

Eradication of *Helicobacter pylori* Restores Glutathione S-Transferase Activity and Glutathione Levels in Antral Mucosa

Arnoud H. A. M. van Oijen, Marie-Louise Verhulst, Hennie M. J. Roelofs, Wilbert H. M. Peters,¹ Wink A. de Boer and Jan B. M. J. Jansen

Departments of Gastroenterology, University Medical Center, PO Box 9101, 6500 HB Nijmegen, The Netherlands

Glutathione S-transferases (GST) and glutathione peroxidases (GPO) are important in detoxification. GST activity in the mucosa of the gastrointestinal tract is inversely correlated with the development of gastrointestinal cancer. *Helicobacter pylori* (*H. pylori*) infection has been associated with gastric cancer. We studied GST activity and the substrate glutathione (GSH) in patients with *H. pylori*-associated gastritis. GST activity and isoenzyme levels, GPO activity and GSH levels were studied in antral biopsies of 38 *H. pylori*-positive patients, before and after eradication treatment. In 31 patients in whom *H. pylori* was successfully eradicated, antral GST enzyme activity before therapy was 532 (465–598) nmol/mg protein·min (mean and 95% confidence interval) and that after therapy was 759 (682–836) nmol/mg protein·min ($P<0.0001$). Correspondingly, levels of GST α and GST-P1 were higher after eradication ($P<0.001$). GSH concentration significantly increased: 21.2 (16.2–26.2) nmol/mg protein before and 27.1 (23.6–30.6) nmol/mg protein after therapy ($P<0.05$). In 7 patients in whom *H. pylori* was not eradicated, GST activity was 671 (520–823) nmol/mg protein·min and 599 (348–850) nmol/mg protein before and after treatment respectively ($P=0.32$). GSH levels were 17.4 (9.0–25.7) nmol/mg protein and 18.2 (9.1–27.3) nmol/mg protein, respectively ($P=0.84$). No differences in antral GPO enzyme activity, both of selenium (Se)-dependent and total GPO, before and after successful treatment were found. Eradication of *H. pylori* infection increases GST activity and GSH levels in antral mucosa. Low GST activity and GSH concentration due to *H. pylori* infection might play a role in gastric carcinogenesis.

Key words: Glutathione — *Helicobacter pylori* — Antral mucosa — Glutathione S-transferase — Glutathione peroxidase

Although our knowledge about the role of infection with *Helicobacter pylori* in the pathogenesis of peptic ulceration and gastric carcinoma and lymphoma is increasing, the mechanisms responsible are only partially understood. An important role in the pathogenesis seems to be played by reactive oxygen species (free radicals produced by the reduction of oxygen to water).^{1–5} Production of these species by neutrophils is greatly increased in *H. pylori* infection.^{6,7} The lifespan of these free radicals *in vivo* is very short, due to their high reactivity, which has a detrimental effect on biological molecules and structures, including cellular membranes and DNA.⁸ Free radicals have also been linked to activation of oncogenes and deactivation of tumor suppressor genes.^{9,10}

The human body utilizes several lines of defense. These defenses include non-enzymatic compounds such as vitamins A, E and C, and enzymatic pathways such as superoxide dismutase, glutathione peroxidase (GPO) and glutathione S-transferase (GST), the latter two enzymes using glutathione (GSH) as a cofactor. GSH is a tripeptide (gluta-

mine-cysteine-glycine) and is the most important source of intracellular thiol molecules.¹¹ It is a very effective radical scavenger, readily reacting with reactive oxygen species and free radicals, and rendering them less toxic. Thus, GSH is involved in both enzymatic and non-enzymatic reactions to counter oxidative stress and many other toxic or carcinogenic compounds.

Two types of GPO enzymes exist, non-seleno- and selenoproteins, but both use GSH as a cofactor. The non-selenium (Se)-dependent GPO enzyme activity occurring in the gastrointestinal tract originates mainly from GST enzymes. The family of human GST enzymes includes four main classes; α , π , μ and θ , each consisting of one or more isoenzymes with different substrate specificity. These enzymes utilize GSH to detoxify reactive oxygen species, but also to detoxify various other compounds with known mutagenic and carcinogenic properties, including polycyclic aromatic hydrocarbons and N-nitroso compounds.^{11,12} In the past, we have shown that low activity of GST enzymes in the mucosa of the human digestive tract correlates highly with cancer incidence, possibly reflecting decreased capacity of the involved mucosa to detoxify toxins and carcinogens.¹³

¹ To whom correspondence should be addressed.
E-mail: w.peters@gastro.azn.nl

Partial deficiency of GST isoenzymes has been linked to the development of various malignancies, including gastric and colorectal carcinoma.^{14–20} In lymphocytes deficient in GST M1, increased cytogenetic damage has been shown.^{21, 22} In a former study we demonstrated that *H. pylori* infection was associated with a low GSH content and GST activity in the antral mucosa.²³ In this controlled study we have investigated the effect of *H. pylori* eradication on gastric mucosal GSH concentration, GST and GPO enzyme activity, as well as GST isoenzyme levels.

