ELSEVIER

Nutrition Research, Vol. 17, No. 3, pp. 463–473, 1997

0271-5317/97 $17.00 + .00

PII S0271-5317(97)00007-9

GLUTATHIONE LEVELS IN CHRONIC INFLAMMATORY DISORDERS OF THE HUMAN COLON

Eduardo A. Ruan, M.D., Simbasiva Rao, M.D., J. Steven Burdick, M.D., Steven J. Stryker, M.D., Gordon L. Telford, M.D., Mary F. Otterson, M.D., Emmanuel C. Opara, Ph.D., and Timothy R. Koch, M.D.*

Departments of Medicine and Surgery, Zablocki VA Medical Center, Medical College of Wisconsin, Milwaukee, Wisconsin; Departments of Surgery and Pathology, Northwestern University Medical School, Chicago, Illinois; and Duke University Medical Center, Durham, North Carolina

ABSTRACT

Glutathione depletion has been described in tissues obtained from several chronic diseases. Increased free radical production by inflammatory cells occurs in inflammatory bowel disease. We hypothesized that this could induce depletion of gut antioxidants. In this study, we examined the potential relationship between chronic inflammation and colonic glutathione levels. Using a validated assay, glutathione levels were determined in the mucosal-submucosal layer and the muscularis externa layer in surgical colonic specimens from 26 patients with ulcerative colitis, 14 patients with Crohn's colitis, and 10 patients who underwent partial colectomy for non-obstructive neoplasia. Inflammation was graded histologically. Glutathione levels were decreased in the muscularis externa and in the mucosal-submucosal layers from both ulcerative colitis and Crohn's colitis (both $p<0.05$). There were parallel declines of glutathione levels in the muscularis externa layer compared to the mucosal-submucosal layer from individual colonic specimens. In ulcerative colitis, glutathione levels were reduced in histologically active disease compared to inactive disease (in the mucosal-submucosal layers: Mean±SEM were 214±68 nmol/g wet tissue and 808±30, respectively; in the muscularis externa layers: 333±97 and 890±340; both $p<0.05$). In Crohn's colitis, there were no significant differences between histologically active and inactive disease (in the mucosal-submucosal layers: 114±53 and 461±206; in the muscularis externa layers: 105±59 and 553±211; both $p>0.05$). This study provides evidence that chronic inflammatory disorders of the colon are associated with glutathione depletion. In ulcerative colitis, there was a relationship between the severity of inflammation and glutathione depletion. By contrast, this relationship was not significant in Crohn's colitis. The results suggest that there could be a primary defect in glutathione production in Crohn's colitis, or a difference in the relative levels of free radical production by inflammatory cells present in these two disorders of colonic inflammation.

Key Words: Glutathione, Colon, Crohn's disease, Ulcerative colitis, Antioxidant, Inflammatory bowel disease.

---

*Corresponding Author: Dr. Timothy R. Koch, Chief, Section of Gastroenterology, Robert C. Byrd Health Sciences Center of West Virginia University, P.O. Box 9161, Morgantown, West Virginia 26506-9161.

INTRODUCTION

Glutathione is a tripeptide-thiol present in most mammalian cells. It has been reported that tissue levels of this non-enzymatic antioxidant are depleted in several chronic disorders including idiopathic pulmonary fibrosis, human immunodeficiency virus-related disease, and respiratory distress syndrome (*1*). Identification of acquired glutathione deficiency states could be helpful in predicting the potential benefit of glutathione supplementation in human diseases.

Free radical production has emerged as a common pathway of tissue injury in a variety of diseases. There is evidence supporting increased free radical production by inflammatory cells in the intestinal mucosa from patients with chronic inflammatory bowel disease (*2-5*). Unfortunately, it has been difficult to discriminate between an etiologic role for free radicals as opposed to their presence as a reflection of injury caused by other potential agents (*6*).

