Role of reactive oxygen species in mercapto-methylimidazole-induced gastric acid secretion and stress-induced gastric ulceration

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The objective of the present study is to delineate the role of reactive oxygen species in drug-induced gastric hyperacidity and stress-induced gastric ulceration. We reported earlier that mercaptomethylimidazole (MMI), an antithyroid drug, induces gastric acid (HCl) secretion partially through H_2 receptor activation of the parietal cell by histamine release and partially through an intracellular mechanism. While studying the latter, MMI-induced acid secretion was found to correlate well with the inactivation of the peroxidase, an important H_2O_2 metabolizing enzyme of the mucosa. MMI activates the isolated parietal cell for acid secretion, which is sensitive to omeprazole. Peroxidase and catalase activity of the isolated cell is also irreversibly inactivated by MMI. It thus creates a favourable condition for endogenous accumulation of H_2O_2. Acid secretion by gastric gland preparation or isolated gastric mucosa is stimulated by exogenous H_2O_2, which is inhibited by omeprazole. Studies indicate that H_2O_2 inactivates the prostaglandin synthetase and removes the inhibitory influence of prostaglandin on acid secretion. MMI thus stimulates acid secretion not only through H_2 receptor activation but also through the stimulation of the parietal cell by intracellular generation of H_2O_2 following inactivation of the peroxidase–catalase system.

Among the various factors responsible for gastric ulceration, stress was found to cause severe haemorrhagic lesions mainly through oxidative damage of the mucosa as indicated by increased lipid peroxidation, increased protein carbonyl content, and decreased glutathione level. The severity of ulcer correlates well with the time-dependent induction of superoxide dismutase and inactivation of peroxidase, a condition favourable for accumulation of endogenous H_2O_2. Desferrioxamine prevents stress ulcer, indicating involvement of transition metal ion in the process. Studies indicate that severity of stress ulcer is dependent on the concurrent generation of hydroxyl radical ($^{\bullet}OH$) formed through metal-catalysed Haber–Weiss reaction between O_2^- and H_2O_2.

OXYGEN is vital for maintenance of the aerobic life processes. However, partial reduction of O_2, instead of resulting in reduction to water, under certain conditions such as ischaemia, leads to the generation of some reactive oxygen species (ROS) namely O_2^-, H_2O_2 and $^{\bullet}OH$. When these oxygen metabolites overwhelm the antioxidant defence of the cell, these create 'oxidative stress'[1,2]. The oxidative stress may lead to DNA damage and mutagenesis, carcinogenesis; lipid peroxidation and membrane damage; and protein and carbohydrate oxidation and metabolic disorders[3,4]. ROS have thus been regarded as highly toxic agents responsible for a wide variety of tissue damage[3]. Recently, interest has been focused on the role of ROS in gastroduodenal pathogenesis related to gastric hypersecretion and gastroduodenal mucosal damage. ROS has been implicated in ischaemia-reperfusion-induced gastric mucosal injury[8,9], and also in gastric mucosal damage by ethanol[10], nonsteroidal anti-inflammatory drugs[11], by *H. pylori*[12], and by stress[13]. The main objective of the present study is to delineate the role of ROS in gastric ulceration caused by stress[14] and gastric hyperacidity caused by drugs, such as mercaptomethylimidazole, a potent inducer of acid secretion[15].

Acid secretion is stimulated by physiological secretagogues such as histamine, acetylcholine and gastrin, which act through the specific receptor on the parietal cell[16]. The stimulus–secretion coupling takes place through the involvement of a second messenger such as cAMP, Ca^{2+} or inositol trisphosphate which activates various protein kinases[17]. The message is transmitted to the terminal proton pumping H^+–K^+–ATPase[18] which actively transports H^+ in exchange with K^+. Prostaglandin (PG) acts as a natural inhibitor of acid secretion by modulating the activity of the adenyl cyclase and cAMP formation via the inhibitory GTP-binding (Gi) protein[19,20].

Various drugs can induce acid secretion, the mechanism of which is poorly understood. Nonsteroidal anti-inflammatory drugs such as aspirin, indomethacin and phenylbutazone are known to stimulate acid secretion by inhibiting the activity of the prostaglandin synthetase to synthesize prostaglandin[21]. We have observed that mercaptomethylimidazole (MMI), an antithyroid drug of thionamide group, used to control hyperthyroidism, is a potent inducer of gastric acid secretion[15], the mode of its action is being studied for the last few years.

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MMI-induced acid secretion was found to be partially sensitive to cimetidine, indicating activation of H_2-receptor by histamine release[15,22]. However, partial cimetidine sensitivity indicates a plausible involvement of an intracellular mechanism for the action of MMI. Evidence has been presented to show that MMI activates the parietal cell through generation of intracellular H_2O_2 by inhibiting the peroxidase and catalase activity of the parietal cell. H_2O_2 acts as an inhibitor of PG-synthetase and thus plays an important role in MMI-induced acid secretion by removing the inhibitory effect of prostaglandin.

Although the mechanism of acid secretion from the parietal cell and the regulation of acid secretion are fairly known[16–21], the mechanism of gastric ulceration caused by various factors such as stress, *H. pylori* infection, alcohol consumption, and use of steroidal and nonsteroidal anti-inflammatory drugs is not quite clear. Although *H. pylori* itself creates a stressful situation, the mechanism of *H. pylori*-induced gastric ulceration is far more complex[23,24].

Stress can arise from prolonged anxiety, tension and emotion, severe physical discomfort, haemorrhagic and surgical shock, burns and trauma, thereby resulting in severe gastric ulceration: the mechanism of gastric ulceration is poorly understood[25]. Recently, we had shown that restraint-cold stress causes severe haemorrhagic ulcer through derangement of the mucosal antioxidant enzymes, such as superoxide dismutase and peroxidase[14]. This is the stress condition arising mainly from the physiological discomfort and the mechanism of ulceration caused in this case should be different from ulcers caused due to other factors. Here we present direct evidence to show that stress generates highly reactive $^{\bullet}OH$ that causes oxidative damage of the gastric mucosa and that the radical is formed by metal-catalysed Haber–Weiss reaction between O_2^- and H_2O_2 following induction of the superoxide dismutase and oxidative damage of the gastric peroxidase.

