Regional antioxidant status in the gastrointestinal tract and the possible role of reactive oxygen-derived substances in peptic ulcer disease

by

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We accept this thesis as conforming to the required standard

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Abstract

1. Enzymatic and non-enzymatic antioxidant profiles in the various segments of the gastrointestinal tract of male and female rats have been investigated and found to exhibit significant differences (P<0.05). In both sexes, the levels of basal glutathione in the gastric and colonic mucosa are comparable, but lower than those in the proximal and distal segments of the small intestine. The activities of glutathione reductase in various portions of gastrointestinal tract were similar. Glutathione peroxidase showed higher activity in the gastric mucosa than that in other parts of the gastrointestinal tract. No significant differences were found in the activity of superoxide dismutase among the various segments examined.

2. Enzymatic and non-enzymatic antioxidant profiles in the gastric and duodenal mucosa of rabbit, quail, cat, pig and rat have been investigated and found to exhibit significant differences (P<0.05). The levels of basal glutathione were highest in the rat gastric and duodenal mucosa when compared with those in other species. In the duodenal mucosa of all species investigated, the activity of glutathione reductase was higher than that in the corresponding gastric mucosa. The activity of glutathione peroxidase was higher in the gastric mucosa than that in other species examined. Superoxide dismutase showed higher activity in quail duodenal mucosa than in any of the other species studied.

3. The effects of 8% and undiluted ethanol administered by gavage on lesion formation and antioxidant components of the gastric and duodenal
mucosa of male and female rats have been examined. Undiluted ethanol produced macroscopic lesions in the body of the stomach in association with decreases in the activity of glutathione reductase and in the level of basal glutathione and an increase in the activity of glutathione peroxidase. Eight percent ethanol produced a small but significant increase (12%) in the level of basal glutathione in rat gastric mucosa when compared with controls.

4. The effects of chronic intermittent stress on the appearance and antioxidant components of the gastric and duodenal mucosa of rats have been examined. No differences in the antioxidant profiles or evidence of macroscopic lesion formation were found.
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LIST OF ABBREVIATIONS

ANOVA Analysis of variance
A. M. ante meridiem
5-ASA 5-aminosalicylic acid
α Alfa
cm centimeter
CAT catalase
Cu copper
DDC diethylthiocarbamate
DEM diethylmaleate
DMSO dimethyl sulfoxide
dsi distal part of small intestine
DNA deoxyribonucleic acid
DTNB 5,5'-dithiobis-2-thiobenzoic acid
EDTA ethylenediaminetetraacetic acid
e. g. for example
Fe iron
GPX glutathione peroxidase
GRD glutathione reductase
GSH glutathione
H₂O₂ hydrogen peroxide
H⁺ hydrogen ion
HCl hydrochloric acid
Abbreviations (cont'd)

hr  hour
i.e.  that is
i. p.  intrapertionially
kg  kilogram
µ  micro
mg  milligram
min  minute
ml  milliliter
mmHg  millimeter of mercury
mM  milliMolar
MTDQ-DA  kontrad
n  nano
N  normal
NEM  N-ethylmaleimide
nm  nanometer
NSAID  non-steroidal anti-inflammatory drug
O₂  oxygen
O₂⁻  superoxide radical
OH⁻  hydroxyl radical
pH  hydrogen ion concentration
P. M.  post meridiem
psi  proximal part of small intestine
RODS  reactive oxygen-derived substances
Abbreviations (cont'd)

SAM       S-adenosylmethionine
s. c.      subcutaneously
SE        standard error
SH        sulfhydryl group
SOD       superoxide dismutase
TCA       trichloroacetic acid
UBC       The University of British Columbia
VGH       Vancouver General Hospital
w/v       weight per volume
Zn        zinc
<         less than
>         greater than
°C        degrees Celsius
±          plus or minus
%         percent
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DEDICATION

This thesis is dedicated to my wife and my son.
1. INTRODUCTION

1.1. Reactive oxygen-derived sustances in pathological and physiological conditions

It is well established that molecular oxygen at increased partial pressure can be harmful and partially reduced oxygen species are the probable agents responsible for this toxicity (1). Reactive oxygen-derived substances (RODS) have been implicated in a variety of pathological conditions, most notably ischemia/reperfusion injury (2-4), radiation therapy (5) and in the toxic effects of certain drugs and chemicals, including anthracyclines (6), mitomycin and bleomycin (7).

Under *in vivo* conditions, oxygen usually undergoes a step-wise, four-electron reduction to water. The three most important partially reduced forms of molecular oxygen are superoxide radical ($\textit{O}_2^-$), hydrogen peroxide ($\textit{H}_2\textit{O}_2$), and hydroxyl radical ($\textit{OH}^-$) (8). These reactive metabolites can be produced by the activity of oxidative enzymes in various intracellular compartments, such as the cytosol, mitochondria, lysosomes, peroxisomes and plasma membranes. Superoxide radicals can arise during the course of mitochondrial electron transport or as a result of the action of such intracellular enzymes as xanthine oxidase or cytochrome $\textit{P}_{450}$-dependent oxidases.

Once produced, superoxide radicals can undergo dismutation by the action of superoxide dismutase (SOD) to produce hydrogen peroxide (9).
\[ 2O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2 \]

Hydrogen peroxide, as produced either by the above reaction or directly by oxidases present in peroxisomes, can undergo either the Fenton reaction

\[ \text{Fe}^{2+} + H_2O_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{OH}^- \]

or the Haber-Weiss reaction.

\[ H_2O_2 + O_2^- \rightarrow \text{OH}^- + \text{OH}^- + O_2 \]

to produce hydroxyl radicals.

The main effects of these reactive species are on unsaturated linkages of membrane lipids (10), sulphydryl groups of proteins (11) and nucleotides of DNA (12). The peroxidation of lipids in biological membranes can cause damage to organelles such as mitochondria, endoplasmic reticulum, lysosomes and microsomal components. Interactions with DNA may result in mutagenesis or carcinogenesis (13). Lipid peroxidation is one well-studied mechanism of reactive oxygen metabolite injury (14-16). It can be initiated by hydroxyl radicals which react with unsaturated fatty acids within the membranes to generate organic free radicals, which, in turn, react rapidly with oxygen to form peroxides. Peroxides themselves then generate free radicals, initiating an autocatalytic chain reaction, resulting in further loss of unsaturated fatty acids and in extensive membrane damage (17).

Once the reactive metabolites are formed, they may spontaneously decay. For example, superoxide radicals can decay spontaneously into oxygen and hydrogen peroxide. There are, however, several systems that are involved
in the direct inactivation of these reactive substances. Enzymatic and non-enzymatic antioxidant systems such as vitamin E, glutathione, ceruloplasmin, glutathione peroxidase (GPX), glutathione reductase (GRD), catalase (CAT) and superoxide dismutase (SOD) can scavenge and detoxify reactive oxygen substances (18).

\[
\begin{align*}
\text{O}_2^- \overset{\text{SOD}}{\longrightarrow} & \quad \text{H}_2\text{O}_2 \\
2\text{H}_2\text{O}_2 \overset{\text{CAT}}{\longrightarrow} & \quad \text{O}_2 + 2\text{H}_2\text{O} \\
2\text{OH}^- + 2\text{GSH} \overset{\text{GPX}}{\longrightarrow} & \quad 2\text{H}_2\text{O} + \text{GSSG} \\
\text{H}_2\text{O}_2 + 2\text{GSH} \overset{\text{GPX}}{\longrightarrow} & \quad 2\text{H}_2\text{O} + \text{GSSG} \\
\text{GSSG} \overset{\text{GRD}}{\longrightarrow} & \quad \text{GSH}
\end{align*}
\]

In many pathologic processes, the final effects of stimulus-inducing free radicals depend on the net balance between free radical formation and inactivation (19).

1.2. Pathophysiology of peptic ulcer disease

Peptic ulcer disease can be generally defined as a disorder of the gastrointestinal tract in which an unfavorable imbalance between damaging agents and defense mechanisms is present. This imbalance eventually results in injury to the mucosal tissue and development of peptic ulceration. Not all peptic ulcers share the same pathophysiology. For example, acute and chronic ulcers can arise under different clinical circumstances, follow a different clinical course and respond differently to treatment. However, all peptic ulcers share a common feature, i.e., their occurrence in mucosa exposed to acid and pepsin.
Recent studies have demonstrated that the notion "no acid-no ulcer" is not always applicable to gastric ulcers. Chronic gastric ulcers are usually associated with low acid secretion and the role of acid in the pathophysiology of acute gastric ulcers is unresolved. Patients with duodenal ulcers almost always show a marked increase in both acid secretion and pepsin activity compared with control subjects (20). One might suggest that the enterogastric reflexes may be impaired due to duodenal ulceration.