Mammalian tissues contain a number of different free radical scavenging systems including enzymes (i.e., catalase), copper/Zinc (Cu/Zn) containing proteins (i.e., superoxide dismutase) and tissue micronutrients, such as ascorbate and glutathione. There is a paucity of information available about the status of these defenses in colon obtained from chronic inflammatory conditions (*7-9*). Decreased glutathione levels have been reported in the terminal ileal mucosa from patients with active Crohn's disease compared to areas without active inflammation and compared to controls without inflammatory bowel disease. This suggests increased consumption of this antioxidant by the inflammatory process (*8*).

It has been proposed that the therapeutic effect of 5-aminosalicylic acid in the inflammatory bowel diseases is related to its free radical scavenging action (*10*). There has been interest in developing new pharmacological approaches for the control of tissue damage induced by free radical production in the chronic inflammatory bowel diseases. Uncontrolled trials of superoxide dismutase have shown some promise (*11*). However, this enzyme requires a special delivery system that permits it to reach its therapeutic target before being destroyed. Since glutathione, given orally, is absorbed intact by the intestine (*12*), it constitutes an attractive possibility for therapeutic trials in inflammatory bowel disease.

Based on these previous studies, we hypothesized that increased free radical production by inflammatory cells in the inflammatory bowel diseases could induce colonic depletion of the antioxidant, glutathione. In this study, we measured tissue glutathione levels in surgical specimens of colon from patients with ulcerative colitis and Crohn's colitis, and from patients with non-obstructing colonic neoplasia as controls. The presence of and severity of inflammation was determined by histopathology in order to examine the potential relationship between inflammation and colonic glutathione levels.

METHODS

Tissue Specimens. This study was approved by the Human Research Review Committee of the Medical College of Wisconsin. Colonic specimens were obtained at surgery from patients with Crohn's colitis (n = 14), ulcerative colitis (n = 26), and in patients with non-obstructing colonic neoplasia who served as controls (n = 10). Among the controls, specimens of grossly normal colonic tissue were obtained from the anti-mesenteric border at least 3 cm distance from the tumor margin and from the stapled ends. Colonic specimens were extracted as rapidly as possible by clamping the blood supply to the colon before removal from the abdominal cavity in order to minimize ischemia. All specimens were quick-frozen and stored at -76°C. The mucosa-submucosa and muscularis externa layers were separated prior to assay by microdissection in ice-cold 0.9% NaCl.

Glutathione Determination. Extracts of each layer were prepared by homogenizing tissue in a suspension of 4.31% sulfosalicylic acid containing 0.25% Disodium-EDTA, and then centrifuging at 8,000 g for 30 minutes at 4° C. Determination of glutathione levels was performed as previously described (*13*) using the Beutler spectrophotometric assay as modified by Lang (*14*). It is known that tissue protein levels are higher in the gut wall in Crohn's disease (*15*). In prior studies of lyophilized colonic tissue, we have shown that normal and abnormal (ulcerative colitis and Crohn's disease) colonic specimens did not differ in water content (*16*). Consequently, glutathione results in this present study were expressed as nmoles/g wet tissue.

Histology. Transmural sections of the specimens were stained with the hematoxylin and eosin method, and then examined by a pathologist (blinded). On histologic examination, inflammation in tissues from patients with Crohn's disease was classified as "inactive" and "active", and in tissues from patients with ulcerative colitis as "inactive", "mild or moderately active", or "severely active", using standardized criteria (*17*).

Statistical Analysis. Quantitative variables were expressed as Mean±1SEM. Differences in glutathione levels between the groups were studied using non-parametric analysis (Kruskal-Wallis one-way ANOVA on ranks), and all pairwise multiple comparisons were then carried out using Dunn's method. To examine the potential relationship between glutathione levels in the two colonic layers, linear correlation analysis was utilized. The relationships between tissue glutathione levels and patients age or duration of disease were tested by Pearson correlation. A p value < 0.05 was considered statistically significant in this study.

RESULTS

Clinical Characteristics. Patients with inflammatory bowel disease included 14 patients with Crohn's colitis (8 males and 6 females; 40.5±3.3 years-old; age range: 25 to 67 years-old) and 26 patients with ulcerative colitis (16 males and 10 females; 38.2±2.7 years-old, age range: 16 to 75 years-old) who underwent colonic resection (see Table). The control group included 10 patients that underwent partial colectomy for non-obstructing colonic neoplasia (8 males and 2 females; 64.3±5.1 years-old; age range: 41 to 80 years-old). The disease duration in patients with ulcerative colitis was 10.6±2 years, and was 14.5±3 years in patients with Crohn's colitis. Two patients with ulcerative colitis had colon cancer and 1 patient had severe dysplasia.