Experimental procedures

For *in vivo* measurement of acid secretion in control, and drug-treated (i.p.) group[15,16], gastric fluid was collected from male rats by flushing out of the stomach cavity with 2 ml of 0.9% NaCl. The clear supernatant after centrifugation was titrated with 1 mM NaOH to pH 6.5. The result was expressed as the total amount of HCl (μmole) secreted in the gastric fluid. For measurement of acid secretion *in vitro* from a chambered gastric mucosal preparation[26], the mucosa was tied over one end of a plastic tube with mucosal surface facing out. It was immediately immersed in a small beaker containing 5 ml of unbuffered Hank's balanced salt solution (HBSS) that lacked phosphate and bicarbonates and acid secretion was monitored after adding the drug in the serosal solution (HBSS) bubbled with 100% O_2. Acid output was expressed as nmole of H^+ secreted. To prepare stress ulcer[14], the rats were immobilized in a supine position and were kept for 2 h at 4°C. This resulted in severe haemorrhagic ulcer which was scored as ulcer index as described before[14]. The mitochondrial Mn-SOD activity was measured by the xanthine–xanthine oxidase-cytochrome *c* reduction method[14]. The peroxidase activity of the mitochondrial fraction was assayed using iodide as an electron donor or by peroxidase catalysed $^{131}I^-$ organification into the protein of the isolated cell[26–28]. Parietal cell from rat gastric mucosa was isolated by controlled digestion with protease followed by Percoll density gradient centrifugation[26]. O_2 consumption in the isolated parietal cell was studied according to Berglindh[29]. In brief, 10^6 cells were added to the oxygraph (Gilson) flask containing HBSS with bovine serum albumin (BSA) and Ca^{2+} (1 mM) in a final volume of 2 ml. O_2 consumption was measured in presence or absence of drugs and is expressed as nmoles of O_2 consumed per minute per 10^6 cells. For *in vitro* acid secretion in isolated gastric gland, ^{14}C-aminopyrine uptake was measured as described[29]. Briefly, finely chopped gastric mucosa was digested at 37°C with 20 mg collagenase in a 50 ml incubation mixture containing 140 mM NaCl, 1.2 mM $MgSO_4$, 1 mM $CaCl_2$, 10 mM Hepes, 5.4 mM KOH, 100 μM cimetidine, 25 mg glucose and 100 mg BSA. After incubating for 40 min, the glands were allowed to settle, washed thrice with cimetidine-free incubation medium and finally suspended in the same medium before assaying the ^{14}C-aminopyrine uptake[29]. Assay of the prostaglandin synthetase activity and measurement of lipid peroxidation, thiol content and protein carbonyl content have been reported earlier[14,30]. Quantitation of the tissue level of $^{\bullet}OH$ was carried out according to the method of Babbs and Steiner[31]. In brief, $^{\bullet}OH$ formed in the fundic stomach was allowed to react with injected DMSO as a $^{\bullet}OH$ scavenger to form a stable product, methanesulfinic acid (MSA), which was extracted and allowed to form a complex with fast blue BB salt to yield a yellow reaction product that was measured spectrophotometrically at 425 nm using benzenesulfinic acid as the standard[31].

Results

Role of endogenous H_2O_2 on mercaptomethylimidazole-induced gastric acid secretion

Mercaptomethylimidazole stimulates gastric acid secretion with concomitant inhibition of gastric peroxidase activity: Mercaptomethylimidazole, *in vivo* induced gastric acid secretion dose dependently with concomitant inhibition of the gastric peroxidase (GPO) activity (Figure 1 *a*). MMI also dose dependently stimulated acid

secretion in isolated gastric mucosal preparation with concurrent inhibition of GPO activity (Figure 1 *b*). In both the cases, acid secretion and peroxidase inhibition were well correlated. Parietal cells are activated while secreting acid and this activation can be measured by

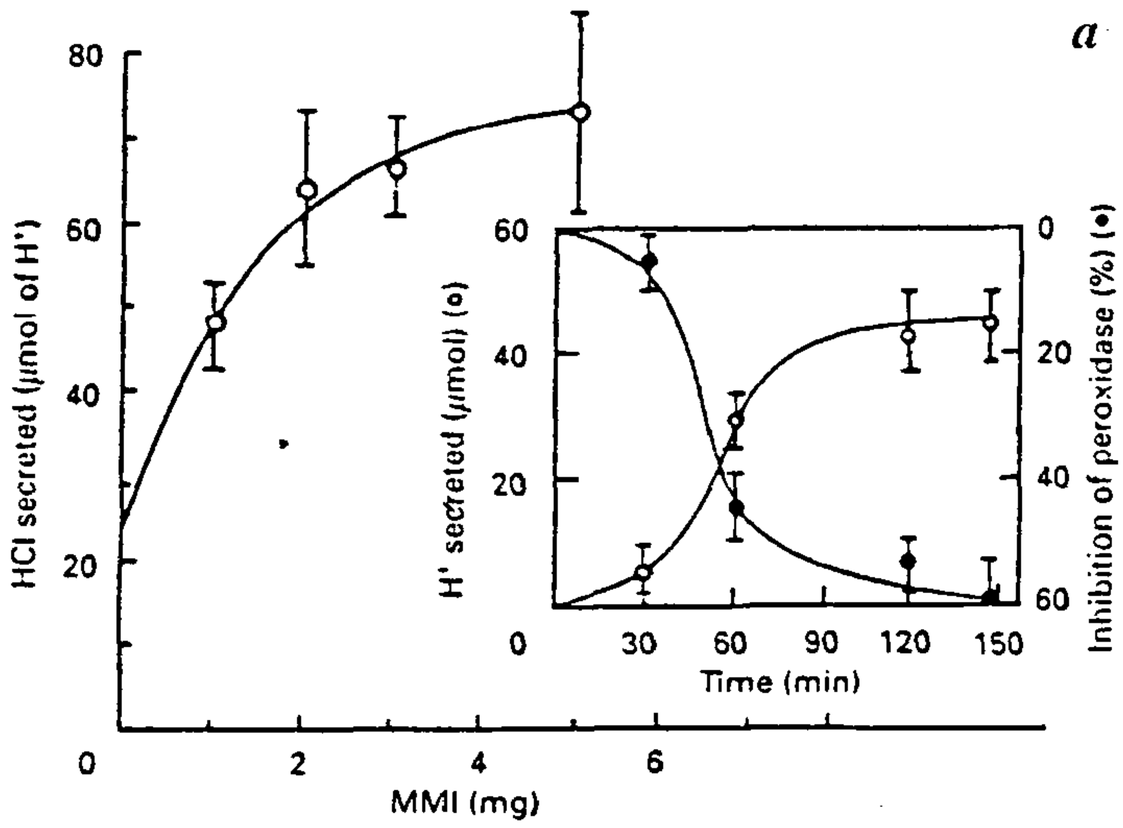


Figure 1. *a*, Effect of MMI *in vivo* on gastric acid secretion and peroxidase activity. Various doses of MMI were administered intraperitoneally to 100–150 g male rats fasted for 24 h. Gastric fluid collected after 2.5 h was assayed for HCl secreted. The inset shows the correlation of acid secretion with peroxidase activity against time after administration of 3 mg of MMI. The values for acid secretion were plotted after correction for basal secretion. Peroxidase activity was assayed in the mitochondrial fraction using iodide as an electron donor. $n = 8$. *b*, Effect of MMI *in vitro* on acid secretion and peroxidase activity of isolated gastric mucosa. Acid secretion was measured in the mucosal solution after addition of various concentrations of MMI in serosal solution. The values presented are the mean of two experiments. The inset represents the correlation between acid secretion and peroxidase activity against time after addition of 450 μM MMI in the serosal solution. Each value of acid secretion was corrected for basal acid output at the time indicated. $n = 6$.

O_2 consumption[28]. Table 1 shows that MMI directly activated the parietal cell for O_2 consumption which was prevented by proton-pump inhibitor, omeprazole, indicating that the activation was due to increased acid secretion through the proton pumping H^+–K^+–ATPase. However, insensitivity to cimetidine suggests that MMI effect was not mediated through its direct interaction with the H_2-receptor. The activation also correlated well with the concurrent inactivation of the parietal cell peroxidase (Figure 2). Table 2 further shows that the catalytically active peroxidase (in presence of H_2O_2) was irreversibly inactivated by increasing concentration of MMI. Catalase activity of the parietal cell, although low when compared to the liver (data not shown), was also lost in presence of MMI. The effect was irreversible as the activity could not be recovered on dilution or dialysis. Thus by inactivating the peroxidase–catalase system, MMI helps elevation of the intracellular H_2O_2.