The main defense mechanism is the so-called "mucus-bicarbonate" barrier, across which bicarbonate diffuses towards the lumen and neutralizes acid that is slowly diffusing towards the epithelium. Several factors are involved in this protective mechanism. Among them, one can mention the existence of a viscid mucus gel at the surface of epithelial cells, the secretion of bicarbonate and additional buffer (so-called "alkaline tide") and the ability of the gastric mucosa to increase mucosal blood flow to buffer and/or to remove excessive intramucosal acid.

Several conditions may predispose to the development of acute gastric mucosal lesions. These include severe illnesses, burns, central nervous system trauma, acute and severe stressful conditions and ingestion of certain drugs or alcohol. Injury to the central nervous system usually causes acute gastric mucosal lesions referred to as "Cushing's ulcers". Endoscopic studies have demonstrated that the incidence of acute gastric mucosal lesions in patients with head injuries was 50-75%, while that in patients with spinal cord injury it was
reported to be about 3%. In such patients with damage to the central nervous system, an increase in gastric acid secretion and high levels of serum gastrin were reported. Patients with burns to 35% or more of the body surface have a tendency to develop acute gastric mucosal lesions, these being referred to as "Curling's ulcers" (21).

Selye (22) has defined stress as a "nonspecific response of the body to any demand upon it." This condition is considered to involve a complex interaction involving the central nervous system, the endocrine system and the immune system of the body. Stress may affect the limbic system, most notably the hypothalamus, which is the regulatory center for both the autonomic nervous system and the neuroendocrine system.

It has been postulated that there are three pathways involved in stress-induced ulceration: the anteriohypothalamus through the vagus nerve, the posteriohypothalamus via the sympathetic axis and the posterior hypothalamo-pituitary-adrenal system. Stress may cause some alterations in gastric motility, gastric secretion and gastric mucosal blood flow. These kinds of alterations could result in erosions or ulcers, so that the more severe the stress, the higher the incidence of lesions.

Certain kinds of drugs, such as non-steroidal anti-inflammatory drugs, usually after long-term/high dose therapy, as in the treatment of rheumatoid arthritis, can cause acute gastric mucosal lesions. It has been reported that the gastric toxicity of the following non-steroidal anti-inflammatory drugs is: aspirin >
indomethacin > phenylbutazone > ibuprofen (23). These drugs do inhibit cyclooxygenase, a key enzyme in the biosynthesis of prostaglandins; prostaglandin E2 plays an important role in gastric mucosal protection against a variety of damaging agents. This prostaglandin can protect the gastric mucosa by a number of mechanisms, such as stimulation of mucus and bicarbonate secretion and/or increase in mucosal blood flow. Other possible mechanisms for ulcer formation by this category of drugs could involve associated changes in gastric motility and/or in gastric mucosal blood flow, as Takeuchi et al. (24) have suggested for indomethacin-induced gastric lesions.

Alcohol is another damaging agent. Administration of low concentrations (8-10%) of alcohol can prevent gastric lesions by processes referred to as "adaptive cytoprotection". However, administration of high concentrations of alcohol are definitely ulcerogenic. This damaging behavior is probably due to the ability of alcohol to alter the integrity of the mucus barrier.

A variety of factors may significantly affect the incidence, severity and localization of gastrointestinal mucosal lesions. Among these factors one can consider: species, regional tissue differences, sex, genetics and age.

1.2.1. Species

Butterfield (25) reported differences in incidence rates of lesion formation in six different species of experimental animals. The incidence of gastric ulceration induced by restraint for 24 hours in mice, rats, guinea pigs and
hamsters was reported to be 92%, 86%, 46% and 4%, respectively. Gastric lesions could not be induced in monkeys or rabbits under these conditions.

1.2.2. Regional tissue differences

Gastric and intestinal mucosa may differ in their susceptibilities to lesion formation. In experimental models, duodenum is more resistant to stress ulceration, so that cold-restraint stress did not produce duodenal ulcers in rats (23). Also, Kaplan (26) has reported that there is no direct evidence that non-steroidal anti-inflammatory drugs or alcohol can produce duodenal ulcers in humans.

1.2.3. Sex

In humans, duodenal ulcers have a male:female ratio of 4:1 while the ratio for gastric ulcers is 3 or 4:1 (27). Another source (28) claims that one in every ten men (10%) and one in every 25 women (4%) may have a peptic ulcer during the course of his or her life. Pregnant women and nursing mothers are somehow resistant to ulcer formation. Peptic ulcer is rare in females prior to menopause.

1.2.4. Genetics

Peptic ulcers are 2 to 3 times more common in first degree relatives of patients with ulcers. Twenty to fifty percent of patients with a duodenal ulcer have a positive family history of duodenal ulcers. Individuals with blood group O have about a 30% increase in risk of duodenal ulcers compared with persons of other blood types (29). Duodenal ulceration is more common in monozygotic
than dizygotic twins. This suggests a role of genetics in influencing susceptibility to ulcer formation.

1.2.5. Age

Age is another important factor determining susceptibility to lesion formation. Most gastrointestinal lesions appear for the first time in the third to fourth decade of life in man (27). In children, gastric ulcers are extremely rare, except in the case of serious brain injury. The estimated annual incidence of gastric lesions is reported to be 0.002-0.005% in children under age 15, as compared with 0.2% in adults (28).

1.3. Factors involved in the pathogenesis of peptic ulcer disease

1.3.1. Gastric acid secretion

Gastric acid is necessary for ulcer formation. There is an increase in gastric acid secretion in patients with duodenal ulcer, but it is normal or decreased in patients with gastric ulcer. Patients admitted into a critical care unit are at greatly increased risk of gastric ulceration. One study of gastric acid secretion in such individuals has shown it to be normal or below normal, even though the initiation of gastric mucosal injuries might be apparent endoscopically (28). Therefore, one can conclude that for production of acute gastric mucosal lesions an increase in gastric acid secretion does not play a role, although hydrochloric acid is necessary for induction of these lesions.
1.3.2. Pepsin activity

Pepsinogen is a polypeptide secreted by chief cells and has a molecular weight of 42,500. The main stimulus for pepsinogen secretion is acetylcholine. Hydrochloric acid may indirectly stimulate pepsinogen secretion as well. In the presence of hydrochloric acid, pepsinogen is converted into pepsin, which has a molecular weight of 35,000 and has proteolytic activity. In patients with duodenal ulcer, the mean activity of pepsin in endoscopically obtained biopsy samples from gastric and duodenal mucosa was found to be increased (29). Also, it is reported that all patients with endoscopically proven active duodenal ulcers had a higher pepsin output than control subjects (30). Several different types of pepsinogens (e.g., pepsinogen I and pepsinogen II) are secreted by peptic and mucous cells of the gastric glands. Kolster et al. (31) reported highly significant differences between serum pepsinogen I levels in patients with duodenal ulcers as compared with those in control subjects. It is reported that hyperpepsinogenemia I is a reliable subclinical marker of the genetic predisposition to duodenal ulceration (32). These findings suggest that pepsin has a significant role in the pathophysiology of peptic ulcer disease.

1.3.3. Helicobacter pylori

*Helicobacter pylori* is a bacterium found in association with gastric mucosa. *H. pylori* infection has been found in the antrum of approximately two-thirds of patients with gastric and/or duodenal ulcers. It has been suggested that this bacterium has a role in peptic ulcer disease by causing mucus depletion.
The organism is usually located in the superficial mucus layer, where it tends to be attached to the epithelium at the site of intercellular junctions (34). This location provides protection from acid gastric contents, but permits hydrolytic enzymes produced by the organism to damage gastric epithelium. It is suggested that the organism damages the mucosa, initiating a progressive disease and in many cases culminating in atrophy and intestinal metaplasia (35). It is generally accepted that infection with this bacterium is closely associated with peptic ulceration (34). The role of this organism in the pathogenesis of gastric ulceration, however, is still controversial.

1.3.4. Reactive oxygen-derived substances

Production of reactive oxygen-derived substances (RODS) seems to be an important factor in the pathophysiology of peptic ulcer disease. These reactive substances can alter cellular function by causing oxidative damage to proteins, nucleic acids and lipids, the latter resulting in loss of cell membrane integrity. Gastric mucosal cells, which are normally relatively impermeable to H+ and Na+ despite the presence of high concentration gradients, could thereby become permeable to these ions. There is considerable evidence that RODS can play a role in the induction of gastrointestinal mucosal lesions, as discussed below. Niida et al. (36) were able to induce duodenal ulcers in rats by the administration of diethyldithiocarbamate (DDC), a metal chelator and SOD inhibitor, at a dose of 750 mg/kg s.c. given daily for 4 days. The DDC-induced lesions were prevented by either allopurinol, a xanthine oxidase inhibitor and
hydroxyl radical scavenger, or superoxide dismutase (SOD), an endogenous superoxide radical scavenger, at doses of 50 mg/kg s.c. and 50,000 unit/kg s.c., respectively. Since the administration of DDC was shown to cause a decrease in SOD activity in the duodenal mucosa, it can be postulated that RODS can play a role in DDC-induced duodenal mucosal lesions in rats. Oka et al. (37) were able to induce antral ulcers in anesthetized pylorus-ligated rats by injection of DDC (800 mg/kg s.c.), followed by oral administration of one milliliter of 0.1 N HCl. Administration of Cu,Zn-SOD (60,000 unit/kg s.c.) significantly reduced the HCl-DDC-induced ulcer index.

