at −70°C.

For another set of studies colonic specimens from the ascending colon were obtained from individuals ($n = 10$) donating organs for transplantation at the same time as other organs were harvested, i.e., the colon was perfused at the time of removal. It was determined that the time of warm ischemia was <45 min, which is shorter than for surgically removed specimens (9). These specimens were dissected free of underlying intestinal tissue, and cut into 2- to 5-mm^2 pieces. Tissue was washed in calcium- and magnesium-free Hanks' balanced salt solution (HBSS) containing 1 mg/ml ticarcillin (Beecham Labs), 0.5 mg/ml amikacin (Briston Saybrook Co., Old Saybrook, Connecticut), 0.4% Bactrim (Roche Diagonstics, Nutley, New Jersey), 2 μg/ml Fungizone (Gibco), 10 mM HEPES, and NaOH to pH 7.4 (medium I). Epithelial cells were removed by repeated washings (45 min on magnetic stirring plate) in medium I containing 0.75 mM EDTA and 5% heat-inactivated pooled human serum (8). The epithelial cells from the colon were obtained as sheets of cells in which single cells as well as entire crypts were observed. Identity of the cells as epithelial cells was corroborated by fixing and histologic staining of cytospin preparations of the cells. Viability was ascertained by trypan blue exclusion. We consistently obtained cell preparations that were greater than 99% epithelial cells, in which only occasional (1 in 500) cells were lymphocyte in origin, and in which greater than 95% of the cells were viable. The epithelial cells were immediately washed, centrifuged, and frozen at −70°C for subsequent biochemical analysis. Liver specimens were obtained from two patients undergoing elective surgery for adenocarcinoma and for biliary cirrhosis. Pieces of uninvolved tissue were excised from the margin of the specimens, rinsed in ice-cold saline, blotted dry, and frozen at −70°C.

CaCo-2 Cell Culture. CaCo-2 cells were obtained from Dr. Jeffery Field, Department of Medicine, University of Iowa. Cells were grown in DME with 4.5 g/liter glucose and L-glutamine, to which we added MEM nonessential amino acids (1%; 10 mM), penicillin-streptomycin (1%; units/mg), L-glutamine (1%; 200 mM), gentamicin (0.1%; 50 mg/ml), and fetal calf serum (20%) (10). Cells in a 85-cm^3 tissue culture flask were fed three times a week with 20 ml medium, and split at 70–80% confluence (about five to seven days) with 3 ml trypsin–EDTA (Gibco). Cells were harvested at confluence for assay.

Enzyme Assays. Tissue or cells were homogenized (10% w/v) in ice-cold 20 mM potassium phosphate buffer (pH 7.4) containing 0.1 mM EDTA and 0.1% Triton X-100. Homogenates were centrifuged 10,000 *g* for 20 min at 4°C and the supernatant saved on ice. Catalase activity was measured by the method of Aebi (11) in which the disappearance of H_2O_2 was monitored at 240 nm. SOD activity was determined by the method of Crapo et al. (12) in which 10 μM NaCN was included in the reaction volume to inhibit cytochrome oxidase. The SOD activity determined by this method represents the total SOD activity of the tissue, i.e., the sum of the Cu–Zn and Mn isozymes. GSH peroxidase was measured using a modification of the method of Paglia and Valentine (13) in which aliquots of supernatants were preincubated with 1 mM GSH for 10 min at 37°C before analysis as described by Blanchflower et al. (14). GSH peroxidase maybe reversibly inactivated upon freezing for several days, and the enzyme can be reactivated by preincubation with GSH (14). Tissue-associated and erythrocyte hemoglobin were determined by the method of Tentori and Salvati (15). Human erythrocyte SOD, catalase, and GSH peroxidase were expressed per milligram hemoglobin and tissue levels of these antioxidant enzymes were corrected for blood contamination. Protein was determined using the BioRad Laboratories protein assay kit and bovine serum albumin as the standard.