Effect of H_2O_2 on gastric acid secretion: In order to investigate the plausible role of endogenous H_2O_2 on acid secretion, experiments were carried out *in vitro* on

Table 1. Effect of cimetidine and omeprazole on mercaptomethylimidazole-induced activation of the parietal cell

	Oxygen consumption (nmol/10^6 cell/min) Mean ± SEM
Control	9.2 ± 0.3
+ MMI	20.0 ± 1.6**
+ Omeprazole + MMI	10.2 ± 2.0**
+ Cimetidine + MMI	18.2 ± 1.3*

Parietal cell (10^6) was incubated in absence or presence of omeprazole or cimetidine (0.1 mM) in the oxygraph vessel for 20 min in 2 ml of Hank's balanced salt solution before the addition of 0.1 mM MMI. $n = 4$. *Not significant. **$P < 0.001$.

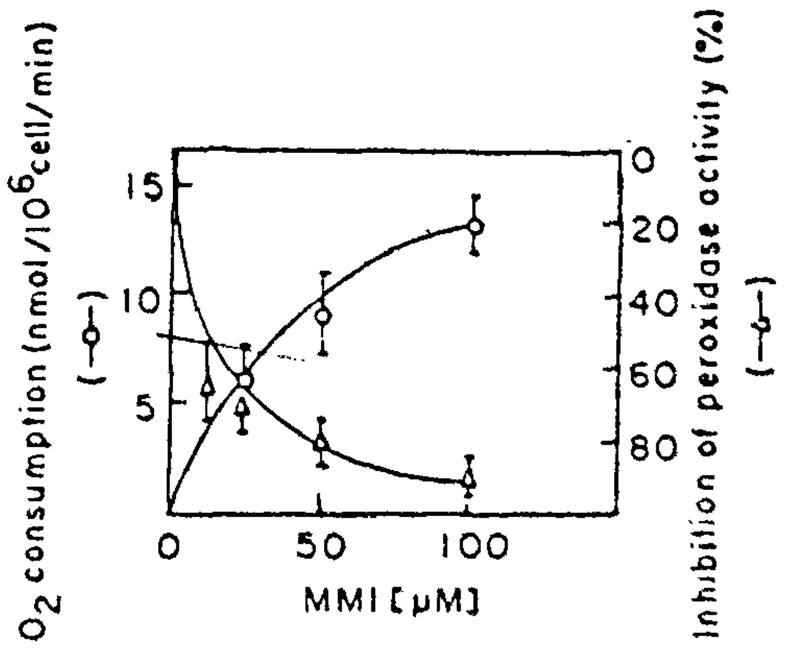


Figure 2. Effect of MMI *in vitro* on O_2 consumption and peroxidase activity as measured by radioiodide organification in proteins of isolated parietal cell. The correlation of O_2 consumption and peroxidase activity was done against varying concentrations of MMI 15 min after addition. The values for O_2 consumption were plotted after correction for basal value. $n = 3$.

the effect of exogenous H_2O_2 on acid secretion in isolated gastric mucosa and gastric gland preparation. H_2O_2 dose-dependently stimulated acid secretion in isolated mucosal preparation as shown in Figure 3 *a*. Addition of H_2O_2 from 5–20 µM stimulated acid secretion, showing a plateau at 40 µM. However, further increasing the H_2O_2 concentration to 60 µM caused inhibition of the stimulated secretion. H_2O_2-induced acid secretion was prevented when the mucosa was pretreated with omeprazole or when omeprazole was added when the mucosa secreted acid at a steady rate under the influence of H_2O_2 (Figure 3 *b*). The effect of H_2O_2 was further tested in isolated gastric gland preparation by monitoring ^{14}C-aminopyrine uptake which accumulates in the acid space and acts as a measure of acid secretion. Table 3 shows that both MMI and H_2O_2 significantly stimulated ^{14}C-aminopyrine accumulation in the parietal cell of the gastric gland preparation. Maximum stimulation with MMI occurred at 200 µM, and at 500 µM, the stimulation decreased to some extent. However, H_2O_2 up to concentration of 1 µM significantly stimulated acid secretion which was somewhat inhibited by 10 µM probably due to damage and loss of cellular integrity at higher concentration.

Effect of H_2O_2 on prostaglandin synthetase activity: As prostaglandin acts as a natural inhibitor of acid secretion, the effect of H_2O_2 was studied on the PG-synthetase activity of the microsomal preparation from the parietal cell. Figure 4 shows that cyclooxygenase activity of the PG-synthetase gradually decreased when preincubated with increasing concentration of H_2O_2, showing nearly 70% inactivation at 10 µM. Thus H_2O_2 is capable of modulating the acid secretion by limiting the biosynthesis of prostaglandin by PG-synthetase.