Takeuchi et al. (24) demonstrated an increase in both amplitude and frequency of gastric contractions 30 minutes after subcutaneous administration of indomethacin at a dose of 25 mg/kg in rats. The authors demonstrated a relationship of indomethacin-induced gastric hypermotility to mucosal hemodynamics, lipid peroxidation and vascular permeability changes in the pathophysiology of indomethacin-induced gastric lesions. Pretreatment of the animals with allopurinol (50 mg/kg), SOD (15,000 units/kg/hr), or dimethyl sulfoxide (DMSO; 30 mg/kg), which reacts with hydroxyl radicals to produce methane sulfinic acid, caused an attenuation of the indomethacin-induced gastric mucosal lesions. It can, therefore, be concluded that RODS are likely to play a role in the development of indomethacin-induced gastric mucosal ulceration.
Salim et al. (38) induced gastric mucosal injury in rats by injection of either reserpine (5 mg/kg i.p.) or serotonin (50 mg/kg i.p.). Parenteral administration of high doses of reserpine causes gastric mucosal vasoconstriction by vagal sympathetic fibers, leading to ischemic mucosal injury. Serotonin also produces gastric mucosal injury by vasoconstriction. Pretreatment with either 1 ml/day of 2% allopurinol or DMSO by gastric gavage for 2 days significantly reduced reserpine- or serotonin-induced gastric lesions in rats. Since both allopurinol and DMSO share the properties of scavenging RODS and each of them could individually prevent gastric mucosal lesion formation, one can conclude that RODS have a role in the pathophysiology of reserpine- or serotonin-induced gastric mucosal lesions.

Zollei et al. (39) instilled 1 ml of 0.1 N HCl into the stomach of anesthetized rats and animals were bled to reduce blood pressure to 30 mmHg for 20 minutes. The shed blood was re-transfused after 20 minutes and, after another 20 minutes, rat stomachs were removed and gastric mucosal lesions were measured. In the same experiment, pretreatment with either allopurinol (50 mg/kg) or kontrad (MTDQ-DA, 6,6-methylene bis 2,2 dimethyl-4-methane sulfinic acid; 50 mg/kg i.p.), a synthetic antioxidant of the dihydroquinoline type, protected the animals against hemorrhagic shock-induced gastric mucosal lesions. These results again suggest that RODS may play an important role in the pathophysiology of gastric mucosal lesions produced under these conditions.
Granger et al. (40) reported the protective effects of the xanthine oxidase inhibitor, allopurinol, on ileal vascular permeability during ischemia/reperfusion in ileal segment of cats. The authors concluded that xanthine oxidase is a source of RODS due to ischemia/reperfusion in the small intestine in cats.

Kvietys et al. (41) investigated the effects of 5-aminosalicylic acid (5-ASA), a hydroxyl radical scavenger, on ischemia/reperfusion-induced gastric bleeding in rats. Rat stomachs were perfused with 30 mM 5-ASA in 0.1 N HCl and systemic blood pressure was then reduced to 25-30 mmHg by withdrawing blood from the femoral artery. After 30 minutes, the shed blood was reinfused via the femoral vein. Gastric lesions in animals receiving 5-ASA were significantly less severe than those in animals that had not received 5-ASA. Therefore, hydroxyl radicals appear to play a role in the production of gastric lesions induced by ischemia/reperfusion in rat stomach.

Boyd et al. (42) were able to induce acute gastric mucosal lesions in rats by subcutaneous administration of diethylmaleate (DEM), a tissue glutathione depletor. On this basis, the authors concluded that glutathione may have a possible role in maintaining the normal homeostasis and integrity of the gastric mucosa. Laudanno (43) investigated the effects of S-adenosylmethionine (SAM) on ethanol- aspirin- or stress-induced gastric lesions. SAM significantly increased non-protein sulphydryl groups in gastric mucosa. SAM at a dose of 100 mg/kg s.c. protected gastric mucosa against the damaging effects of aspirin, ethanol or stress. Therefore, it can be concluded that the above mentioned
irritants may cause gastric lesions by depleting glutathione levels in gastric mucosa of rat stomach.

Despite the abundance of evidence concerning the effects of antioxidants in the prevention of peptic ulcers, there are a few studies suggesting there is no relationship between gastric glutathione levels and ulcer formation. Thus, Ushima et al. (44) investigated the role of gastric mucosal sulfhydryl groups (SH) in the pathophysiology of indomethacin-induced lesions in rats. They found these lesions could be prevented by prior s.c. administration of cysteamine, glutathione or diethylmaleate, irrespective of the fact that mucosal SH levels were increased by the former two agents but reduced by the latter agent. Therefore, the authors concluded that gastric mucosal SH levels have no relation to the ulcerogenic effects of indomethacin in gastric mucosa in rats.

Takeuchi et al. (45) investigated the role of gastric mucosal glutathione on lesions induced by 1 ml of 50% ethanol in rats. Induction of these lesions was inhibited by pretreatment of animals with either diethylmaleate (DEM; 1ml/kg, s.c.) or cysteamine (100 mg/kg, s.c.). Since DEM decreased gastric glutathione levels and cysteamine increased them, the authors concluded that gastric mucosal glutathione levels do not relate to ethanol-induced gastric lesions in rats.

Tariq (46) studied the effects of the endogenous lipophilic chain terminating antioxidant, vitamin E, on gastric mucosal damage induced by cold-restraint stress, indomethacin, reserpine, 0.6M HCl, hypertonic sodium chloride
or ethanol in rats. He reported that pretreatment of 36-hr fasted animals with an oral dose of 100 mg/kg of vitamin E 30 minutes before subjecting animals to cold-restraint stress significantly reduced the production of gastric lesions by the above mentioned agents. The results suggest a possible role of lipid peroxidation in the pathophysiology of acute gastric mucosal lesions induced by the above mentioned agents.

Tatsumi et al. (47) reported that the simultaneous addition of both xanthine and xanthine oxidase to a homogenate of rat gastric mucosa caused a significant reduction in the activity of glucosamine synthetase. This effect was counteracted by catalase (an endogenous enzymatic antioxidant) but not by SOD. Hydrogen peroxide could also decrease the activity of this enzyme. This effect of hydrogen peroxide was counteracted by either catalase or dithiothreitol. Since glucosamine synthetase is important in protecting the gastric mucosa, one might conclude that RODS may have a role in the destruction of the mucus barrier resulting in lesion formation.

Schoenberg (48) induced ischemia of the small intestine in cats by partial occlusion of the superior mesenteric artery. Specimens obtained during and after the ischemia showed the presence of mucosal lesions. Administration of SOD during the ischemia could prevent mucosal damage. Therefore, it could be concluded that generation of RODS due to local ischemia might play a role in ischemia-induced lesions in the small intestine in cats.
Grisham et al. (49) demonstrated that ischemia/reperfusion in the small intestine of cats could result in a marked increase in neutrophil infiltration and a concurrent decrease in reduced glutathione levels and SOD activity. Pretreatment with allopurinol (50 mg/kg p.o. for 2 days) or administration of SOD (injected in the superior mesentric artery prior to ischemia) prevented the influx of neutrophils and the decreases in reduced glutathione concentration and SOD activity. Since inflammatory neutrophils are potential sources of reactive oxidants, such as superoxide and hypochlorous acid, these data again suggest that RODS may have a role in ischemia/reperfusion-induced injury in the small intestine in cats.

Victor et al. (50) reported that topical administration of either a mild irritant (8% ethanol) or prostaglandin E₂ (1 µg/ml) could increase gastric mucosal glutathione levels by 20% compared with control values in dogs. Both agents individually could protect gastric mucosa against the effects of 40% ethanol, while administration of N-ethylmaleimide (NEM; 50 mg/kg), a glutathione depleting agent, prevented the protective effects of both 8% ethanol and prostaglandin E₂. Therefore, one can conclude that gastric mucosal glutathione levels may play a role in the process referred to as "adaptive cytoprotection".