TABLE

Clinical Characteristics of Patients with Crohn's Colitis, Ulcerative Colitis, and Controls

	Crohn's Colitis	Ulcerative Colitis	Controls
Number of Patients	14	26	10
Age*	40.5±3.3	38.2±2.7	64.3±5.1
Sex (Male/Female)	8/6	16/10	8/2
Disease Duration*		14.5±3	10.6±2

*Years: Mean±SEM

Glutathione Levels in IBD. In ulcerative colitis, tissue glutathione levels were significantly reduced both in the mucosal-submucosal layer (Mean±SEM: 283±71 nmoles/g wet tissue, pANOVA<0.05) and in the muscularis externa layer (398±98, pANOVA<0.05) compared to the mucosal-submucosal layer (614±126) and the muscularis externa layer (837±130) from controls. Glutathione was undetectable in the mucosal-submucosal layer from 13 of 26 patients and in the muscularis externa layer from 14 of 26 patients with ulcerative colitis, but colonic glutathione was measurable in all of the controls.

In Crohn's colitis, tissue glutathione levels were significantly reduced both in the mucosal-submucosal layer (337±139, pANOVA<0.05) and in the muscularis externa layer (393±147, pANOVA<0.05) compared to the controls. Similar to ulcerative colitis, glutathione was undetectable in the mucosal-submucosal layer from 7 of 14 patients and in the muscularis externa layer from 6 of 14 patients with Crohn's colitis.

Tissue glutathione levels were not significantly different between patients with ulcerative colitis and Crohn's colitis in either the mucosal-submucosal or the muscularis externa layers (Dunn's method: $p>0.05$).

In examination of tissue glutathione levels in the two colonic layers, in ulcerative colitis there was a positive linear correlation ($r=0.76$, $p<0.001$) between tissue glutathione levels in the mucosal-submucosal and the muscularis externa layers (Figure 1). Similarly, in Crohn's colitis there was a positive linear correlation ($r=0.86$, $p<0.001$) between tissue glutathione levels in the mucosal-submucosal and the muscularis externa layers (Figure 2).

Relationship Between Glutathione Levels and Inflammation. In patients with ulcerative colitis, tissue glutathione levels in the mucosal-submucosal layers were different among the controls, patients with inactive disease, and patients with active inflammation: mild/moderately active and severely active (pANOVA on Ranks<0.05) (Figure 3). Similarly, in patients with ulcerative colitis, tissue glutathione levels in the muscularis externa were different among the controls, patients with inactive disease, and patients with active inflammation: mild/moderately active and severely active (pANOVA on Ranks<0.05) (Figure 4).

When all pairwise comparisons were examined by Dunn's method in ulcerative colitis, tissue glutathione levels in patients with inactive disease were not different from controls, either in the mucosal-submucosal layer (808±30, $p>0.05$) or in the muscularis externa layer (890±340, $p>0.05$). However, tissue glutathione levels were decreased in patients with active inflammation in the mucosal-submucosal layer (214±68, $p<0.05$) and in the muscularis externa layer (333±97, $p<0.05$) compared to controls. Tissue glutathione levels in patients with mild/moderate inflammation were not significantly different from those patients with severe inflammation (in the mucosal-submucosal layer: 100±56 and 391±134, respectively; $p>0.05$; in the muscularis externa layer: 125±79 and 657±169, respectively; $p>0.05$).

In patients with Crohn's colitis, there was a trend toward a difference in tissue glutathione levels in mucosal-submucosal layers from controls, patients with inactive disease, and patients with active inflammation (pANOVA on Ranks=0.08) (Figure 5). Similarly, in patients with Crohn's colitis, there was a strong trend toward a difference in tissue glutathione levels in muscularis externa layers from controls, patients with inactive disease, and patients with active inflammation (pANOVA on Ranks=0.05) (Figure 6). In patients with either ulcerative colitis or Crohn's colitis, there were no significant correlations (defined by: $r > 0.50$ or < -0.50) between tissue glutathione levels and patient age or duration of disease.