RESULTS

Figures 1–3 illustrate the antioxidant enzyme activities from the different layers of the normal human colon as well as from the liver (for comparison). We found that the colonic mucosa contained significantly more SOD activity when compared to the muscularis/serosa (Figure 1), whereas there was no significant difference in catalase activity in the three layers of the colon (Figure 2). In addition, the colonic mucosa contained significantly more GSH peroxidase activity when compared to either the submucosa or muscularis/serosa (Figure 3). Although significant levels of all three antioxidant enzymes were detected

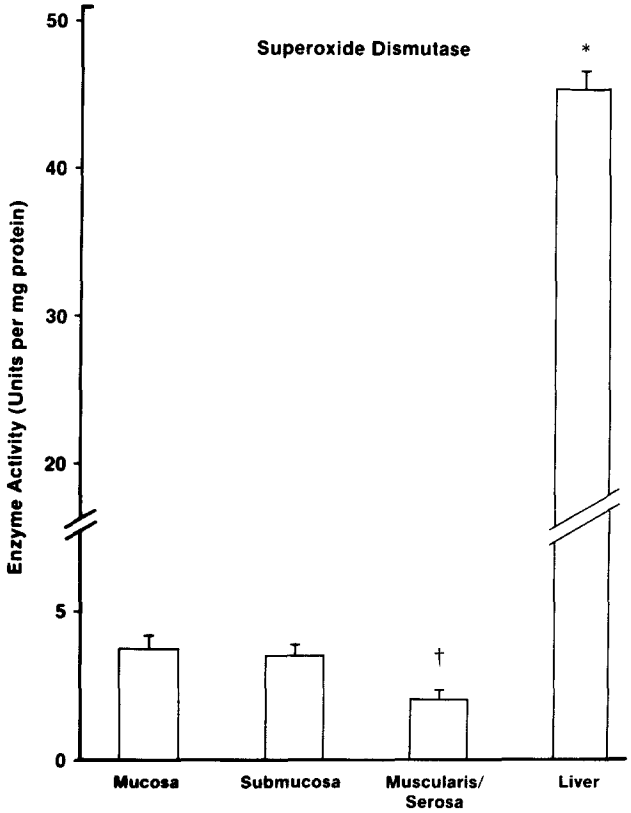

Fig. 1. Superoxide dismutase (SOD) activity in the different regions of the colon and in the liver. Data are corrected for contributions made by blood contamination of the tissue and are expressed as units per milligram protein. Data represent the mean ± *SEM* for duplicate samples from six individual specimens, except for liver which represents the mean ± *SEM* from three individual specimens. † and * represent $P < 0.007$ and 0.008, respectively, compared to mucosa.

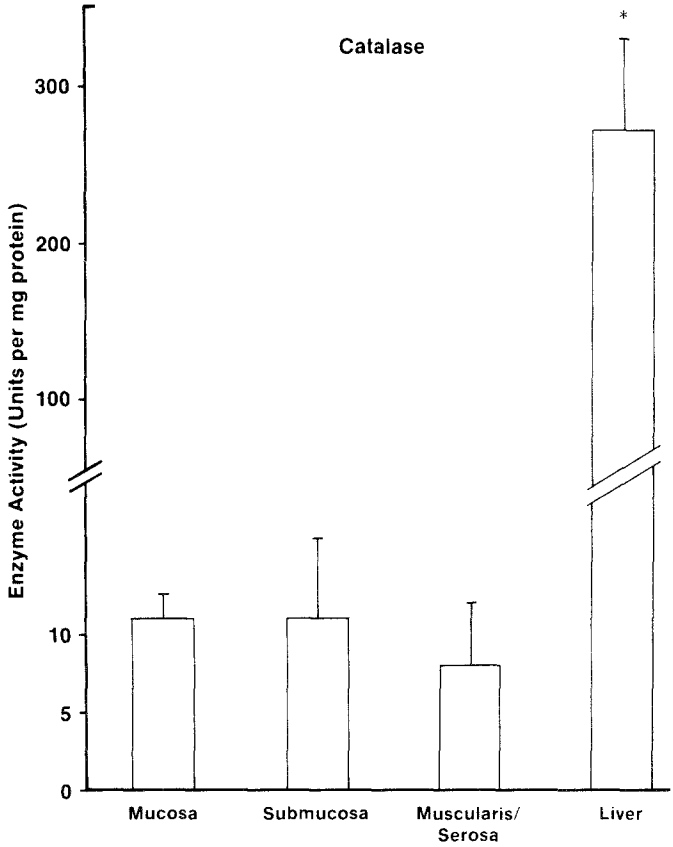

Fig. 2. Catalase activity in the different layers of the colon and in the liver. Data are corrected for contributions made by blood contamination of the tissue and are expressed as units per milligram protein. Data represent the mean ± *SEM* for duplicate samples from six individual specimens, except for liver which represents the mean ± *SEM* from three individual specimens. * represents $P < 0.05$ compared to mucosa.