Pasechnikov et al. (51) studied the role of lipid peroxidation and the activities of SOD, glutathione peroxidase, glutathione reductase and glutathione levels in gastric mucosa obtained endoscopically from 60 patients with peptic ulcer disease. The authors concluded that lipid peroxidation and a drop in the
activities of the above mentioned antioxidants may be important pathogenic factors leading to a chronic and recurrent course of peptic ulcers.

1.4. The role of oxidative stress in gastrointestinal disorders

Like other organs, the gastrointestinal tract may be exposed to conditions leading to oxidative stress (52). One such condition is ischemia/reperfusion (53, 54). A relatively subtle indicator of ischemic damage to tissue is enhanced capillary permeability which may result in edema. More severe damage may cause cell death and tissue necrosis. Granger et al. (55) were able to show that feline intestinal ischemia can lead to accumulation of hypoxanthine as a result of extensive breakdown of ATP. When oxygen is readmitted during reperfusion, hypoxanthine is oxidized to uric acid catalyzed by xanthine oxidase, with generation of superoxide radical. It has been reported that xanthine oxidase inhibitors could decrease ischemia-induced tissue damage in the feline small bowel (55). Therefore, it might be concluded that xanthine oxidase is a potential source of mucosal damage during reperfusion in intestinal ischemia. Younes et al. (53) have observed that during ischemia substrates of xanthine oxidase accumulate in the feline small intestine. These can be rapidly metabolized resulting in the generation of reactive oxidants that are capable of initiating lipid peroxidation with resulting tissue damage. Moreover, Niida et al. (36) were able to produce duodenal ulcers in rats by administration of diethyldithiocarbamate (DDC 750 mg/kg), an SOD inhibitor. Thus, it might be concluded that impairment
of antioxidant capacity of the duodenal mucosa may be involved in the pathogenesis of DDC-induced duodenal ulcers.

There is much evidence in the literature that reactive oxygen metabolites might be involved in the pathogenesis of another gastrointestinal disorder, inflammatory bowel disease (IBD). Verspaget et al. (56) have reviewed the role of RODS in the pathogenesis of colitis. In general, scavenging of superoxide radicals by SOD or prevention of their formation by inhibition of xanthine oxidase by allopurinol, have been found most effective in the prevention of experimental colitis. Salim (57) has reported in a double-blind investigation that the inclusion of the xanthine oxidase inhibitor, allopurinol, with sulfasalazine could reduce the recurrence of ulcerative colitis attacks as compared with administration of sulfasalazine alone. He has concluded that RODS may be involved in the mechanism of ulcerative colitis and that antioxidant measures may be useful both in the treatment of attacks and in preventing their recurrence.

RODS may also be involved in the occurrence of gastrointestinal malignancy. Babbs (58) has hypothesized that high concentrations of iron in feces and the presence of bacteria can lead to the production of large quantities of highly reactive hydroxyl radicals in the colon. Intracolonic free radical formation, therefore, may be responsible for the production of colon cancer. These reactive metabolites may have a pivotal role in carcinogenesis by affecting the genes and chromosomes resulting in abnormalities in cell division. Bostick et al. (59) have reported a strong inverse association between vitamin E
intake and colon cancer in a prospective Iowa women's health study. Since vitamin E is a lipid-soluble chain-terminating antioxidant agent, these data suggest that RODS and lipid peroxidation may be involved in the pathogenesis of colon cancer. Cahill et al. (60) have also reported a strong inverse correlation between serum concentrations of antioxidant vitamins A and E and incidence of colon cancer in humans. Nomura et al. (61) have reported significantly lower serum selenium concentrations in individuals with gastrointestinal malignancies compared with controls. Diets low in copper have been shown to increase the incidence of 1,2 dimethylhydrazine-induced colon tumors in rats when compared with diets high in copper (62).

Peptic ulcer disease is another gastrointestinal disorder. This disease is a general public health problem all over the world. Statistical information indicates that 10% or more of the western population may be afflicted by this disease at some time in their lives. About 10% of all adult admissions to general hospitals are patients with peptic ulcer disease. This disease accounts for about 1% of all deaths in the general population. The health care cost of peptic ulcer disease was estimated to be 3,224 million dollars in the United States of America in 1977 (63).

Peptic ulcers are lesions that are induced by gastric acid and pepsin. These lesions are typically found in the distal third part of the esophagus, in the stomach and in the duodenum. Two types of lesions can be distinguished, namely erosions and ulcers. By definition, lesions that involve only mucosa are
called "erosions," and if they fail to heal and tissue destruction progresses and penetrates beyond the muscularis mucosa "ulcers" are formed. Gastric and duodenal lesions can be subclassified into two groups: acute and chronic.

Acute ulcers usually occur in the body (glandular portion) of the stomach—less commonly in the duodenum—in patients with any condition associated with severe stress, such as a major surgical procedure, sepsis, respiratory failure, uremia, or hypotension (23). These acute lesions (which may be erosions or ulcers) are frequently multiple in number, superficial and a few millimeters in diameter. This condition is also known by a variety of names such as "stress ulceration," "acute stress ulceration," "acute gastric mucosal lesions," "stress ulcer syndrome," "stress ulcer diathesis" or "acute hemorrhagic gastritis" (26).

Central nervous system injury-induced ulcers (Cushing's ulcers), burn-induced ulcers (Curling's ulcer) and drug-induced ulcers exhibit similar macroscopic and microscopic features as other forms of acute ulceration. Cushing's ulcers can also be found in the esophagus and duodenum (23).

Acute lesions usually develop within hours. If damaging agents are removed, lesions can heal without scar formation within a few days by epithelial cell regeneration and they do not recur (64). The main complication with these lesions is upper gastrointestinal bleeding due to congestion of submucosal blood vessels. There is usually no pain accompanying these lesions.

Histologically, acute inflammatory exudate and necrotic debris can be found in the ulcer crater; however, there is no granulation tissue. Gross and
histological features of acute gastric lesions were described in my previous histopathological study (65) of the gastric mucosa in rats exposed to fasting, psychological stress or physical activities.

A chronic peptic ulcer of the stomach or the duodenum is an ulcer that has failed to heal within a period of time (2 weeks or more) and shows histological evidence of tissue repair, i.e., granulation tissue that will give rise to scar formation when the ulcer heals. Chronic ulcers are deeper and larger than acute ones and they usually occur singly and have a round or oval shape. Chronic ulceration is less common in the stomach than in the duodenum and the former most frequently appears as a single lesion along the lesser curvature of the stomach. The occurrence of chronic gastric ulcers is uncommon in the body or the greater curvature of the stomach. Chronic duodenal ulceration is about 8 times more common than chronic gastric ulceration and its site is usually in the duodenal bulb (66).

Usually, when the terms "peptic ulcer," "gastric ulcer" or "duodenal ulcer" are used without further qualification, a chronic ulcer is implied (28). One of the most distinctive features of chronic peptic ulcers is their tendency to heal and recur months or years later.

Another type of peptic ulcer might be classified as "hormonally-induced ulceration". This is seen in patients with the Zollinger-Ellison syndrome that is associated with greatly enhanced secretion of gastrin by an Islet cell tumor.
1.5. Hypothesis

RODS generation may play an important role in the pathophysiology of some gastrointestinal diseases. There are several lines of evidence that support this hypothesis. It is proposed that differences in susceptibility to ulceration may be determined by species-, sex-, age-, and tissue-related differences in gastrointestinal mucosal antioxidants. Species-related antioxidant enzyme differences in tissues other than gastrointestinal mucosa have been reported by Godin and Garnett (67) and sex- and age-related differences in the plasma of normal persons by Olinescu et al. (68) and age-related antioxidant capacity in rat tissues by Matsuo et al. (69). Preliminary work in our laboratory has shown differences in gastric and intestinal mucosal antioxidants of rats, rabbits and quail.

1.6. Rationale and Objectives

The previously cited literature studies did not directly measure the enzymatic and non-enzymatic antioxidant systems in gastric and intestinal mucosa. Moreover, we are unaware of any reports studying the activity of antioxidant systems in gastric and duodenal mucosa of different species. In order to demonstrate any associated changes in the activity of antioxidant status in normal and ulcerative gastric and duodenal mucosa, a number of studies will be conducted. Specific objectives are:
1.6.1. Experiment I

To measure the activities of superoxide dismutase, glutathione peroxidase, glutathione reductase and the levels of basal glutathione in normal gastric, proximal small intestine (psi), distal small intestine (dsi) and colonic mucosa in both male and female rats.

1.6.2. Experiment II

To measure the activity of the above mentioned enzymes and the levels of basal glutathione in the gastric and duodenal mucosa in several other species, namely rabbit, pig, quail and cat. These experiments will include both sexes.