DISCUSSION

This study provides evidence that chronic inflammatory disorders of the colon are associated with glutathione depletion. Ulcerative colitis and Crohn's colitis can therefore be added to the list of diseases with acquired glutathione depletion. In this present study, tissue glutathione levels were decreased in colon from patients with Crohn's colitis and ulcerative colitis in both the mucosal-submucosal and the muscularis externa layers. In ulcerative colitis, glutathione levels were lower in patients with active inflammation compared to those with inactive disease. By contrast, in patients with Crohn's colitis, there was no significant difference in tissue glutathione levels among patients with active inflammation, patients with inactive disease, and controls.

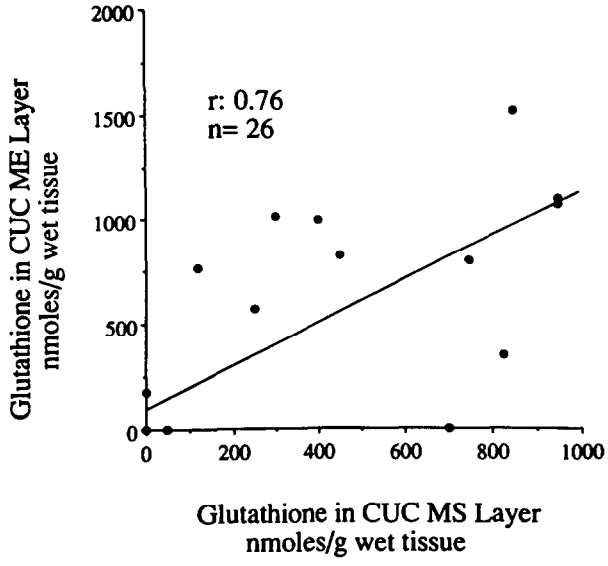

FIG 1. Linear Correlation Analysis Revealed a Positive Correlation between Glutathione Tissue Levels in Mucosal-Submucosal and Muscularis Externa Layers from Ulcerative Colitis ($p<0.001$).

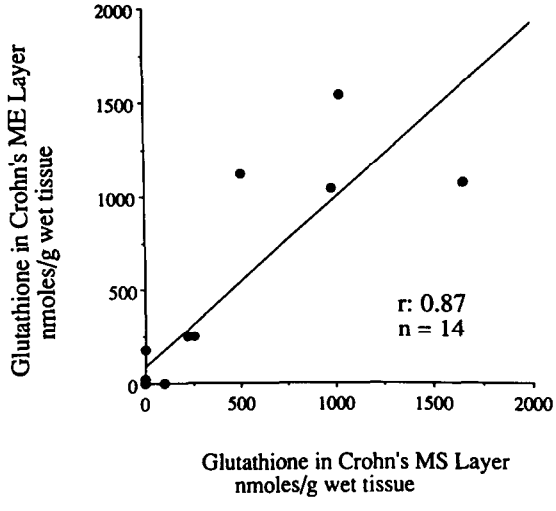

FIG 2. Linear Correlation Analysis Revealed a Positive Correlation between Glutathione Tissue Levels in Mucosal-Submucosal and Muscularis Externa Layers from Crohn's Colitis ($p<0.001$).