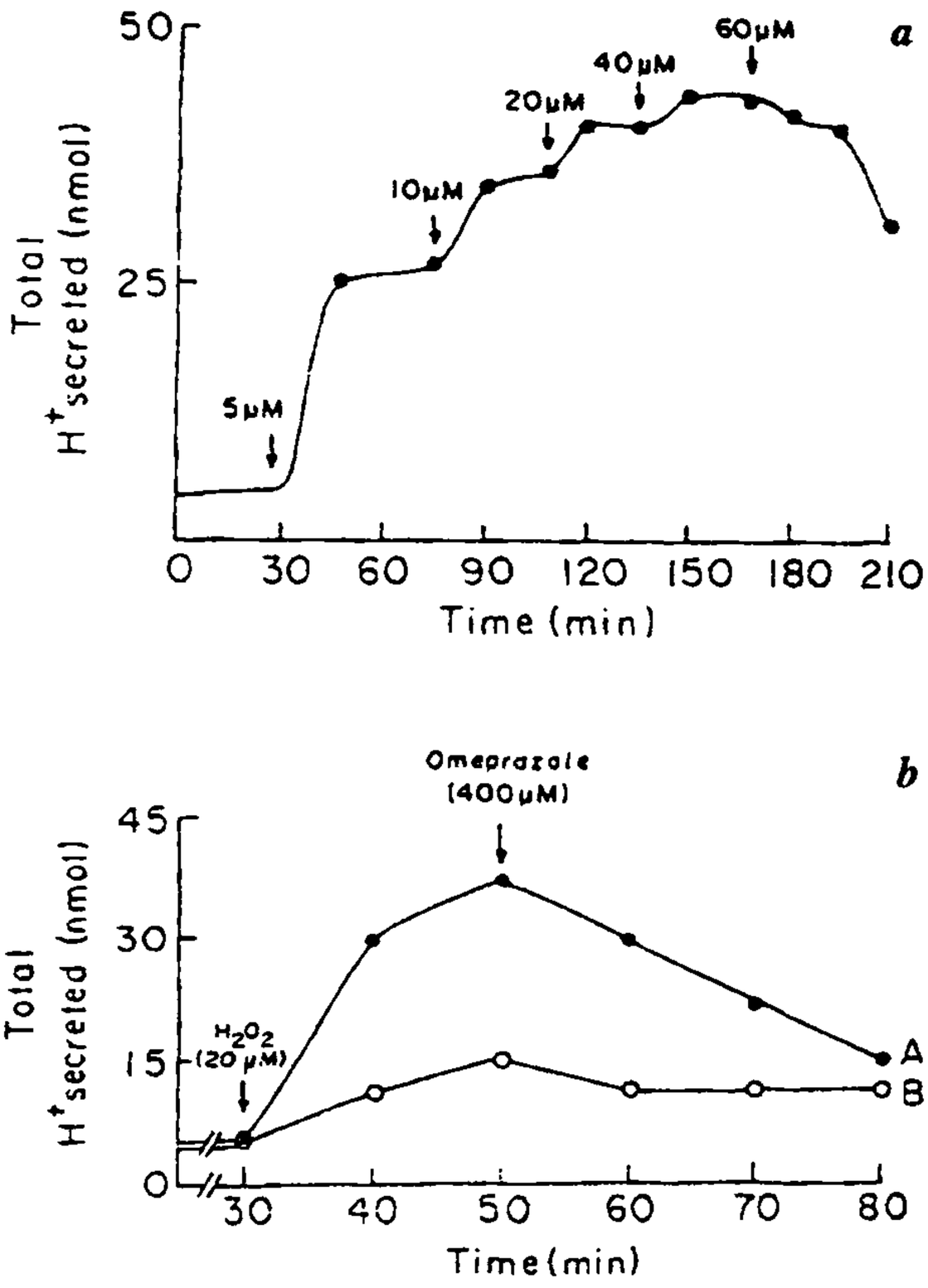


Figure 3. *a*, Dose-dependent effect of H_2O_2 on gastric acid secretion in isolated gastric mucosa. H^+ secretion in the isolated gastric mucosa was assayed after addition of H_2O_2 to the serosal solution as indicated by the arrows. Acid secretion in the mucosal solution was quantitated by titration. This is the result of a typical experiment and has been verified by two more experiments. *b*, Effect of omeprazole on H_2O_2 stimulated H^+ secretion in isolated gastric mucosa. H_2O_2 (20 µM) was added to the control and omeprazole (400 µM)-pretreated gastric mucosa and acid secretion was measured as described. (*a*) control; (*b*) omeprazole-pretreated. This is the result of a typical experiment and has been verified by two more experiments.

Table 2. Effect of MMI on peroxidase and catalase activity of the parietal cell

	Peroxidase activity units/mg	Catalase activity O_2 evolved/min/mg
Control	75 ± 8	170 ± 10
+ MMI 50 µM	36 ± 5*	150 ± 7
100 µM	15 ± 3***	120 ± 10*
200 µM	7 ± 2***	100 ± 8**
400 µM	—	60 ± 3**
600 µM	—	30 ± 3***

Peroxidase activity of an aliquot of cell-free homogenate was assayed using iodide as an electron donor after preincubation for 3 min with varying concentrations of MMI in presence of 100 µM H_2O_2 in 50 mM Tris-HCl buffer pH 8.0 in a final volume of 100 µl. For catalase assay, an aliquot of the cell-free homogenate was preincubated for 5 min with 100 µM H_2O_2 in presence of varying concentration of MMI in a final volume of 100 µl containing 50 mM sodium phosphate buffer pH 7.2. It was transferred to the oxygraph flask containing 2 ml of 50 mM phosphate buffer pH 7.2 and the reaction was started by adding 100 µM H_2O_2 to assay the O_2 evolution.
$n = 4$, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Table 3. Effect of MMI and H_2O_2 on ^{14}C-aminopyrine uptake in gastric gland preparation

	^{14}C-aminopyrine uptake CPM
Control	450 ± 42
+ MMI 50 µM	926 ± 78***
100 µM	1006 ± 85**
200 µM	1089 ± 100**
500 µM	781 ± 80**
+ H_2O_2 0.5 µM	1240 ± 20***
1.0 µM	1446 ± 40***
10.0 µM	800 ± 38***

Gastric gland was incubated for 30 min with varying concentrations of MMI or H_2O_2 in presence of 1 µCi ^{14}C-aminopyrine. The uptake was measured as described in ref. 28.
$n = 4$, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Role of reactive oxygen species in stress-induced gastric ulceration

Stress-induced oxidative damage and the status of antioxidant enzymes: To study the mechanism of mucosal damage by stress, ulcer was developed by restraint-cold stress in rat and three important parameters such as lipid peroxidation, protein carbonyl content and mucosal glutathione (antioxidant) level were evaluated as established indicators of oxidative damage of tissue caused by ROS. Stress caused significant increase in lipid peroxidation and protein carbonyl content with significant decrease in the mucosal glutathione level (Table 4), indicating that lesions were due to oxidative damage by stress. Since ROS develops due to defective oxygen metabolism as a result of the alteration of some antioxidant enzymes, the effect of stress was studied on the activity of superoxide dismutase and peroxidase, the two important antioxidant enzymes of the mucosa. The severity of ulceration as measured by ulcer index significantly increased with the increase of stress from 30 min to 120 min and this was associated with concurrent stimulation of the superoxide dismutase and inhibition of the peroxidase activity (Figure 5). Increased superoxide dismutase activity could be completely prevented by prior administration of α-amanitin, a specific inhibitor of RNA polymerase (Table 5) indicating that stress induces superoxide dismutase by increased transcriptional synthesis. Pretreatment with antioxidants such as glutathione or vitamine E prevented the inactivation of the gastric peroxidase (Table 5), suggesting stress-induced oxidative damage of the enzyme. Stress ulcer was also significantly blocked by these antioxidants, indicating the role of ROS in ulcer generation. Inactivation of peroxidase and stress ulcer was also prevented by desferrioxamine, a nontoxic metal ion chealator, indicating that the oxidative damage of the enzyme and the mucosa needs the presence of some divalent transition metal. Dimethylsulfoxide, an established $^{\bullet}OH$ scavenger and α-phenyltert-butylnitrone, a