1.6.3. Experiment III

To measure the activities of the above mentioned enzymes and basal glutathione in the gastric and duodenal mucosa of rats treated with 8% or undiluted ethanol. The results of these experiments would indicate if any possible changes in the activities of the antioxidant systems occur in either of the above regions following the exposure to different concentrations of ethanol when compared with those in control rats.

1.6.4. Experiment IV

To measure the activity of the above mentioned enzymes and the levels of basal glutathione in the gastric and duodenal mucosa of rats exposed to chronic intermittent stress.
2. EXPERIMENT I

Analysis of the antioxidant status of gastric, proximal small intestine, distal small intestine and colonic mucosa in male and female rats.

2.1. Materials and Methods

Eight male and 8 female Sprague Dawley rats weighing 245-310 grams were purchased from the animal unit, U.B.C., and kept for 2-3 days in the animal room under usual conditions in the Department of Pharmacology and Therapeutics. Animals were anesthetized with an i.p. injection of pentobarbital (60 mg/kg), and killed by cardiac excision at about 10:00 A.M. Different segments of the gastrointestinal tract, including stomach, the first 10-15 cm of the small bowel (proximal small intestine), 10-15 cm of the small intestine about 30 cm proximal to ileo-cecal connection (distal small intestine) and the colon were removed and placed in cold normal saline. The stomach was opened by cutting through the greater curvature. All intestinal segments were flushed with cold normal saline and opened by cutting along the opposite line of the mesentery. All mucosal surfaces were rinsed with cold normal saline. All specimens were placed on a metal plate placed on ice and examined for any macroscopic lesions. If any macroscopic lesions were found in any portion of the gastrointestinal tract, all tissue specimens obtained from that animal were discarded. The excess normal saline on the mucosa was removed by blotting with a piece of tissue paper. The mucosa was gently scraped using a blunt scalpel. The mucosal material obtained was weighed (0.15-0.3 grams) and
homogenized on ice in 50 mM Tris-0.1 mM EDTA (ethylenediaminetetraacetic acid), pH 7.6 (10% w/v) using two 15 second bursts of a Polytron (Brinkmann, Westbury, N.Y.) at 25% maximal speed. Two hundred milliliters of homogenate were used for the estimation of basal glutathione and the reminder of the homogenate was centrifuged for 15 minutes at 12,000 x g in an Eppendorf microcentrifuge at 4° C. The supernatant fraction was assayed for the activities of the antioxidant enzymes glutathione peroxidase, glutathione reductase and superoxide dismutase.

Basal glutathione (GSH) levels were measured by adding 0.2 ml of 0.9% saline/azide followed by 0.1 ml of 25% TCA to the homogenate on ice. The samples were mixed and centrifuged for 5 minutes at 12,000 x g. The resulting supernatants were assayed for acid-soluble sulfhydryl groups by adding 0.04 ml of 3 mM 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and the absorbance at 412 nm was measured at 10 minutes. Since in most tissues more than 90% of acid-soluble sulfhydryl groups is glutathione, herein the term basal glutathione indicates acid-soluble sulfhydryl groups. The activities of glutathione reductase (GRD) glutathione peroxidase (GPX) and Cu-Zn-superoxide dismutase (SOD) were measured as described by Godin et. al. (70). A Cecil spectrophotometer (CE 292, digital ultraviolet spectrophotometer) was employed in the measurement of SOD activity while all other assays were performed using a Perkin-Elmer model Lambda 6B dual beam spectrophotometer.
Results were analyzed using single factor ANOVA at a significance level of $P<0.05$, followed by the application of the Tukey test to assess the significance of specific inter-group differences.

2.2. Results

Figures 1-12 show levels of endogenous antioxidant components in various segments of the gastrointestinal tract of male and female rats. Figures 1 and 5 show the levels of basal glutathione (GSH) in the gastric, proximal small intestine (psi), distal small intestine (dsi) and colon of male and female animals, respectively. In both sexes, the levels of this non-enzymatic antioxidant are comparable in the gastric and colonic mucosa but statistically ($P<0.05$) lower than levels in the proximal and distal segments of small intestine (Figure 9). Figures 2 and 6 show the activities of glutathione reductase (GRD) in the gastrointestinal tract of male and female rats, respectively. Different parts of male gastrointestinal tract showed similar activities of this enzyme, whereas colonic mucosa of female rats showed significantly ($P<0.05$) higher activity when compared with that in the other parts of either male or female animals (Figure 10). Figure 3 shows the activity of glutathione peroxidase (GPX) in the gastrointestinal tract of male rats. The activity of this enzyme is significantly ($P<0.05$) higher in the gastric and distal small intestinal mucosa than that in the other parts of this organ. Figure 7 shows that the activity of GPX in the gastrointestinal tract of female animals is much higher in the gastric mucosa than in the other parts of this organ. Comparison of the activity of this enzyme in
males and females indicate that it was significantly higher in the female gastric and colonic mucosa and male distal small intestine mucosa as compared with the corresponding segment of the other sex (Figure 11). No marked differences were found in the activity of superoxide dismutase among the various segments examined or between males and females (Figures 4, 8 and 12).
Figure 1: The levels of basal glutathione in gastric, proximal small intestine (psi), distal small intestine (dsi) and colon of male rats (mean ± SE). [In this and all subsequent Figures, values denoted by different letters (a, b, c, etc.) are significantly different (P<0.05) from each other; n=8.]
Figure 2: The activity of glutathione reductase in various regions of the gastrointestinal tract of male rats (mean ± SD). [Abbreviations, expression of statistical significance and number of animals are as in Figure 1.]
Figure 3: The activity of glutathione peroxidase in various regions of the gastrointestinal tract of male rats (mean ± SE). [Abbreviations, expression of statistical significance and number of animals are as in Figure 1.]
Figure 4: The activity of superoxide dismutase in various regions of the gastrointestinal tract of male rats (mean ± SE). [Abbreviations, expression of statistical significance and number of animals are as in Figure 1; the value denoted by b is not significantly (P<0.05) different from that of psi.]
Figure 5: The levels of basal glutathione in various regions of the gastrointestinal tract of female rats (mean ± SE). [Abbreviations, expression of statistical significance and number of animals are as in Figure 1.]
Figure 6: The activity of glutathione reductase in various regions of the gastrointestinal tract of female rats (mean ± SE). [Abbreviations, expression of statistical significance and number of animals are as in Figure 1.]
Figure 7: The activity of glutathione peroxidase in various regions of the gastrointestinal tract of female rats (mean ± SE). [Abbreviations, expression of statistical significance and number of animals are as in Figure 1.]
Figure 8: The activity of superoxide dismutase in various regions of the gastrointestinal tract of female rats (mean ± SE). [Abbreviations, expression of statistical significance and number of animals are as in Figure 1.]
Figure 9: Comparison of basal glutathione levels in various regions of the gastrointestinal tract of male and female rats (mean ± SE). [Abbreviations, expression of statistical significance and number of animals are as in Figure 1.]
Figure 10: Comparison of the activity of glutathione reductase in various regions of the gastrointestinal tract of male and female rats (mean ± SE). [Abbreviations, expression of statistical significance and number of animals are as in Figure 1.]
nMoles / min /mg tissue

male

female

nMoles / min /mg tissue

8

7

6

5

4

3

2

1

0

gastric

psi

dsi

colon
Figure 11: Comparison of the activity of glutathione peroxidase in various regions of the gastrointestinal tract of male and female rats (mean ± SE).

[Abbreviations, expression of statistical significance and number of animals are as in Figure 1.]
Figure 12: Comparison of the activity of superoxide dismutase in various regions of the gastrointestinal tract of male and female rats (mean ± SE). [Abbreviations, expression of statistical significance and number of animals are as in Figure 1; the value denoted by b is not significantly (P<0.05) different from that of psi.]
3. EXPERIMENT II

Comparative study of antioxidant status in the gastric and proximal small intestinal mucosa of rat, rabbit, quail, cat and pig.

3.1. Materials and Methods

In this experiment, rat data from the previous experiment were used, while those for the other species were obtained from untreated "control" animals being used for other purposes in the Department or elsewhere, as indicated below.

For the data on the gastric and proximal portion of the small intestine in rats, results from the female rats in Experiment I were used. The stomach and the proximal portion of the small bowel of 6 female rabbits are from animals being used for other purposes in the Department of Pharmacology and Therapeutics, U.B.C. The proximal segments of the small bowel of eight male quail were from animals which had been sacrificed by decapitation. The stomach and the duodenum of female cats were obtained from the Department of Ophthalmology, VGH. The stomach and the duodenum of female pigs were obtained from the Department of Surgery, VGH. All other methods and materials were the same as described in Experiment I.