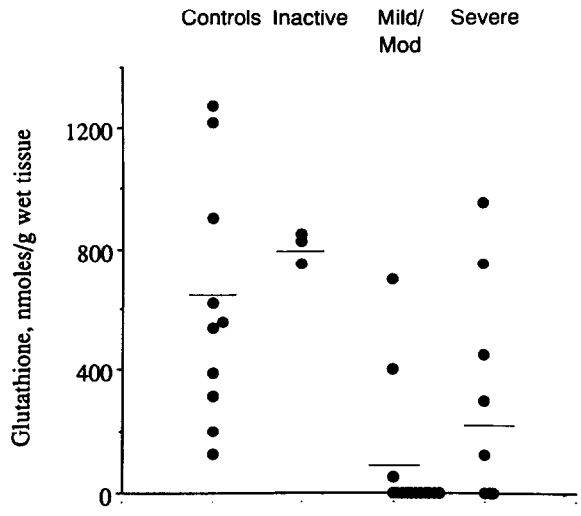

FIG 3. Glutathione Tissue Levels in Mucosal-Submucosal Layers from Controls and from Ulcerative Colitis with Inactive Disease, Mild/Moderate Inflammation, or Severe Inflammation. orizontal Bars Represent Mean Values. Each Point Represents the Level for One Patient. *(Kruskal-Wallis One-Way pANOVA on Ranks <.05)

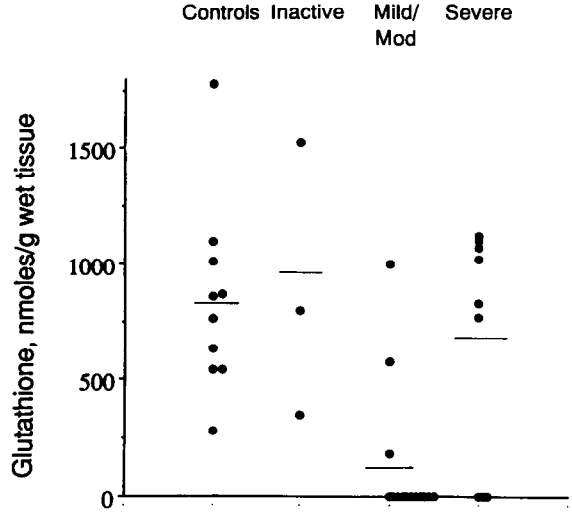

FIG 4. Glutathione Tissue Levels in Muscularis Externa Layer from Controls and from Ulcerative Colitis with Inactive Disease, Mild/Moderate Inflammation, or Severe Inflammation. Horizontal Bars Represent Mean Values. Each Point Represents the Level for One Patient. *(Kruskal-Wallis One-Way pANOVA on Ranks <.05)

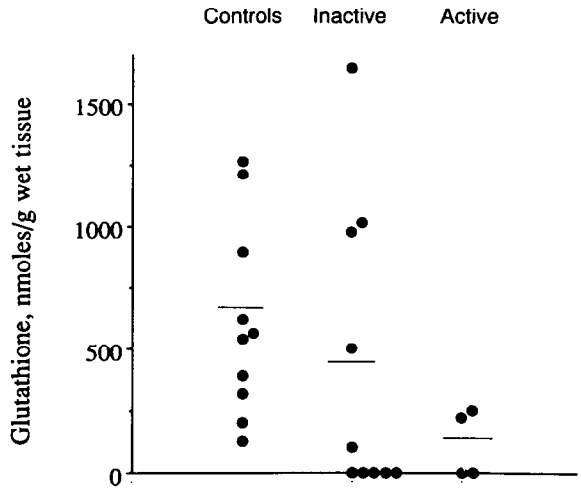

FIG 5. Glutathione Tissue Levels in Mucosal-Submucosal Layers from Controls and from Crohn's Colitis with Inactive Disease or Active Inflammation. Horizontal Bars Represent Mean Values. Each Point Represents the Level for One Patient. *(Kruskal-Wallis One-Way pANOVA on Ranks =.08)

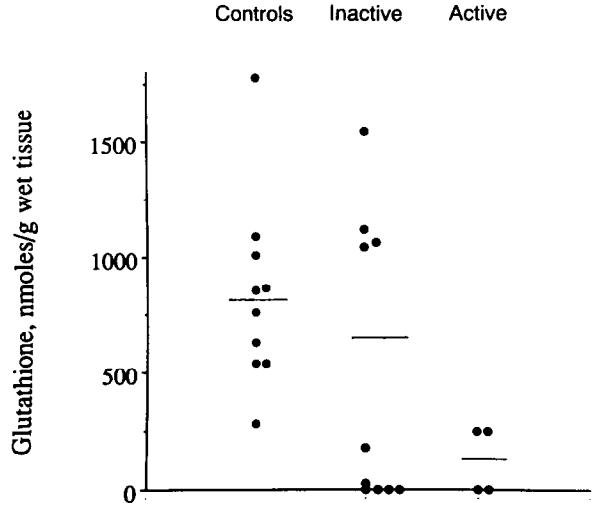

FIG 6. Glutathione Tissue Levels in Muscularis Externa Layers from Controls and from Crohn's Colitis with Inactive Disease or Active Inflammation. Horizontal Bars Represent Mean Values. Each Point Represents the Level for One Patient. *(Kruskal-Wallis One-Way pANOVA on Ranks = .05)