in human colon, mucosal levels of SOD, catalase, and GSH peroxidase represented only, 8%, 4%, and 45%, of those values found in liver, respectively. Figures 4–6 show the antioxidant enzyme activity profile for colonic epithelial cells, CaCo-2 cells, and erythrocytes. Colonic epithelial cells exhibited significantly higher specific activities of SOD and catalase when compared to mucosal levels of these enzymes, whereas the specific activity of GSH peroxidase in colonic epithelial cells was similar to that found in the mucosa. In addition, the specific activities for SOD and catalase from colonic epithelial cells were two- and fourfold higher than the levels found in CaCo-2 cells, respectively (Figures

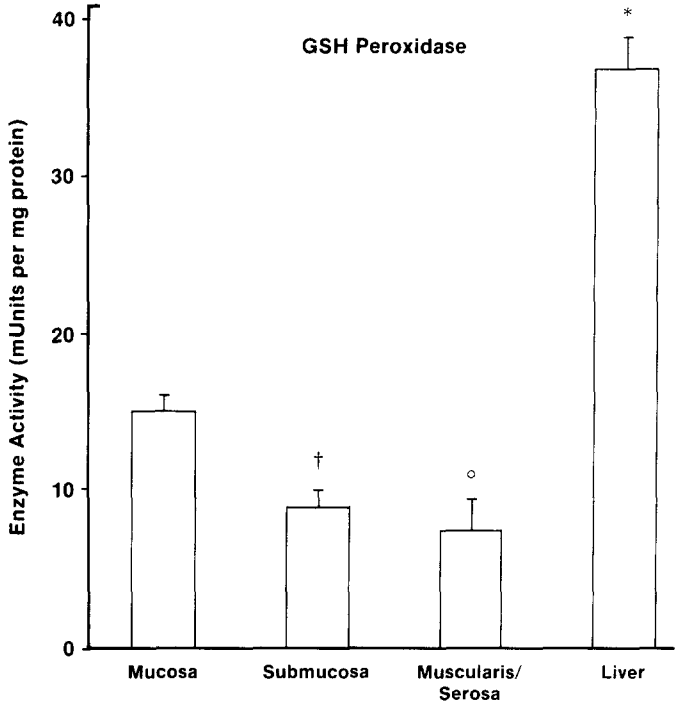

Fig. 3. GSH peroxidase activity in the different layers of the colon and in the liver. Data are corrected for contributions made by blood contamination of the tissue and are expressed as mU per milligram protein. Data represent the mean ± *SEM* for duplicate samples from six individual specimens, except for liver which represents the mean ± *SEM* from three individual specimens. †, °, and * represent $P < 0.03$, 0.01, and 0.001, respectively, compared to mucosa.

4 and 5), while GSH peroxidase activity was similar in both cell types (Figure 6). Colonic epithelial cell SOD activity was four times that of erythrocytes (Figure 4), whereas epithelial cell catalase activity represented only 14% of the activity found in erythrocytes (Figure 5). GSH peroxidase activity was similar for both cell types (Figure 6).

DISCUSSION

The intestinal mucosa is particularly well endowed with the enzymatic machinery necessary to produce large quantities of reactive oxygen metabolites (16). These reactive species have been shown to play an important role in

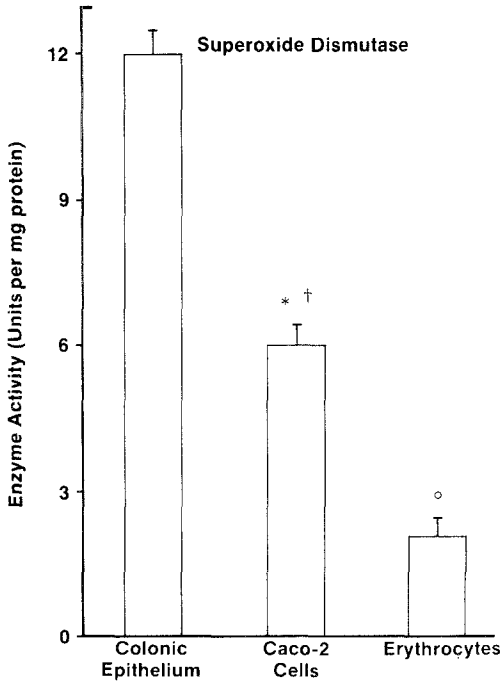

Fig. 4. SOD activity in various cell types. Data are expressed as units per milligram protein and represent the mean ± *SEM* for duplicate samples from 10 specimens of colonic epithelium, 0.5 g of confluent CaCo-2 cells, and six for human erythrocytes. * and ° represent $P < 0.05$ and 0.001, respectively, compared to colonic epithelium. † represents $P < 0.001$ compared to erythrocytes.