3.2. Results

Figures 13-16 show the levels of the non-enzymatic antioxidant glutathione and the activities of the enzymatic antioxidant components in the gastric and duodenal mucosa of rabbit, cat, pig and rat and the proximal part of
small intestine of quail. In the gastric mucosa, the level of basal glutathione was
highest in the rat, whereas the levels in the duodenal mucosa were greater in
quail. Rabbit tissues, both gastric and duodenal mucosa, showed significantly
higher basal glutathione levels than those of cat or pig. The gastric mucosa of
cat and pig showed similar contents of basal glutathione, while that of the pig
duodenal mucosa was much higher than that of cat (Fig. 13). The highest activity
of GRD was found in both tissues of rat. Generally, the activity of this enzyme
was significantly higher in the duodenal mucosa when compared to that in
gastric mucosa in all species investigated (Fig. 14).

Similarly, the activity of GPX in rat gastric mucosa was much higher when
compared with that of other species. However, the activities of this enzyme in the
duodenal mucosa of rat, pig and rabbit were almost the same, and higher than
that of quail or pig (Fig. 15). The activities of SOD in the gastric and duodenal
mucosa of pig were lower than those in rat and rabbit. The activity of this
enzyme in the quail duodenal mucosa was much higher than that of any of the
other species studied (Fig. 16).
Figure 13: Basal glutathione levels in the gastric and duodenal mucosa of various species (mean ± SE). [Abbreviations and expression of statistical significance are as in Figure 1; n=8, 8, 6, 4, and 2 for rat, quail, rabbit, cat and pig, respectively.]
Figure 14: Activity of glutathione reductase in the gastric and duodenal mucosa of various species (mean ± SE). [Abbreviations and expression of statistical significance are as in Figure 1; n=8, 8, 6, 4, and 2 for rat, quail, rabbit, cat and pig, respectively.]
Figure 15: Activity of glutathione peroxidase in the gastric and duodenal mucosa of various species (mean ± SE). [Abbreviations and expression of statistical significance are as in Figure 1; n=8, 8, 6, 4, and 2 for rat, quail, rabbit, cat and pig, respectively.]
The diagram compares the nMoles/min/mg tissue of gastric and duodenal mucosa across various species: rabbit, cat, pig, rat, and quail. The bar heights represent the activity level for each species in each mucosal type. For gastric mucosa, rabbit and cat have the highest activity levels, labeled 'c'. For duodenal mucosa, rat and quail show lower activity, labeled 'e'.
Figure 16: Activity of superoxide dismutase in the gastric and duodenal mucosa of various species (mean ± SE). [Abbreviations and expression of statistical significance are as in Figure 1; n=8, 8, 6, 4, and 2 for rat, quail, rabbit, cat and pig, respectively.]
4. EXPERIMENT III

The effects of 8% and undiluted ethanol on lesion development and the antioxidant status of gastric and proximal small intestinal mucosa of male and female rats.

4.1. Materials and Methods

Twenty one male and 21 female Sprague Dawley rats weighing 220-365 grams were purchased from the animal unit, UBC. About eighteen hours before the experiment, each animal was randomly assigned to one of the experimental groups and placed in an individual cage and food was removed, although animals had free access to water. Ethanol was purchased from chemical stores, U.B.C., and used as undiluted ethanol or diluted with double distilled water to make an 8% solution. At 10:00 A.M on the day of experiment., animals were anesthetized using a mixture of 4% halothane and oxygen. One milliliter of double distilled water, 1 ml of undiluted ethanol or 1 ml of 8% ethanol was administered to anesthetized animals by gastric gavage. Animals receiving double distilled water were used as controls. One hour after administration of water or ethanol, animals were again anesthetized and sacrificed by cardiac excision. The stomach and the proximal portion of the small intestine were removed, opened and rinsed, as described previously. The mucosa was carefully examined for macroscopic lesions. The mucosa was then scraped and all assays performed as described earlier. The results obtained from male animals were compared with those of females.
4.2. **Results**

Figures 17-24 show the effects of 1 ml of 8% or undiluted ethanol on the antioxidant components of gastric and duodenal mucosa in male and female rats. Generally, administration of 1 ml of 8% ethanol to rats by gavage caused an increase in the activity of enzymatic antioxidant systems and basal glutathione levels in both gastric and duodenal mucosa. On the other hand, administration of 1 ml undiluted ethanol produced a general decrease in the antioxidant systems in both tissues, with the exception of an increase in the activity of GPX.

Figures 25-33 show macroscopic and microscopic changes found in the stomachs of rats treated with undiluted ethanol. All of these rats developed visible gastric mucosal lesions in the body of the stomach. Macroscopic alterations observed included distention of the stomach and duodenum, with increased luminal fluid volume, as well as strips of redness on the surface of the stomach indicative of gastric mucosal vascular changes. Other stomachs and duodenums appeared normal with no visible lesions. Gastric epithelial necrosis was found in gastric specimens of both male and female rats treated with undiluted ethanol. Tissue damage was more profound in females than that in males.

Significant alterations in the activities of mucosal antioxidant enzymes and levels of basal glutathione following the administration of ethanol are summarized in Table 1.
Figure 17: Basal glutathione levels in the gastric mucosa of male and female control and treated rats (mean ± SE). [Abbreviations and expression of statistical significance are as in Figure 1; n=7 for both male and female.]
Figure 18: The activity of glutathione reductase in the gastric mucosa of male and female control and treated rats (mean ± SE). [Abbreviations and expression of statistical significance are as in Figure 1; n=7 for both male and female.]
Figure 19: The activity of glutathione peroxidase in the gastric mucosa of male and female control and treated rats (mean ± SE). [Abbreviations and expression of statistical significance are as in Figure 1; n=7 for both male and female.]
Figure 20: The activity of superoxide dismutase in the gastric mucosa of male and female control and treated rats (mean ± SE). [Abbreviations and expression of statistical significance are as in Figure 1; n=7 for both male and female.]
Figure 21: Basal glutathione levels in the duodenal mucosa of male and female control and treated rats (mean ± SE). [Abbreviations and expression of statistical significance are as in Figure 1; n=7 for both male and female.]
The graph shows the nMoles/mg tissue for duodenal samples from both males and females in different groups: control, 8% ethanol, and 100% ethanol.

- For duodenal (male) samples:
  - Control and 8% ethanol groups are indicated by 'a' and 'b' respectively.

- For duodenal (female) samples:
  - Control and 8% ethanol groups are indicated by 'a' and 'b' respectively.
Figure 22: The activity of glutathione reductase in the duodenal mucosa of male and female control and treated rats (mean ± SE). [Abbreviations and expression of statistical significance are as in Figure 1; n=7 for both male and female.]
Figure 23: The activity of glutathione peroxidase in the duodenal mucosa of male and female control and treated rats (mean ± SE). [Abbreviations and expression of statistical significance are as in Figure 1; n=7 for both male and female.]
Figure 24: The activity of superoxide dismutase in the duodenal mucosa of male and female control and treated rats (mean ± SE). [Abbreviations and expression of statistical significance are as in Figure 1; n=7 for both male and female.]
Table 1: Presence of macroscopic lesions (+) and average percent increases (+) or decreases (-) in levels endogenous antioxidant components of gastric (G) and duodenum (D) of male (M) and female (F) rats following administration of double distilled water (A), 8% ethanol (B) or undiluted ethanol (C). [Data from Figures 17-24.]

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lesion</th>
<th>GSH</th>
<th>GPX</th>
<th>GRD</th>
<th>SOD</th>
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Figure 25: Gross appearance of stomach and duodenum in the abdomen of a male rat treated with 8% ethanol. [The stomach and duodenum of control animals were indistinguishable in appearance from those in the present Figure].
ethanol (8%)
Figure 26: Distended stomach and duodenum with increased luminal fluid volume in the abdomen of a male rat treated with undiluted ethanol.
ethanol (undiluted)
Figure 27: Comparison of appearance of gastric and duodenal mucosa of male rats treated with double distilled water, ethanol (8%) or ethanol (undiluted). [Note the appearance of red strips in the body of the stomach of the animal treated with undiluted ethanol].
Figure 28: Histology of gastric mucosa of a male control rat (longitudinal section, gastric glands and pits). [In this and all subsequent Figures tissues were stained with Haemotoxylin and Eosin; prints are produced from slides that were taken of tissue sections by using Zeiss Photomicroscope II (Mag. X25).]
Figure 29: Appearance of gastric mucosa of a control male rat (transverse section).
Figure 30: Superficial necrosis of gastric epithelium of a male rat treated with undiluted ethanol.
Figure 31: Gastric epithelial necrosis surrounded by normal looking mucosa in a female rat treated with undiluted ethanol.
Figure 32: Deep gastric epithelial necrosis in a female rat treated with undiluted ethanol.
Figure 33: Severe gastric mucosal necrosis following administration of undiluted ethanol to a female rat.
5. EXPERIMENT IV

The effect of chronic intermittent stress on the antioxidant status of the gastric and proximal small intestinal mucosa of male rats.