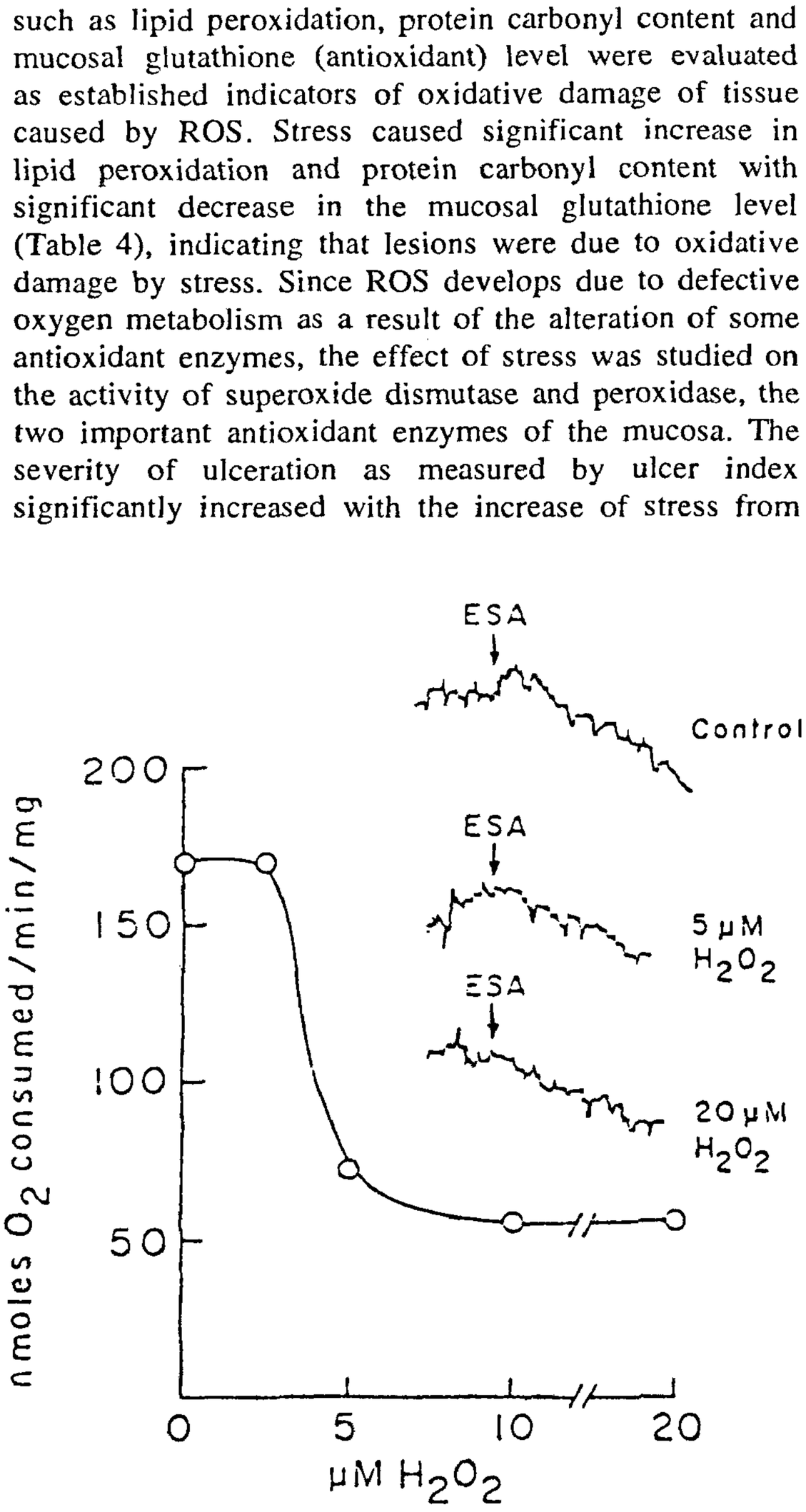


Figure 4. Effect of varying concentrations of H_2O_2 on the cyclooxygenase activity of the prostaglandin synthetase of the parietal cell. The microsomal fraction of the isolated parietal cell was preincubated with H_2O_2 for 5 min before monitoring the cyclooxygenase activity by O_2 consumption in the oxygraph. The assay system contained in a final volume of 2 ml 0.2 M Tris-HCl buffer pH 8, 0.5 mM epinephrine and a suitable amount of the microsomal fraction as the enzyme. The reaction was started by the addition of 75 μg eicosatrienoic acid (ESA). The inset shows the typical oxygraphic record of O_2 consumption in absence and presence of H_2O_2.

Table 4. Effect of stress on lipid peroxidation, protein carbonyl content and glutathione level of the gastric mucosa

	Lipid peroxidation malondialdehyde content (nmol/mg)	Protein carbonyl content (nmol/mg)	Glutathione content (nmol/mg)
Control	0.43 ± 0.06	1.6 ± 0.22	60 ± 3.5
+ Stress	0.65 ± 0.02***	2.7 ± 0.45*	38 ± 2.0**

Lipid peroxidation product in the mitochondrial membrane fraction of gastric homogenate was assayed as thiobarbituric acid reactive species[14]. Protein carbonyl content was determined according to Levine *et al.*[44]. Reduced glutathione content was determined by its reaction with DTNB[45].
n = 8–10, *P < 0.05, **P < 0.001, ***P < 0.0001.

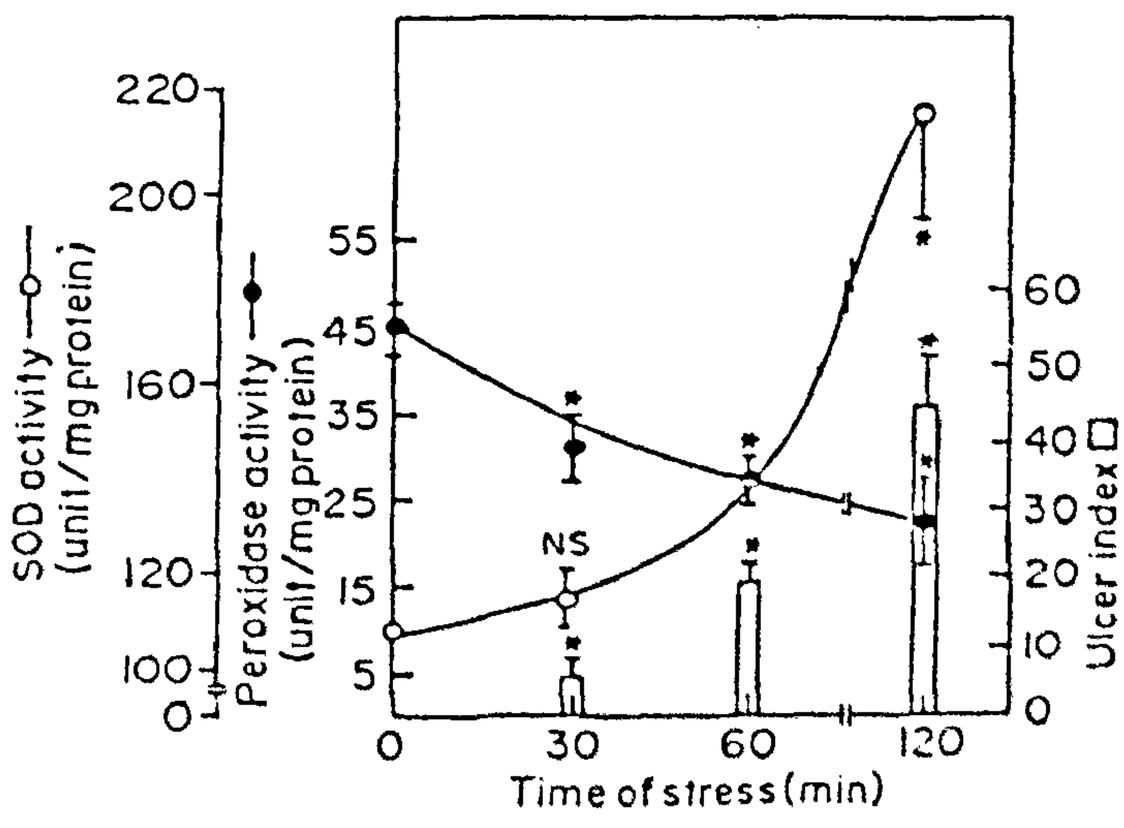


Figure 5. Effect of stress on the antioxidant enzymes and the ulcer index. SOD (Mn-SOD) activity was measured in the mitochondrial fraction by cytochrome c reduction in xanthine–xanthine oxidase system. Peroxidase activity was measured in the same fraction using iodide as an electron donor. The control SOD activity was 108 ± 10 units/mg protein. n = 8–14. *P < 0.001.

Table 5. Effect of various compounds on superoxide dismutase, peroxidase and ulcer index

	SOD activity (units/ml)	GPO activity (units/ml)	Ulcer index
Control	108 ± 10	45 ± 2.7	0
+ stress	213 ± 15*	25 ± 2.3*	45 ± 5.0*
+ α-amanitin + stress	83.1 ± 15*		
+ glutathione + stress		42.7 ± 2.8*	21 ± 3.5*
+ vitamin E + stress		39 ± 2.8*	5 ± 1.6*
+ desferrioxamine + stress		46.7 ± 5.1*	6.0 ± 1.0*
+ dimethylsulfoxide + stress			9.0 ± 3.0*
+ α-phenyl tert-butyl-nitrone + stress			10 ± 2.0*

SOD activity in the mitochondrial fraction was measured by cytochrome *c* reduction in xanthine–xanthine oxidase system and peroxidase activity was measured in the same fraction by using iodide as an electron donor. The severity of ulcer was expressed as ulcer index after two hours of restraint-cold stress. α-amanitin was injected (i.p.) at the dose of 25 µg/100 g body weight 30 min before the onset of stress. Reduced glutathione was injected twice (i.p.) at the dose of 15 mg/100 g body weight 20 h and 1 h before the onset of stress. Desferrioxamine was injected (i.p.) at the dose of 80 mg/kg, dimethylsulfoxide 0.4 ml of 25% in normal saline/100 g body weight and α-phenyl tert-butyl nitrone dissolved in normal saline containing 15% ethanol was injected (i.p.) at the dose of 20 mg/100 g body weight 30 min before the onset of stress.
$n = 8–20$, $^*P < 0.01$.

well-known spin (radical) trap significantly blocked stress ulcer, suggesting involvement of $^{\bullet}OH$ in oxidative damage of the mucosa. This is confirmed by direct measurement of the tissue level of $^{\bullet}OH$ which was elevated by more than six-fold over the control value at 2 h of stress (Figure 6). The increased generation of $^{\bullet}OH$ correlated well with the increase in the ulcer index with the progress of stress. This indicates that $^{\bullet}OH$ is the major causative factor of stress ulcer.