PATIENTS AND METHODS

Patients Thirty-eight *Helicobacter*-positive patients [15 men, 23 women, mean age 44 (range 22–75) years] with non-ulcer dyspepsia were studied. At gastroscopy 4 biopsies each were taken from antrum and corpus, for histological and bacteriological examination to assess *H. pylori* status. For GSH, GPO and GST determination 3 additional biopsies were taken from the antral mucosa, within 2 cm of the pyloric ring. Patients who had used proton pump inhibitors, H₂ receptor antagonists, antibiotics, NSAID's or bismuth preparations within 4 weeks prior to endoscopic examination were excluded from the study.

After confirmation of *H. pylori* infection, all patients were treated with a proton pump inhibitor (either omeprazole 20 mg bid or lansoprazole 30 mg bid) and antibiotics (amoxicillin 1 g bid and metronidazole 500 mg bid) for a period of 2 weeks. Thereafter, treatment was stopped and patients did not receive any other medication until endoscopic re-evaluation, 6 weeks after the end of treatment. Biopsies were taken according to the aforementioned method. Treatment was considered to be successful if both bacteriological and histological assessment did not show the presence of *H. pylori*.

Written informed consent was obtained from all patients prior to inclusion. The study was approved by the local ethical committees.

GSH, GST and GPO determinations Biopsies from antral mucosa for GSH and GST/GPO determination were immediately frozen in liquid nitrogen and stored at –80°C until further analysis. For preparation of cytosolic fractions, biopsies were quickly thawed in cold running water and homogenized in 0.25 M saccharose, 20 mM Tris/HCl and 1 mM dithiothreitol pH 7.4 using disposable polypropylene pestles and micro tubes (Kontes, van Oortmerssen, Rijswijk, The Netherlands). The homogenate was centrifuged at 150 000g at 4°C for 1 h. Aliquots of post centrifugation supernatant cytosolic fraction were frozen in liquid nitrogen and stored at –80°C until use. For determination of GSH content, cytosol was immediately diluted (1/10) with a solution of 10% trichloroacetic acid. GST enzyme and isoenzyme activity were determined as described before.¹³ Se-dependent GPO activity with hydro-

gen peroxide as a substrate and non Se-dependent activity with *t*-butyl hydroperoxide as a substrate were measured essentially as described by Howie *et al.*,²⁴ as follows: reduction in absorption at 340 nm (37°C) was recorded on a Lambda 12 spectrophotometer (Perkin Elmer, Langen, Germany) at intervals of 1 min for a total analysis time of 5 min. The assay solution contained 60 mM Tris/HCl buffer pH 7.6, 0.12 mM EDTA, 1 mM sodium azide, 0.33 mM NADPH, 1.3 mM reduced GSH and 1.3 U GSH reductase. The reaction was started with the addition of substrate and the final concentrations of the substrates were 1.2 mM for *t*-butyl hydroperoxide and 0.6 mM for hydrogen peroxide. All measurements were performed in triplicate. GSH content was assayed by high-performance liquid chromatography after reaction with monobromobimane as described in detail before.²⁵

Statistical analysis All results are given as means (±95% confidence interval) unless otherwise specified. Graphical data are displayed as Box-Whisker plots, showing median, 25th and 75th percentile and range. Statistical analysis was performed using the *t* test for paired samples after log transformation of the data. Differences with a *P* value <0.05 were considered to be statistically significant in all analyses.

RESULTS

Of the 38 patients included in the study, in 31 patients *H. pylori* was eradicated (group A) and 7 patients remained *H. pylori*-positive (group B).