Glutathione is synthesized in the cytoplasm of most mammalian cells using as substrates glutamine, cysteine, and glycine (*18*). The rate limiting enzyme in glutathione biosynthesis is γ-glutamylcysteine synthetase. The free radical scavenging action of glutathione is catalyzed by the enzyme, glutathione peroxidase. The reaction between glutathione and a free radical yields oxidized glutathione. Oxidized glutathione can be converted to its reduced form by the enzyme glutathione reductase using electrons derived from the pentose-phosphate pathway. In the presence of large quantities of free radicals, derived for example from inflammatory cells, the recovery pathway may be overwhelmed causing oxidized glutathione to accumulate in the cytoplasm. The cell membrane is relatively permeable to oxidized glutathione, and this form of glutathione readily diffuses outside the cell resulting in depletion of tissue glutathione.

Glutathione homeostasis may be related to the dietary supply of amino acid precursors, by the rates of oxidation and reduction during its function as a free radical scavenger, and by an interaction between glutathione and ascorbate (*18*). Ascorbate conserves cellular reduced glutathione. Reduced glutathione in turn is important for reduction of dehydroascorbate to ascorbate (also a free radical scavenger), thus bypassing breakdown of dehydroascorbate and allowing maintenance of cellular ascorbate levels. The availability of glutathione to function as an antioxidant is dependent upon the relationship between circulating blood glutathione, the major source of which is the liver (*1*), and tissue glutathione production. The bioavailability of blood glutathione could affect colonic glutathione levels. However, hypervascularity has been reported in both Crohn's Disease and ulcerative colitis *(19)*, and blood flow is increased in ulcerative colitis *(20)*. These results would suggest increased delivery of blood glutathione to the colon. Other human disorders involving glutathione depletion include adult respiratory distress syndrome, idiopathic pulmonary fibrosis, and acquired immunodeficiency syndrome (*1*).

In an animal model of glutathione deficiency, rats treated with an inhibitor of glutathione synthesis develop severe intestinal epithelial degeneration and diarrhea, suggesting that glutathione is essential in the protection of the intestine against luminal oxidants (*21*). Defects in the intestinal antioxidant defenses could be involved in the pathogenesis of the inflammatory bowel diseases since cellular damage may be related to an imbalance between high levels of free radicals and decreased tissue antioxidants (*22*).

In our present study, in patients with ulcerative colitis, there was an association between decreased glutathione and active inflammation. Tissue glutathione levels were not different between controls and patients with inactive ulcerative colitis. These results are consistent with depletion of tissue antioxidants due to excess production of free radicals derived from inflammatory cells. Our present findings are in agreement with a report of decreased levels of reduced ascorbate in colonic mucosa from patients with active IBD compared to non-inflamed mucosa (*9*). Since a recent experimental rat model has suggested that glutathione depletion may impair T-cell and macrophage function (*23*), development of glutathione depletion in the inflammatory bowel diseases could lead to further alteration of gut immune function.

The explanation for simultaneous declines in glutathione levels in the muscularis externa layer and the mucosal-submucosal layer from patients with ulcerative colitis is unclear. Since there is no increase in inflammatory cells in the muscularis externa layer from ulcerative colitis, it is unlikely that increased free radical production by inflammatory cells is the sole explanation. Although our results could be related to excessive production of free radicals in the muscularis externa from a source distinct from inflammatory cells, there could in addition be a primary underlying defect in glutathione biosynthesis.