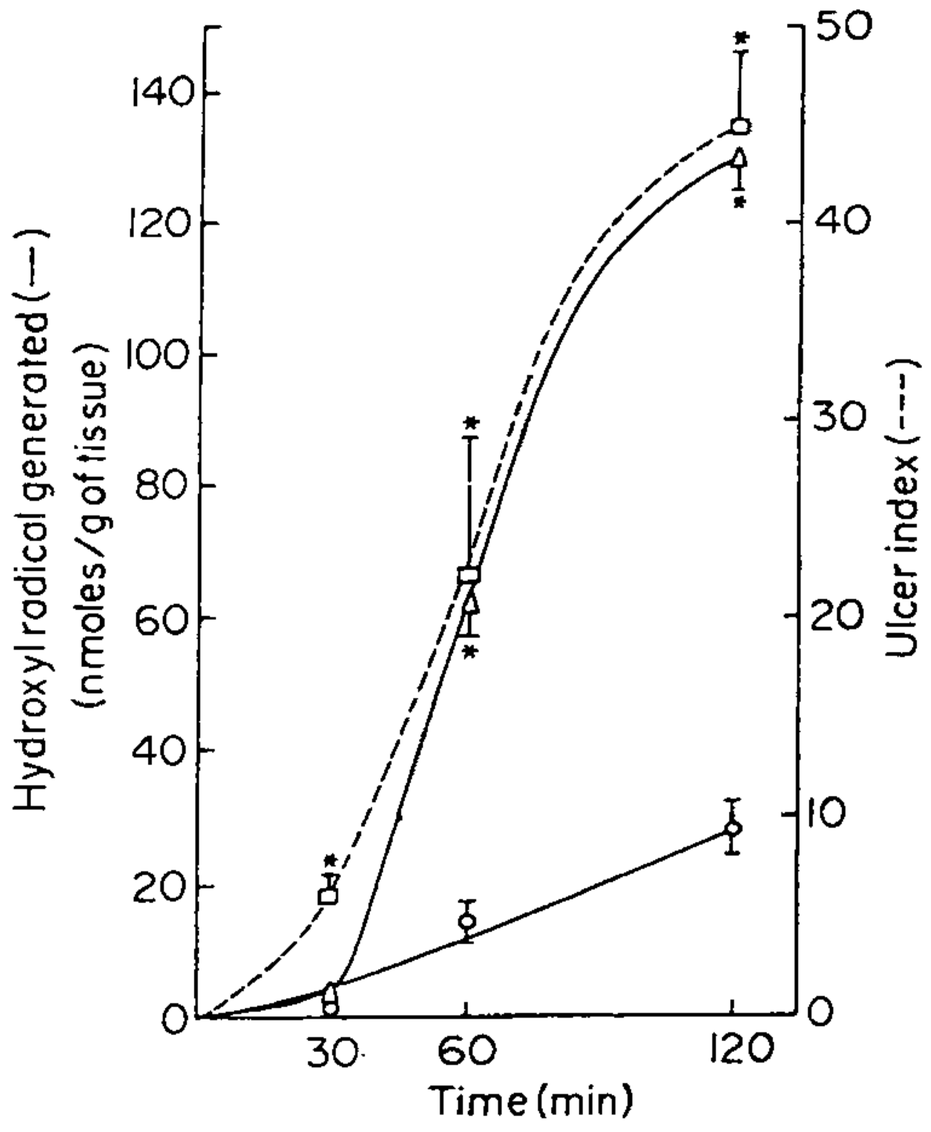


Figure 6. Direct measurement of hydroxyl radical at different times of stress. Hydroxyl radical with (Δ) and without (o) stress was measured as described in the text. $n = 9–12$. $^*P < 0.001$.

Discussion

Many drugs are now known to cause gastric hyperacidity and ulceration through increased acid secretion and decreasing the natural gastroprotection against some endogenous or exogenous damaging factors. Nonsteroidal anti-inflammatory drugs act through an intracellular mechanism causing inhibition of prostaglandin synthetase which controls the acid secretion through generation of prostaglandin acting through the Gi protein[19,20]. Mercapto-methylimidazole, an antithyroid drug, however induces acid secretion both through H_2-receptor activation[15] and through an intracellular mechanism. Evidence shows that MMI-induced acid secretion correlates well with the concurrent decrease in the intracellular peroxidase activity which normally scavenges endogenous H_2O_2 (ref. 28). As gastric peroxidase is mainly located in the parietal cell[26], its possible involvement in regulating acid secretion was investigated. Our results show that MMI stimulates O_2 consumption in the isolated parietal cell, sensitive to omeprazole, which correlates well with the inhibition of its peroxidase activity. However, MMI-induced O_2 consumption is insensitive to cimetidine, indicating that MMI is not working through the H_2 receptor. However, cimetidine-sensitive acid secretion *in vivo* is due to increased histamine release from the adjacent mast cell either directly by MMI or indirectly through degranulating action on mast cell by H_2O_2 likely to be generated by peroxidase inactivation[22]. The mechanism of peroxidase inactivation by MMI has been extensively studied using purified gastric peroxidase where MMI irreversibly inactivates the enzyme by acting as a suicidal substrate[32]. Further studies showed that MMI-thiyl radical, a peroxidase-catalysed one-electron oxidation product of

MMI interacts at the heme part of the enzyme to cause irreversible inactivation[33]. Although parietal cell contains low catalase activity when compared to liver, it is also irreversibly inactivated by MMI. Thus MMI creates a favourable condition for intracellular accumulation of H_2O_2 by inactivating both peroxidase and catalase. It is interesting to note that exogenous H_2O_2 stimulates acid secretion in isolated gastric mucosa as well as in gastric gland preparations. How H_2O_2 triggers acid secretion is not clear yet. Our results show that H_2O_2 inhibits PG-synthetase activity of the parietal cell and can thus limit the biosynthesis of prostaglandin, an endogenous inhibitor of acid secretion[19,20]. The inhibitory influence of PG on the adenylate cyclase system is thus withdrawn, leading to increased acid secretion. We thus propose that endogenous H_2O_2 plays an important role in MMI-induced acid secretion, as shown in Figure 7. However, the possible role of endogenous H_2O_2 in intracellular Ca^{2+} mobilization[34] or activation of protein kinase C (ref. 35) to stimulate signal transduction for acid secretion[17] cannot be excluded. Evidence is accumulating that intracellular H_2O_2 regulates various cellular functions by acting as a signal transduction messenger or by affecting some steps of the signal transduction cascade[35]. It is likely that MMI changes the redox status of the parietal cell by elevation of the intracellular H_2O_2 which acts as a stimulus for increased acid secretion. It is tempting to speculate that many drugs causing hyperacidity may be oxidized by the peroxidase to a free radical which either kills the enzyme and generates H_2O_2 or reduces O_2 to generate O_2^-. The latter may be dismutated by SOD to H_2O_2 which can stimulate acid secretion by activating the signal transduction mechanism.