mediating the mucosal injury associated with hypoperfusion (ischemia, shock), radiation enteritis, and inflammation (1, 2, 4, 5). We have demonstrated that inhibition of the ROM-generating enzyme xanthine oxidase significantly protects the intestine from the injurious effects of hypoperfusion (e.g., ischemia, endotoxin, and hemorrhagic shock) and remarkably attenuates bacterial translocation (1, 2, 4). In addition to parenchymal sources of ROM, the postischemic and/or inflamed gut contains large numbers of inflammatory phagocytes (granulocytes, macrophages) that have the potential to generate several different, highly reactive oxidants (5). Activated granulocytes release large quantities of cytotoxic oxidants including superoxide (O_2^-), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), and *N*-chloramines (RNHCl). We have estimated, using an enzymatic marker for granulocyte infiltration, that the postischemic intestine and inflamed colon contain approximately 10^7 granulocytes/g wet weight, respectively (17, 18). Assuming that 10^7 fully activated granu-

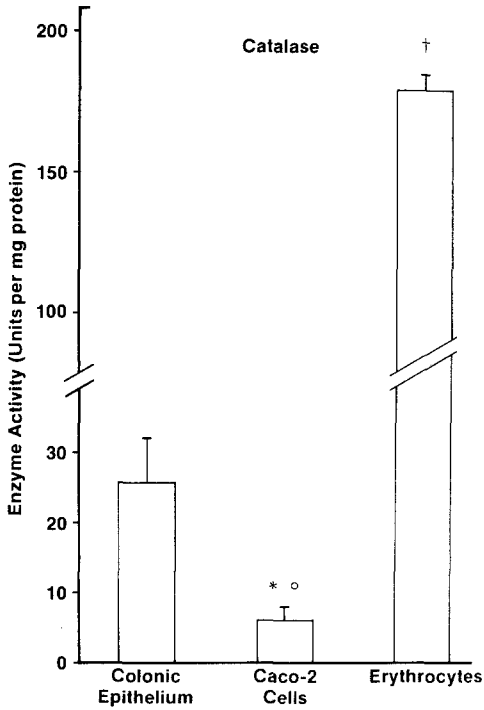

Fig. 5. Catalase activity in various cell types. Data are expressed as units per milligram protein and represent the mean ± *SEM* for duplicate samples from 10 specimens of colonic epithelium, 0.5 g of confluent CaCo-2 cells, and six for human erythrocytes. * and † represent $P < 0.05$ and 0.001, respectively, compared to colonic epithelium. ° represents $P < 0.01$ compared to erythrocytes.

locytes produce 50–60 nmol O_2^-/min (19), of which 5–10 nmol H_2O_2/min (20) and 5–10 nmol HOCl/min (20) are derived, one may speculate that even with adequate oxidant defenses, the tissue may be eventually overwhelmed by these fluxes of oxidants. Indeed, we have demonstrated recently that intestinal epithelial cells may be injured by concentrations of oxidants similar to those calculated above (21). Exposure of the distal bowel or enterocytes to the granulocyte oxidants of H_2O_2, HOCl, and certain RNHCl produced significant increases in mucosal permeability as well as cytotoxicity (21). If intestinal epithelial cells were exposed to sublethal concentrations of H_2O_2 (0.1 mM) for short periods of time (15 min), we found extensive DNA strand scission that appeared to be partially dependent upon iron (22). These cytotoxic and cytogenetic effects of ROM may play an important role in the mucosal alterations observed in patients with numerous, recurrent episodes of inflammation in the