5.1. Materials and Methods

Eleven male Wistar rats weighing 200-250 grams were purchased from the animal unit, U.B.C. Animals were kept in the animal room in the Department of Pharmacology and Therapeutics at U.B.C for 14 days. Beginning on the fifteenth day following their arrival, 5 animals were selected randomly to remain in their home cages for the next 14 days. These animals were used as the control group. The remaining 6 animals were exposed to a stress protocol which involved restraint for one hour twice daily, the first exposure taking place between 9:00 A. M. and 12:00 A. M., and the second between 1:00 P. M. and 4:00 P. M. In order to minimize habituation, the sequence of the restraint stressors was randomized for both the first 7 morning and afternoon exposures. The series was repeated during the second week with the exception that the morning and afternoon sequences were reversed. The stressors used included: 1.) towel wrap secured with tape; 2.) number one, placed in a supine position; 3.) restraint in a plastic box with lid; 4.) restraint in a polyvinlchloride tube closed at either end; 5.) secured to a board with tape; 6.) number 5, placed in a supine position; and 7.) metal bar cage restrainer. Each stressor exposure was conducted in a room remote from the animal facility and animals exposed to stressors were returned to the animal facility 15 minutes following stress
exposure to minimize disturbance to control animals. On the day following the last exposure to stress, animals were killed by decapitation. The stomach and proximal portion of the small intestine were removed and handled as described in the previous sections. The mucosal surfaces were examined carefully for any macroscopic lesions. All other procedures were performed as described earlier. Results were analyzed using a t two-sample test assuming equal variance at a significance level of $P<0.05$.

5.2. **Results**

Figures 34-37 show the activities of antioxidant systems in the gastric and duodenal mucosa of both control and stressed rats. No statistically significant differences between any of the antioxidant components in either gastric or duodenal mucosa of stressed rats were found when comparison was made with those of control animals at a significance level of $P<0.05$. No macroscopically detectable lesions were found in the gastric or duodenal mucosa of stressed or control animals.
Figure 34: Levels of basal glutathione in the gastric and duodenal mucosa of control and stressed rats (mean ± SE). [Abbreviations and expression of statistical significance are as in Figure 1; n=5 and 6 for control and stressed rats, respectively.]
Figure 35: Activity of glutathione reductase in the gastric and duodenal mucosa of control and stressed rats (mean ± SE). [Abbreviations and expression of statistical significance are as in Figure 1; n=5 and 6 for control and stressed rats, respectively.]
nMoles / min / mg tissue

control

stressed

nMoles / min / mg tissue

gastric mucosa

duodenal mucosa

b

a
Figure 36: Activity of glutathione peroxidase in the gastric and duodenal mucosa of control and stressed rats (mean ± SE). [Abbreviations and expression of statistical significance are as in Figure 1; n=5 and 6 for control and stressed rats, respectively.]
control

stressed

gastric mucosa

duodenal mucosa

Moles mg tissue / min

5.00 4.50 4.00 3.50 3.00 2.50 2.00 1.50 1.00 0.50 0.00

a a a a b b
Figure 37: Activity of superoxide dismutase in the gastric and duodenal mucosa of control and stressed rats (mean ± SE). [Abbreviations and expression of statistical significance are as in Figure 1; n=5 and 6 for control and stressed rats, respectively.]
6. GENERAL DISCUSSION

Peptic ulcer disease has a multifactoral etiology that ultimately results in the production of lesions in tissues exposed to gastric acid and pepsin. This disease has 3 forms that differ in their pathophysiology. Gastric and duodenal ulcers are usually classified as recurrent ulcers and are different from each other, and also from the third form called "stress ulceration". The differences among them might be due to differences in the activity of the antioxidant systems in the gastric and duodenal mucosal tissue. Some factors such as species, genetics and age can play important roles in the incidence of this disease, as discussed in the Introduction.

The pathophysiology of "stress ulceration" in experimental animals and man is quite complex. A variety of irritants and stressful conditions can result in "stress ulcer" formation. With regard to the induction of lesions, irritants may well differ in their mechanism of action. However, there is a body of evidence that supports the role of RODS in the pathophysiology of "stress ulcer" production (71-73). With regard to "stress ulceration", susceptibility of the gastric mucosa to ulcer formation seems to be much greater than that of the duodenal mucosa.

The susceptibility to stress-induced ulceration in experimental animals and man increases with age. Administration of enteric-coated aspirin causes a lower incidence of gastric lesions when compared with conventional aspirin. Subjects receiving salicylates by the intravenous route do not demonstrate gastric mucosal lesions, although salicylate blood levels are comparable to
those in subjects receiving the drug orally and in whom gastric lesions usually occur (30). The literature indicates that non-steroidal anti-inflammatory drugs (NSAIDs) induce gastric lesions by at least two different mechanisms. One is the well-characterized effect of these drugs to inhibit biosynthesis of prostaglandin E₂. The second mechanism involves “ion trapping”. In the non-ionized state, which predominates at the low pH of the stomach, these drugs can enter mucosal cells by freely diffusing across the cell membrane. Once inside the cell, these drugs ionize (NSAIDs pKₐ = 3.5-4) and become trapped, creating a concentration gradient that favors the accumulation of the NSAID in the gastric mucosa. Cell permeability alterations are developed resulting in damage caused by hydrogen ion influx, with sodium and potassium ions moving into the gastric lumen (74). Therefore, one can postulate that in NSAID-induced gastric lesions, the direct contact of gastric mucosa with drug seems to be important for lesion formation. Also, the fact that ulceration of the duodenum does not usually occur with the administration of NSAID suggests that differences between gastric and duodenal mucosa may be important.

Results obtained in the present study show that significant differences in antioxidant status exist among different portions of the gastrointestinal tract. It has also been shown that antioxidant systems in the gastrointestinal tract of different species can differ markedly in activity. These results show that the levels of basal glutathione and the activity of GPX in the gastric mucosa of rats are significantly different from those of duodenal mucosa. However, this
information cannot answer the question as to whether or not these differences in antioxidant status are the main factors determining the resistance or susceptibility of gastric and duodenal mucosa to lesion formation. Significant differences in the antioxidant status have been demonstrated in gastric mucosa of different species; however, there does not appear to be a parallel relationship between gastric antioxidant status and susceptibility of corresponding animals to "stress ulceration". For example, the activity of enzymatic antioxidants and the levels of basal glutathione in the gastric mucosa of rats have been shown to be greater than those of rabbits; on the other hand, rabbits are very resistant to "stress ulceration" while rats are more sensitive (25). Also, except for the activity of GPX, which was higher in the gastric mucosa of female rats than in that of male rats, gastric and duodenal muosa of both male and female rats showed similarity in their antioxidant status, but it is reported that gender affects the susceptibility to gastric or duodenal ulcer formation in man so that duodenal ulcers have a male:female ratio of 4:1 (27, 28). Therefore, one might conclude that, at least in laboratory animal models, the antioxidant status is not the only (or the most important) factor determining susceptibility to ulcerogenesis.

It has been found that administration of 1 ml of undiluted ethanol by gastric gavage leads to so-called "watermelon stomach" (34), suggestive of gastric microcirculation injury, with an incidence of 100% in both male and female Sprague Dawley rats within one hour. These macroscopic changes were associated with decreases of 22% and 11% in basal glutathione levels of male
and female rat gastric mucosa, respectively. Lesion formation was also associated with decreases of 14% and 7% in the activity of GRD in males and females, respectively. The susceptibility of gastric mucosa to undiluted ethanol might be influenced by its low reduced glutathione content relative to that of the duodenum. In this regard, it should be noted that the administration of 1 ml of undiluted ethanol orally produced no macroscopically detectable duodenal lesions in either sex. It might be postulated, therefore, that the higher content of reduced glutathione and the higher activity of GRD might contribute to the resistance of duodenal mucosa to ethanol-induced lesion formation. It should be noted, however, that duodenal mucosa did show a decrease in basal glutathione levels and in the activity of GRD despite the absence of macroscopic lesion formation, suggesting that antioxidant status in this region is sufficient to protect the mucosa from the damaging effects of ethanol. Rapid development of gastric lesions following the instillation of undiluted alcohol in the stomach might be due to early gastric vascular damage. One outcome of vascular injury is formation of damaging RODS that might also be responsible for lesion formation in the body of the stomach (39, 75, 76).