The role of ROS in ulcer generation by various factors has recently attracted the attention of many investigators. Our results indicate that the severity of stress ulcer increases with the concurrent induction of SOD and inactivation of the mucosal peroxidase. The derangement of these antioxidant enzymes creates a favourable condition for the accumulation of endogenous H_2O_2. Stress causes ischaemic condition in the gastric mucosa by reducing the blood flow following activation of parasympathetic and sympathetic nervous system, resulting in the constriction of the smooth muscles of the blood vessels and gastric tissue. This causes generation of O_2^- which is dismutated by SOD to form H_2O_2. Increased SOD activity by stress might be explained as due to adaptive response through increased transcription of the enzyme by O_2^- generated during ischaemic stress when mitochondrial electron transport chain remains in the relatively reduced state favouring the leakage of electron for partial reduction of O_2 to O_2^- (ref. 36). Stress-induced inactivation of peroxidase is due to oxidative damage of the protein by some ROS, as it is prevented by prior administration of antioxidants. This has been confirmed by our recent studies that purified gastric peroxidase when incubated with ROS generating system is inactivated due to site-specific generation of $^{\bullet}OH$ at a copper-binding site near heme[37]. Stress ischaemia releases transition metal ions from the metalloproteins[38]. The free metal ions and H_2O_2 developed due to peroxidase inactivation diffuse to the membrane or to the protein, and site-specifically generate $^{\bullet}OH$ by Haber–Weiss reaction[4] to cause lipid peroxidation or protein oxidation. Lipid peroxidation leads to loss of membrane fluidity, ion transport and membrane integrity of the surface epithelial cell and helps to generate gastric lesions. Protein oxidation leads to derangement of cellular metabolic functions. The radical may also cause DNA damage to block cell differentiation and restitution or may oxidatively damage the matrix protein to prevent cell adherence, which may be responsible for the loss of cell renewal required for healing the ulcer. Increased ROS also depletes cellular antioxidants (glutathione, ascorbate, α-tocopherol, etc.) and thus the cell can no longer prevent oxidative damage and shows pathogenesis. Stress also inactivates the mucosal PG synthetase by accumulated H_2O_2 and inhibits the synthesis of prosta-

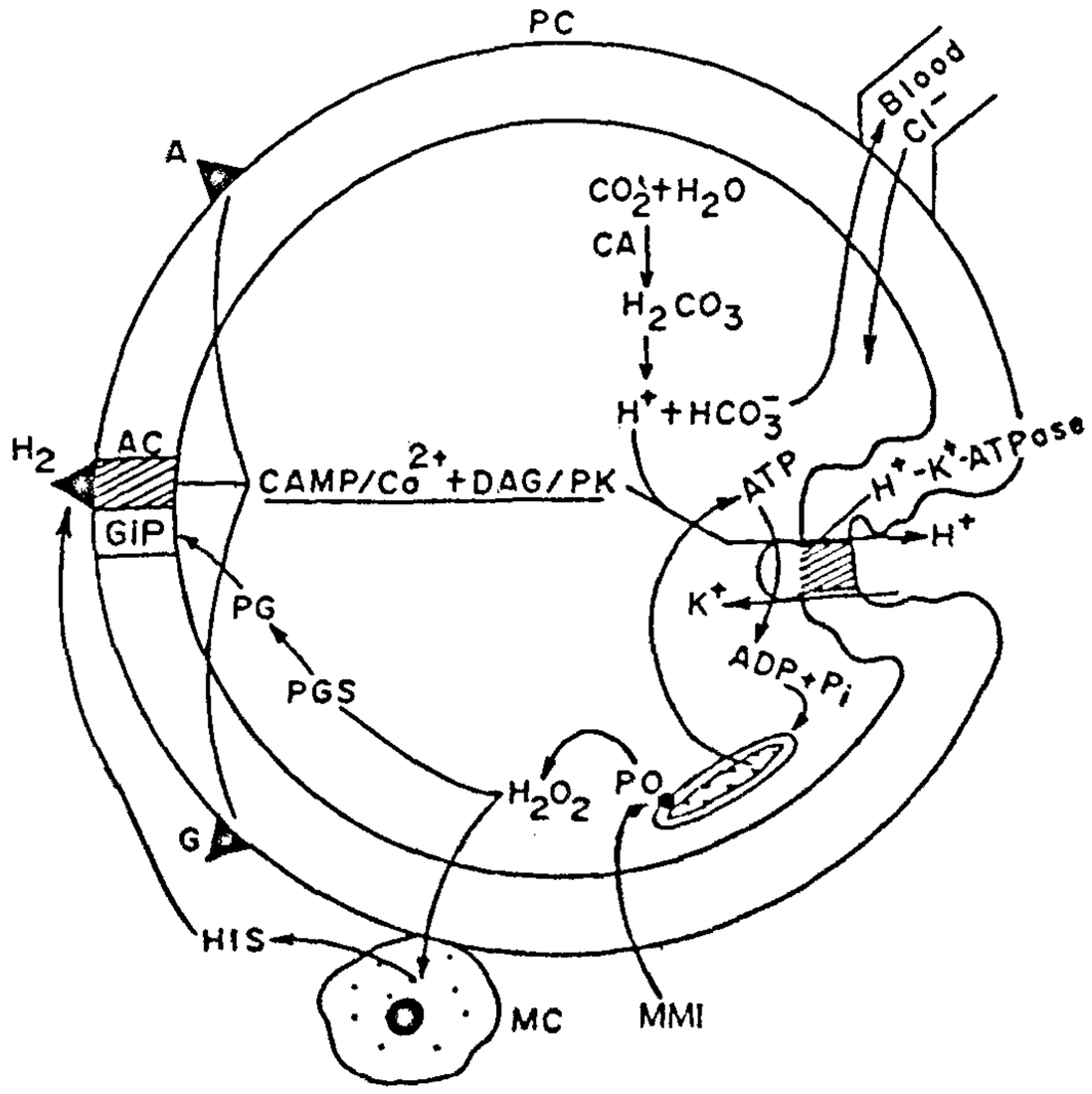
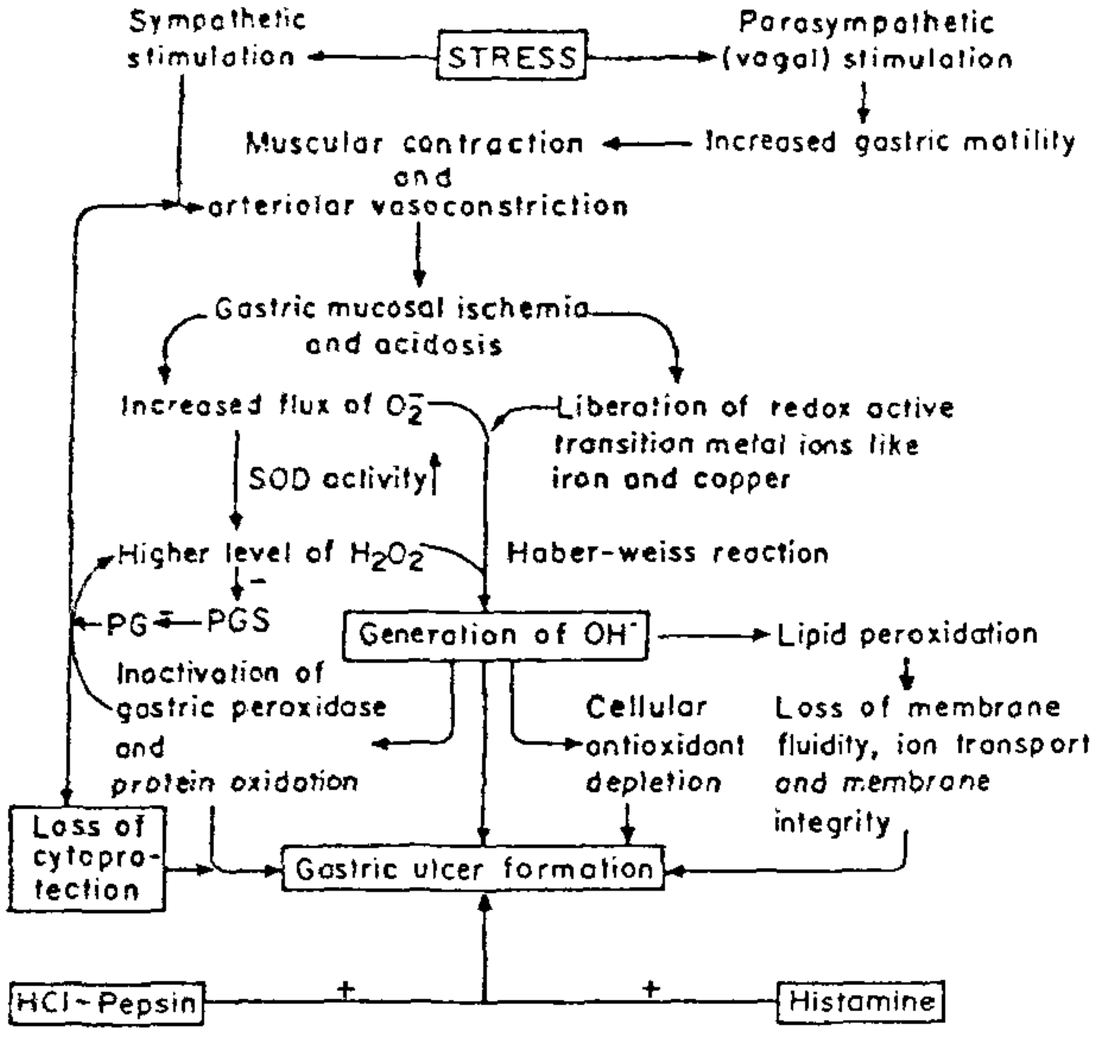