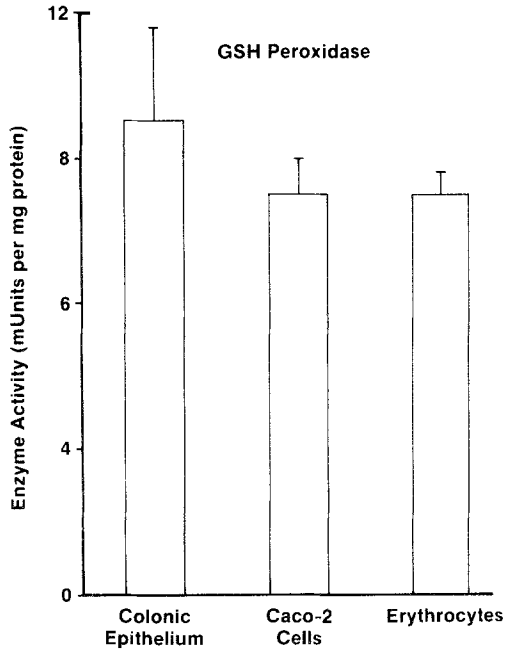

Fig. 6. GSH peroxidase activity in various cell types. Data are expressed as mU per milligram protein and represent the mean ± *SEM* for duplicate samples from 10 specimens of colonic epithelium, 0.5 g of confluent CaCo-2 cells, and six for human erythrocytes.

colon. Reactive oxygen metabolites have recently been implicated as mediators of intestinal injury in a model of necrotizing enterocolitis (23, 24). These investigators found that intravenous infusion of SOD just after induction of inflammation totally prevented the mucosal injury (23).

Some investigators have proposed that the relative lack of antioxidant enzymes in certain tissues may make these tissues especially susceptible to injury induced by a sustained oxidative insult (6). Because of the importance of tissue-associated oxidant defenses, coupled to the fact that virtually nothing is known regarding oxidant protective mechanisms in the colon, we determined the specific activities of SOD, catalase, and GSH peroxidase in the normal human colon.

We found significant levels of all three antioxidant enzymes in the mucosa, submucosa, and muscularis/serosa (Figures 1–3). There appeared to be a gradient of SOD and GSH peroxidase activities with respect to the different layers of the bowel: The mucosa and submucosa contained significantly more SOD activity when compared to the muscularis/mucosa (Figure 1), whereas the

mucosa contained significantly more GSH peroxidase activity compared to the submucosa or muscularis/serosa (Figure 3). Although significant levels of all antioxidant enzymes were detected in human colon, mucosal levels of SOD, catalase, and GSH peroxidase were much lower than the activities found in human liver. This is not surprising when one considers the propensity of each tissue to generate reactive oxygen metabolites. Hepatocytes, for example, are known to possess the enzymatic machinery (cytochrome P-450 system) necessary for the oxidative metabolism of a wide variety of endogenous and exogenous substrates. In the process of this metabolism, relatively large quantities of reduced oxygen species and free radicals are generated as metabolic byproducts. Thus, the liver has evolved an extensive oxidant defense system composed of both enzymatic and nonenzymatic antioxidants. The colon, on the other hand, engages in a less oxidative metabolism, and thus the metabolic pressure to evolve and maintain high concentrations of antioxidants is minimal. These data suggest that colonocytes may be more susceptible to injury induced by an overproduction of oxyradicals generated by parenchymal and/or inflammatory cells. When we separated the colonic epithelium from the mucosa, we found significant increases in specific activities of SOD and catalase. Taken together, our data suggest that the colonic mucosa is relatively deficient in antioxidant enzymes when compared to the liver and that most of the protective enzymes of the mucosa are localized within the epithelial cells. These data agree with an earlier report in which Hernandez et al. (25) showed that SOD and catalase were confined primarily to the epithelium of the cat small bowel.

The fact that epithelial cell specific activities of SOD and catalase were greater than those activities found in whole mucosa suggests that the colonic lamina propria (i.e., mucosal interstitium) may be deficient in, or devoid of oxidant protective mechanisms. This agrees with the recognized deficiency of antioxidant enzymes in the extracellular space (26). Because phagocytic leukocytes accumulate within areas of the mucosa that are deficient in antioxidants (e.g., lamina propria and intraepithelial spaces) in response to ischemia, radiation, and inflammation, one would expect oxidant-induced alterations in the extracellular matrix. The mucosal interstitium is composed primarily of collagen fibrils and mucopolysaccharides. Phagocyte-generated oxidants have been shown to degrade and depolymerize hyaluronic acid (27), collagen (28, 29) and mucin (30). Leukocyte-mediated degradation of these biopolymers in vivo may result in significant changes in mucosal integrity. We have demonstrated that perfusion of intestinal segments with the bacterial tripeptide *N*-formyl-methionyl-leucyl-phenylalanine (FMLP) produces significant increases in mucosal permeability (31). This increased permeability was inhibited either by rendering the animals neutropenic or by addition of a low-molecular-weight SOD mimetic (manganese–Desferal chelate) or by addition of a lipophilic GSH peroxidase analog (PZ-51) (32). These data suggest that FMLP-activated neutrophils pro-