Among patients with Crohn's colitis, although there was a strong trend toward decreased glutathione levels in patients with active inflammation compared with inactive disease, this difference was not statistically significant. This result suggests that an underlying defect in glutathione production may be responsible for glutathione deficiency in Crohn's colitis. In support of this interpretation, alterations in glutathione metabolism

have been reported in the ileal mucosa from patients with Crohn's disease, including decreased γ-glutamylcysteine synthetase activity (*8*). Alternatively, there may be a difference in free radical production by the type of, the number of, or the distribution of inflammatory cells in Crohn's colitis.

Differences in the origin of glutathione depletion in ulcerative colitis compared to Crohn's colitis might help to explain the clinical benefit of nutritional therapy in Crohn's disease as opposed to ulcerative colitis. In ulcerative colitis, inflammatory cells may be avidly consuming glutamine (*24*), an important gut nutrient and a potential substrate for glutathione production. Glutamine consumption by inflammatory cells (*24*) could explain why nutritional therapy has no apparent benefit in patients with ulcerative colitis. By contrast, supplemental nutrients in Crohn's disease may be available for gut metabolism (*25*), including glutathione biosynthesis, and may enhance the gut's protection against free radical-induced tissue damage.

It is presently unclear whether oral glutathione administration would increase mucosal levels of glutathione in inflammatory bowel disease. Glutathione monoester is presently available and is easily transportable across cell membranes *(26)*. Oral glutathione administration could provide several avenues of potential benefit in inflammatory bowel disease. It could be available to reduce intraluminal free radical levels. The digested component amino acids from glutathione could be absorbed into enterocytes and resynthesized into glutathione *(27)*.

A potential limitation of our data is the use of patients with colonic neoplasia as controls. Although glutathione levels have been found to be decreased in malignant colonic tumors, glutathione levels in non-tumorous tissue from patients with colon cancer were not different compared to controls without colon cancer (*28*). However, the controls used in our present study were older than the patients with inflammatory bowel disease. Since circulating glutathione levels decline with age (*14*), the magnitude of tissue glutathione depletion in the inflammatory bowel diseases might be greater if age-matched controls were available.

ACKNOWLEDGMENTS

This work was supported by a research award from the American College of Gastroenterology and by the Advisory Board of the Digestive Disease Research Center at the Medical College of Wisconsin.

REFERENCES

1. White AC, Thannickal VJ, Fanburg BL. Glutathione deficiency in human disease. J Nutr Biochem 1994; 5: 218-226.

2. Bhaskar L, Ramakrishna BS, Balasubramanian KA. Colonic mucosal antioxidant enzymes and lipid peroxide levels in normal subjects and patients with ulcerative colitis. J Gastroent Hepatol 1995; 10: 140-143.

3. Melichar B, Karlicek R, Tichy M. Increased urinary nitrate excretion in inflammatory bowel disease. Eur J Clin Chem Clin Biochem 1994; 32: 3-4.

4. Simmonds NJ, Allen RE, Stevens TRJ, Van Someren RNM, Blake DR, Rampton DS. Chemiluminescence assay of mucosal reactive oxygen metabolites in inflammatory bowel disease. Gastroenterology 1992; 103: 186-196.

5. Boughton-Smith NK, Evans SM, Hawkey CJ, Cole AT, Balsitis M, Whittle BJR, Moncada S. Nitric oxide synthase activity in ulcerative colitis and Crohn's disease. Lancet 1993; 342: 338-340.

6. Harris ML, Schiller HJ, Reilly PM, Donowitz M, Grisham MB, Bulkley GB. Free radicals and other reactive oxygen metabolites in inflammatory bowel disease: cause, consequence, or epiphenomenon? Pharmac Ther 1992; 53: 375-408.

7. Mulder TP, Verspaget HW, Janssens AR, DeBruin PAF, Pena AS, Lamers CBHW. Decrease in two intestinal Cu/Zn containing proteins with antioxidant function in inflammatory bowel disease. Gut 1991; 32: 1146-1150.

8. Iantomasi T, Marraccini P, Favilli F, Vincenzini MT, Ferretti P, Tonelli F. Glutathione metabolism in Crohn's disease. Biochem Med Met Biol 1994; 53: 87-91.