Figure 7. Plausible role of endogenous H_2O_2 in MMI-induced acid secretion by parietal cell. PC, parietal cell; MC, mast cell; His, histamine; G, gastrin receptor; H_2, histamine receptor; A, acetylcholine; AC, adenyl cyclase; GiP, G-inhibitory protein; DAG, diacylglycerol; PK, protein kinase; PO, peroxidase; PGS, prostaglandin synthetase; PG, prostaglandin; and CA, carbonic anhydrase. MMI inactivates the peroxidase and elevates the intracellular H_2O_2, which, by stimulating histamine release from the mast cell and inhibiting the PGS activity, activates the parietal cell for acid secretion by modulating the H_2-receptor-adenyl cyclase-Gip system. However, MMI may also have a direct activating effect on the parietal cell.



Scheme I

Scheme 1. Critical role of reactive oxygen species in stress-induced gastric ulceration.

glandin which offers gastroprotection by various mechanisms[39]. Thus loss of gastroprotection by stress favours ulcer generation by ROS, while acidity and increased pepsin and histamine release[14] aggravate the situation. Although the role of ROS in the generation of various pathological conditions has been well documented[3,4], we are the first to show by direct measurement that stress-induced gastric ulcer is mainly caused by the generation of $^{\bullet}OH$. We thus propose that ROS plays a critical role in stress-induced gastric ulceration as depicted in Scheme 1. It is initiated by stress-induced activation of sympathetic and parasympathetic nervous system causing vascular and smooth muscle constriction of the stomach, leading to ischaemia due to reduced blood supply. Ischaemic stomach generates ROS specially $^{\bullet}OH$ for oxidative damage of the mucosa. A view is emerging that oxidative stress leads to increased intracellular level of H_2O_2 which acts like a second messenger and induces various stress proteins[35]. Studies are now directed to investigate whether stress ulcer is caused by the induction of some stress proteins by increased intracellular H_2O_2.

Apart from stress, *H. pylori* infection is also a major causative factor for gastro-duodenal lesions[23,24]. Although bacterial infection is considered as a stress condition, the mechanism of ulceration by *H. pylori* is more complex[23,24]. Production of ammonia, protease, phospholipase, ROS and lipid peroxides has been implicated in mucosal damage in *H. pylori* infection[23]. *H. pylori* also elaborates Vac A and Cag A cytotoxins for ulcer generation[40,41]. *H. pylori* itself attracts neutrophils which on activation produces ROS to cause oxidative damage. *H. pylori* elaborates a neutrophil-activating protein expressed by *napA* gene and other soluble chemotactic proteins for the generation of ROS[42,43]. In stress condition, ulcer is developed mainly due to oxidative damage by $^{\bullet}OH$ generated from derangement of the antioxidant enzymes. Although ROS plays a role in *H. pylori*-induced oxidative damage, it is mainly contributed by the invading neutrophils. However, it is worth investigating whether *H. pylori*-elaborated toxic products[23,24] can also cause derangement of the antioxidant systems of the gastric mucosa to cause oxidative damage by ROS.

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Shift from a Th1-type response to Th2-type in dengue haemorrhagic fever

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Dengue virus causes a mild febrile illness, dengue fever (DF) and at times a severe illness, dengue haemorrhagic fever (DHF), the pathogenesis of which is not fully known. The present study was undertaken to investigate the profile of Th1- and Th2-type cytokines in the sera of 117 patients of various grades of dengue illness and 21 normal healthy controls. Commercial sandwich ELISA kits were used to assay the serum levels of tumour necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), interleukin (IL)-2, IL-4, IL-6, and IL-10. Serum levels of IFN-γ and IL-2 were the highest in DF while in the most severe cases of DHF (i.e. grade IV) serum levels of IL-6 and IL-10 were maximum. Levels of IL-10 were negligible in patients with DF and levels of IFN-γ were lowest in patients with DHF grade IV. The levels of TNF-α were higher in cases of DHF grades II, III, and IV and did not show the clear association pattern shown by IFN-γ, IL-2, IL-4, IL-6, and IL-10. The levels of IFN-γ, IL-6 and TNF-α increased first while IL-4 and IL-10 levels increased during the 4th to 8th day of the illness. The most significant finding of the present study was a shift of the predominant Th1-type response observed in 66% of DF patients to the Th2-type response seen in the 71% of DHF grade IV patients, thus indicating a possible role for Th2 cells in the pathogenesis of DHF.

DENGUE virus, prevalent in over 100 tropical and sub-tropical countries with about two billion people at risk[1], produces a mild self-limiting acute febrile illness, dengue fever (DF), and a life threatening severe illness, dengue haemorrhagic fever (DHF). DHF has emerged as the most important arbovirus disease in man in the last two decades. It has been estimated that about 100 million cases of DF occur every year with about 250,000 cases of DHF[2]. The frequency of dengue epidemics has markedly increased with hyperendemic transmission and expansion to newer geographical areas. In a number of dengue endemic countries such as Bangladesh, Sri Lanka, and India where DHF was previously unknown, severe epidemics of DHF have occurred[1], (U. C. Chaturvedi, unpublished).

DHF has been classified into four grades on the basis of the clinical presentation and laboratory findings; the mildest is grade I and the most severe is grade IV[3].

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