Both male and female rats developed a large luminal fluid volume in the stomach within one hour following the administration of undiluted ethanol. This finding raises the question as to whether it was a physiologic response to dilute the irritant or merely an exudate due to vascular damage induced by ethanol. We are unaware of the composition of this fluid, but we observed that some
components of the fluid tends to precipitate over time, so that 2 hours following
the administration of undiluted ethanol, we were unable to drain the fluid with a
normal syringe. Instead, we observed a brownish mass, indicative of sloughed
mucosal cells and coagulated protein contents of the aforementioned fluid.

Ethanol-induced gastric mucosal damage was associated with a
significant reduction in reduced glutathione levels of mucosa. Miller et al. (77)
have also observed a similar reduction in glutathione level in gastric mucosa of
dogs treated with concentrated ethanol. This reduction may reflect oxidation of
reduced glutathione by RODS produced by ethanol and/or binding of glutathione
to acetaldehyde generated through the oxidation of ethanol by gastric mucosal
alcohol dehydrogenase. Since we observed increases in the activities of GPX
and SOD in the gastric mucosa of rats treated with undiluted ethanol, the
possibility that the decrease in glutathione contents was due to damaged
mucosa seems to be unlikely. Gastric glutathione content depletion was
significantly different in male and female rats.

In contrast to the similar overall appearance and gross changes in male
and female gastric mucosa, light microscopic examination of gastric mucosa
sections stained with Haemotoxilin and Eosin revealed differences between
males and females. Epithelial necrosis was deeper and more extensive in
females than in males. The activity of gastric alcohol dehydrogenase has been
shown to be significantly lower in women as compared with that of men (78, 79).
If this is also the case in rats, one might conclude that the metabolism of ethanol
in gastric mucosal cells was slower in female rats than in males. Therefore, female gastric mucosal cells were exposed to ethanol and other ethanol-mediated injurious factors for longer period of time, resulting in more irreversible tissue damage.

It is well established that acute and severe stress, such as that associated with major surgery, hemorrhagic shock, massive burns or cold-restraint can lead to acute gastric ulceration (21, 23, 26, 80-83). Restraint is one of the most common experimental methods used to produce stress ulceration. The duration of restraint needed to induce acute gastric lesions varies from 2-24 hours, depending on the environmental temperature, so that the lower the temperature the more rapid the induction of lesions (21, 23, 80-87). Senay and Leviene (82) showed that a brief two hour cold-restraint (4° to 7° C) period produced a high degree of reproducibility and homogeneity of acute gastric lesions in rats. On the other hand, a number of publications (88-93) have reported that mild and chronic irritants, including restraint and water-immersion, can protect gastric mucosa against damaging agents by a process called “adaptive cytoprotection.” Uramoto and Ishihara (94) showed that restraint and water-immersion stress (22 ° C) in rats reduced gastric damage induced by a combination of 60% ethanol and 150 mM HCl. The 15 day chronic intermittent stress protocol used in the present study did not result in any macroscopically visible lesions or in any significant alterations in the antioxidant components of either gastric or duodenal mucosa of male rats. Therefore, the fact that we did not find any macroscopic lesions in
the stomach of stressed rats might indicate that either mucosal damage occurred acutely, then healed or the stress conditions resulted in "adaptive cytoprotection" that prevented lesion induction. Since the stress protocol did not alter the antioxidant status in the gastric and duodenal mucosa of rats, it can be suggested that if "adaptive cytoprotection" did occur, it apparently does not involve antioxidant components.

A large body of evidence (38, 43, 44, 47, 75, 76, 95-97) suggests a role of RODS in the pathogenesis of gastric ulceration. Since both stress- and ethanol-induced gastric ulceration have been found to respond similarly to agents such as sulphydryl group-containing compounds and zinc (43, 98), it can be suggested that generation of RODS may play a role in gastric ulceration induced by acute stress or ethanol. On the other hand, the fact that alterations in antioxidant status following the administration of undiluted ethanol, although statistically significant, were relatively small in magnitude suggests that other mechanisms may be more important in the production of ethanol-induced gastric lesions.

The incidence of macroscopic lesions in the body of the stomach resulting from the instillation of 1 ml of dilute ethanol (8%) was zero in both sexes. However, analyses of antioxidant systems in animals so treated showed some significant differences between antioxidant systems in both gastric and duodenal mucosa of both sexes relative to control. For example, increases of 12-13% in basal glutathione levels were noted in gastric mucosa of both male and female rats. These increases in basal glutathione levels were associated with an
increase of approximately 6% in the activity of GRD but no significant changes in the activity of GPX. Víctor et al. (50) have reported a 20% increase in gastric mucosal glutathione levels when 8% ethanol was topically applied to gastric epithelium of anaesthetized mongrel dogs. These investigators have also reported that pretreatment of gastric mucosa with 8% ethanol could protect the gastric mucosa against subsequent damage induced by 40% ethanol. In the foregoing study, 8% ethanol was as effective as PGE$_2$ in protecting the gastric mucosa. Moreover, Glavin and Szabo (99) have postulated that 10-20% ethanol, given before a necrotizing agent, might cause cytoprotection by releasing endogenous prostaglandins in the stomach.

Since our results do show some similarities to those which are reported by other investigators (50, 99), one might conclude that an increase in the level of basal glutathione accompanied by an increase in the activity of GRD and possibly prostaglandin E$_2$ levels might contribute to the cytoprotective effects of low concentrations of ethanol on gastric mucosa. Therefore, one may suggest that administration of SH-containing compounds might be effective in prevention of acute gastric ulceration due to ulcerogenic agents or conditions, including stress. With regard to our findings concerning antioxidant alterations under physiological and pathological conditions, it seems that the two above mentioned antioxidant components might play a significant role in the defense mechanisms of gastrointestinal tract. The mechanism by which the level of basal glutathione and the activity of GRD are increased by low concentrations of
ethanol is still unclear and remains to be elucidated. Since ethanol is absorbed rapidly from gastric mucosa but some changes also occur in antioxidant components of the proximal small intestine, it might be concluded that central effects of ethanol and/or its metabolites might contribute to both gastric cytoprotection and lesion formation.

In addition to antioxidant systems, another element involved in protecting gastric mucosa is the "mucus-bicarbonate" barrier. Due to the existence of this barrier on the gastric mucosa, surface cells are normally relatively protected from damage by acid and pepsin. Cells within gastric pits, such as chief and parietal cells, might also be protected against the aforementioned damaging agents. The apical surface of chief cells seems to be impermeable to hydrogen ions, even at hydrogen ion concentration gradients of about 100,000 fold. Tight junctions may play an important role in this regard. In contrast to parietal cells, chief cells produce and store pepsinogen and secrete it whenever they receive appropriate stimulation, mainly from acetylcholine. RODS could cause damage to membranes, including the tight junctions of chief cells. The consequences of this damage at the membrane level would be an increased permeability to hydrogen ions, the release of almost all stored pepsinogen and, ultimately, death of chief cells. On the other hand, released pepsinogen can be converted to pepsin and act as a potent damaging agent and produce further damage.

Finally, it seems to be a possibility that by stabilizing and increasing the activities of the antioxidant systems in the gastrointestinal mucosa, one might
decrease the risk of formation of peptic ulcers. Therefore, more studies have to be done to gain more information about the role of endogenous antioxidant systems in gastrointestinal ulceration which might lead to the development of improved therapeutic measures to treat, and possibly prevent this common and potentially life-threatening condition.
7. SUMMARY AND CONCLUSIONS

Results obtained in this study show that the activities of enzymatic antioxidants and levels of basal glutathione show significant differences in various portions of the gastrointestinal tract of both male and female Sprague Dawley rats. It has also been shown that various species including rat, rabbit, cat and pig exhibit marked differences in antioxidant profiles in the gastric and duodenal mucosa. Antioxidant status in the mucosa of the proximal portion of the small intestine of quail was different from that in the duodenal mucosa of the aforementioned species, as well.

Administration of 1 ml of undiluted ethanol by gastric gavage to male and female Sprague Dawley rats caused visible lesions in the body of stomach in all rats so treated, while administration of 1 ml of 8% ethanol caused no visible lesions in the stomach of the any of the rats examined. Both undiluted ethanol and dilute ethanol (8%) did, however, produce some changes in the antioxidant status of the gastric and duodenal mucosa of male and female rats. Epithelial tissue necrosis was deeper and more extensive in gastric mucosa of female rats than that in males.

Fifteen day chronic intermittent stress had no effect on either lesion formation or antioxidant status in the gastric and duodenal mucosa of male Wistar rats.

In conclusion, in spite of the existence of marked differences in antioxidant profiles in the gastric and duodenal mucosa of various species, our
findings suggest that antioxidant status is not the only (or the most important) defense mechanism against ulcerogenesis.
8. REFERENCES


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