duce significant levels of O_2^- and H_2O_2, which directly or indirectly mediate the increase in mucosal permeability. Data obtained in this study suggest that inflammation of the colon may produce significant fluxes of oxidants that could overwhelm the relatively low levels of protective enzymes and cause epithelial cell injury as well as alterations in mucosal integrity.

REFERENCES

1. DEITCH, E. A., W. BRIDGES, J. BAKER, J-W. MAY, L. MA, M. B. GRISHAM, D. N. GRANGER, R. D. SPECIAN, and R. BERG. 1988. Hemorrhagic shock-induced bacterial translocation is reduced by xanthine oxidase inhibition or inactivation. *Surgery* **104:**191–198.
2. DEITCH, E. A., L. MA, M. B. GRISHAM, D. N. GRANGER, R. D. SPECIAN, and R. BERG. 1989. Inhibition of endotoxin-induced bacterial translocation in mice. *J. Clin. Invest.* **84:**36–42.
3. WELLS, C. L., M. A. MADDAUS, and R. L. SIMMONS. 1989. Bacterial translocation. *In* Splanchnic Ischemia and Multiple Organ Failure. A. Marston, G. B. Blukley, R. G. Fiddian-Green, and U. H. Haglund, Editors. Edward Arnold, London. 195–204.
4. GRANGER, D. N., 1988. Role of xanthine oxidase and granulocytes in ischemia-reperfusion injury. *Am. J. Physiol.*, **255:**H1269–H1275.
5. GRISHAM, M. B., and D. N. GRANGER, 1990. Mechansims of neutrophil-mediated tissue injury. *In* Inflammatory Bowel Disease. R. MacDermott, and W. Stenson, Editors. Elsevier, New York (in press).
6. DOROSHOW, J. H., G. Y. LOCKER, and C. E. MYERS. 1980. Enzymatic defense of the mouse heart against reactive oxygen metabolites. *J. Clin. Invest.* **65:**128–135.
7. MACDERMOTT, R. P, G. S. NASH, M. J. BERTROVICH, R. F. MOHRMAN, I. J. KODNER, D. L. DELACROIX, and J. P. VAERMAN. 1986. Altered patterns of secretion of monomeric IgA and IgA Subclass 1 by intestinal mononuclear cells in inflammatory bowel disease. *Gastorenterology* **91:**379–385.
8. SCOTT, M. G., M. H. NAHM, K. MACKE, G. S. NASH, M. J. BERTROVICH, and R. P. MACDERMOTT. 1986. Spontaneous secretion of IgG subclasses by intestinal mononuclear cells: Differences between ulcerative colitis, Crohn's disease and controls. *Clin. Exp. Immunol.* **66:**209–215.
9. PETERS, M. G., H. SECRIST, K. R. ANDERS, G. S. NASH, S. R. RICH, and R. P. MACDERMOTT. 1989. Normal human intestinal B lymphocytes, increased activation compared with peripheral blood. *J. Clin. Invest.* **83:**1827–1833.
10. FIELD, F. J., E. ABRIGHT, and S. N. MATHUR. 1987. Regulation of cholesterol esterification by micellar cholesterol in CaCo-2 cells. *J. Lipid Res.* **28:**1057–1066.
11. AEBI, H. 1984. Catalase in vitro. *Methods Enzymol.* **105:**121–126.
12. CRAPO, J. D., J. M. MCCORD, and I. FRIDOVICH. 1987. Preparation and assay of superoxide dismutases. *Methods Enzymol.* **53:**382–387.
13. PALIA, D. E., and W. N. VALENTINE. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.* **70:**158–169.
14. BLANCHFLOWER, W. J., D. A. RICE, and W. B. DAVIDSON. 1986. Blood glutathione peroxidase: A method for measurement and the influence of storage, cyanide, and Drabkin's reagent on enzyme activity. *Biol. Trace Element Res.* **11:**89–100.
15. TENTORI, L., and A. M. SALVATI. 1981. Hemoglobinometry in human blood. *Methods Enzymol.* **76:**707–731.