9. Buffinton GD, Doe WF. Altered ascorbic acid status in the mucosa from inflammatory bowel disease patients. Free Rad Res 1995; 22: 131-143.

10. Ahnfelt-Rønne I, Nielsen OH, Christensen A, Langholz E, Binder V, Riis P. Clinical evidence supporting the radical scavenger mechanism of 5-aminosalicylic acid. Gastroenterology 1990; 98: 1162-1169.

11. Emerit J, Pelletier S, Tosoni-Verlignue D, Mollet M. Phase II trial of copper zinc superoxide dismutase in treatment of Crohn's disease. Free Rad Biol Med 1989; 7: 145-149.

12. Hagen TM, Wierzbicka GT, Bowman BB, Aw TY, Jones DP. Fate of dietary glutathione: disposition in the gastrointestinal tract. Am J Physiol 1990; 259: G530-G535.

13. Ballard TC, Farag A, Branum GD, Akwari OE, Opara EC. Effect of L-glutamine supplementation on impaired glucose regulation during intravenous lipid administration. Nutrition 1996; in press.

14. Lang CA, Naryshkin S, Schneider DL, Mills BJ, Lindeman RD. Low blood glutathione levels in healthy aging adults. J Lab Clin Med 1992; 120: 720-725.

15. Sjolund K, Schaffalitzky De Muckadell OB, Fahrenkrug J, Hakanson R, Peterson BG, Sundler F. Peptide-containing nerve fibres in the gut wall in Crohn's disease. Gut 1983; 24: 724-733.

16. Koch TR, Carney JA., Go VLW. Distribution and quantitation of gut neuropeptides in normal intestine and the inflammatory bowel diseases. Dig Dis Sci 1987; 32: 369-376.

17. Truelove SC, Richards WRD. Biopsy studies in ulcerative colitis. British Med J 1956; i : 1315.

18. Meister A. Glutathione-ascorbic acid antioxidant system in animals. J Biol Chem 1994; 269: 9397-9400.

19. Brahme F, Lindstrom C. A comparative radiographic and pathological study of intestinal vaso-architecture in Crohn's disease and in ulcerative colitis. Gut 1970; 928-940.

20. Srivastava ED, Russell MAH, Feyerabend C, Rhodes J. Effect of ulcerative colitis and smoking on rectal blood flow. Gut 1990; 31:1021-1024.

21. Mårtensson J, Jain A, Meister A. Glutathione is required for intestinal function. Proc Natl Acad Sci USA 1990; 87:1715-1719.

22. Machlin LJ, Bendich A. Free radical tissue damage: protective role of antioxidant nutrients. FASEB J 1987; 1: 441-445.

23. Robinson MK, Rodrick ML, Jacobs DO, Rounds JD, Collins KH, Saporoschetz IB, Mannick JA, Wilmore DW. Glutathione depletion in rats impairs T-cell and macrophage immune function. Arch Surg 1993; 128: 29-35.

24. Souba WW, Kliniberg S, Plumley DA, Salloum RM, Flynn TC, Bland KI, Copeland EM. The role of glutamine in maintaining a healthy gut and supporting the metabolic response to injury and infection. J Surg Res 1990; 48: 383-391.

25. Bragg LE, Thompson JS, Rikkers LF. Influence of nutrient delivery on gut structure and function. Nutrition 1991; 7: 237-243.

26. Robinson MK, Ahn MS, Rounds JD, Cook JA, Jacobs DO, Wilmore DW. Parenteral glutathione monoester enhances tissue antioxidant stores. J Parent Enteral Nutri 1992; 16:413-418.

27. Flagg E W, Coates R J, Eley JW, Jones DP, Gunter EW, Byers TE, Block GS, Greenberg RS. Dietary glutathione intake in humans and the relationship between intake and plasma total glutathione level. Nutr Cancer 1994; 21:33-46.

28. Siegers CP, Böse-Younes ET, Hoppenkamps R, Younes M. Glutathione and GSH dependent enzymes in tumorous and non-tumorous mucosa of the human colon and rectum. J Cancer Res Clin Oncol 1984; 107: 238-241.

Accepted for pubication October 15, 1996.