16. GRANGER, D. N., L. A. HERNANDEZ, and M. B. GRISHAM. 1986. Reactive oxygen metabolites: mediators of cell injury in the digestive system. *Vien Dig. Dis.* **18:**13–16.
17. GRISHAM, M. B., L. A. HERNANDEZ, and D. N. GRANGER. 1986. Xanthine oxidase and neutrophil infiltration in intestinal ischemia. *Am. J. Physiol.* **251:**G567–G574.
18. SEKIZUKA, E., M. B. GRISHAM, M. LI, E. A. DEITCH, and D. N. GRANGER. 1988. Inflammation-induced intestinal hyperemia in the rat: Role of neutrophils. *Gastroenterology* **95:**1528–1534.
19. GRISHAM, M. B., T. D. ENGERSON, J. M. MCCORD, and H. P. JONES. 1985. A comparative study of neutrophils purification and function. *J. Immunol. Methods* **82:**315–320.
20. GRISHAM, M. B., M. N. JEFFERSON, D. F. MELTON, and E. L. THOMAS. 1984. Chlorination of endogenous amines by isolated neutrophils. *J. Biol. Chem.* **259:**10404–10413.
21. GRISHAM, M. B., T. S. GAGINELLA, C. VON RITTER, H. TAMAI, M. BE, and D. N. GRANGER. 1990. The effects of neutrophil-derived oxidants on ileal mucosal permeability and epithelial cell viability. *Inflammation* **14:**531 – 542.
22. GRISHAM, M. B., and P. R. KVIETYS. 1989. Sublethal amounts of hydrogen peroxide produce breaks in double stranded DNA of intestinal epithelial cells and lymphocytes. *Gastroenterology* **96:**A185 (abstract).
23. CLARK, D. A., D. M. FORNABAIO, H. MCNEILL, K. M. MULLANE, S. J. CARAVELLA, and M. J. S. MILLER, 1988. Contribution of oxygen-derived free radicals to experimental necrotizing enterocolitis. *Am. J. Pathol.* **130:**537–542.
24. MILLER, M. J. S., and D. A. CLARK. 1988. Participation of free radicals and eicosanoids in experimental necrotizing enterocolitis, a neonatal inflammatory bowel disease. *In* Inflammatory Bowel Disease: Current Status and Future Approach. R. P. MacDermott, editor. Excerpta Medica, Amsterdam. 267–272.
25. HERNANDEZ, L. A., M. B. GRISHAM, C. VON RITTER, and D. N. GRANGER. 1987. Biochemical localization of xanthine oxidase in the cat small intestine. *Gastroenterology* **92:**1433 (abstract).
26. HALLIWELL, B. and J. MC. GUTTERIDGE. 1986. Oxygen free radicals and iron in relation to biology and medicine: Some problems and concepts. *Arch. Biochem. Biophys.* **246:**501–514.
27. GREENWALD, R. A., and S. A. MOAK. 1986. Degradation of hyaluronic acid by polymorphonuclear leukocytes. Inflammation **10:**15–30.
28. CURRAN, S. F., M. A. AMORUSO, B. D. GOLDSTEIN, and R. A. BERG. 1984. Degradation of soluble collagen by ozone or hydroxyl radicals. *FEBS Lett.* **176:**155–159.
29. GREENWALD, R. A., and W. W. MOY. 1979. Inhibition of collagen gelation by action of the superoxide radical. *Arthritis Rheum.* **22:**251–259.
30. GRISHAM, M. B., C. VON RITTER, B. F. SMITH, J. LAMONT, and D. N. GRANGER. 1987. Interaction between oxygen radicals and gastric mucin. *Am. J. Physiol.* **253:**G93–G96.
31. VON RITTER, C., E. SEKIZUKA, M. B. GRISHAM, and D. N. GRANGER. 1988. The chemotactoc peptide *N*-formyl-methionyl-leucyl-phenylalanine increases mucosal permeability in the distal ileum of the rat. *Gastroenterology* **95:**651–656.
32. VON RITTER, C., M. B. GRISHAM, M. E. HOLLWARTH, W. INAUEN, and D. N. GRANGER. 1989. Neutrophil-derived oxidants mediate F-Met-Leu-Phe-induced ileitis in rats. *Gastroenterology* **97:**778–780.