GST Before treatment there was no significant difference in GST activity between the two patient groups A and B. In group A antral GST activity was 532 (466–599) nmol/

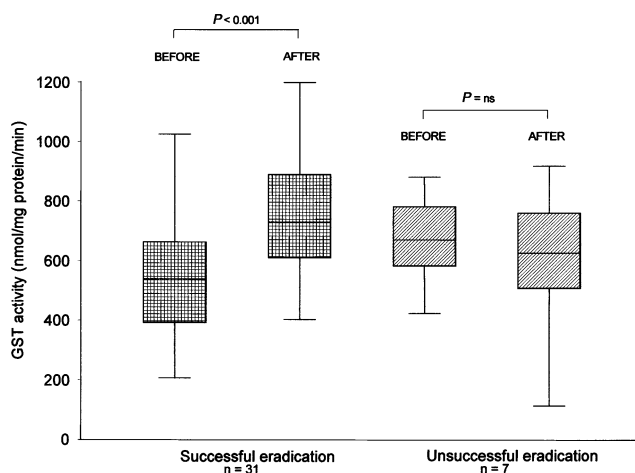


Fig. 1. Glutathione S-transferase activity in antral mucosa before and after *H. pylori* eradication treatment. Box-Whisker plots showing range, 25th and 75th percentile and median.

mg protein·min before and 759 (682–836) after eradication ($P<0.001$). For group B these values were 671 (520–823) nmol/mg protein and 599 (348–850) nmol/mg protein·min, respectively ($P=0.32$). Data are depicted in Fig. 1.

GST isoenzymes GST isoenzyme analyses were performed in 22 patients of group A, for whom sufficient mucosal material was available after initial determination of GSH and GST. GST α isoenzymes were undetectable in one patient and GST μ isoenzymes were undetectable in thirteen patients. GST α isoenzyme levels increased from 2.1 (1.4–2.9) $\mu\text{g}/\text{mg}$ protein before to 3.80 (2.7–4.9) $\mu\text{g}/\text{mg}$ after eradication treatment ($P<0.001$). The data are depicted in Fig. 2. GST π isoenzyme levels increased from 7.0 (5.8–8.1) $\mu\text{g}/\text{mg}$ protein to 8.4 (7.6–9.2) $\mu\text{g}/\text{mg}$ protein ($P<0.001$). GST μ concentration was 2.6 (1.2–3.9) $\mu\text{g}/\text{mg}$ protein before and 3.0 (1.6–4.4) $\mu\text{g}/\text{mg}$ protein after eradication therapy ($P=0.31$).

GPO Total GPO activity was assessed in 17 patients of group A, for whom sufficient mucosal material was available after initial determination of GSH, GST and GST isoenzymes. Total GPO activity was 143 (109–177) nmol/mg protein before and 164 (113–216) nmol/mg protein after treatment ($P=0.28$). Se-dependent GPO activity was assessed in 18 patients, in all of whom *H. pylori* was eradicated. Se-dependent GPO activity was 379 (333–424) nmol/mg protein·min before and 335 (287–383) nmol/mg protein after treatment ($P=0.39$).

GSH There was no significant difference in GSH concentration between the two patient groups before eradication treatment. In group A antral GSH concentration was 21.2 (16.2–26.2) nmol/mg protein before and 27.1 (23.6–30.6)

nmol/mg protein after eradication treatment ($P<0.05$, Fig. 3). In group B these values were 17.4 (9.0–25.7) nmol/mg protein and 18.2 (9.1–27.3) nmol/mg protein, respectively ($P=0.84$).

DISCUSSION

In a former paper, we studied two different groups of patients, either infected or not infected with *H. pylori*. We showed that *H. pylori* infection was associated with low GST enzyme activity and low GSH concentration in the infected antral mucosa.²³ In the present study, we demonstrate that successful eradication of *H. pylori* restores GST activity and tissue GSH concentration, whereas unsuccessful treatment does not. In addition we studied which isoenzymes are responsible for the increase in GST activity after *H. pylori* eradication.

The reason for the low GSH concentration in *H. pylori*-infected antral mucosa is not clear. The following mechanisms might play a role. First, *H. pylori* infection is associated with a strongly increased local production of reactive oxygen species by neutrophil granulocytes.^{6,7} These reactive oxygen species may be actively scavenged by GSH, resulting in the formation of the oxidized form of GSH, GSSG. This oxidized form of GSH is rapidly converted back to GSH, catalyzed by GSH reductase, thereby consuming NADPH. A greatly increased production of reactive oxygen metabolites, however, might overpower the capacity of the tissue to synthesize or regenerate sufficient amounts of GSH, resulting in a decrease in tissue GSH concentration.²⁶ This hypothesis is in line with the

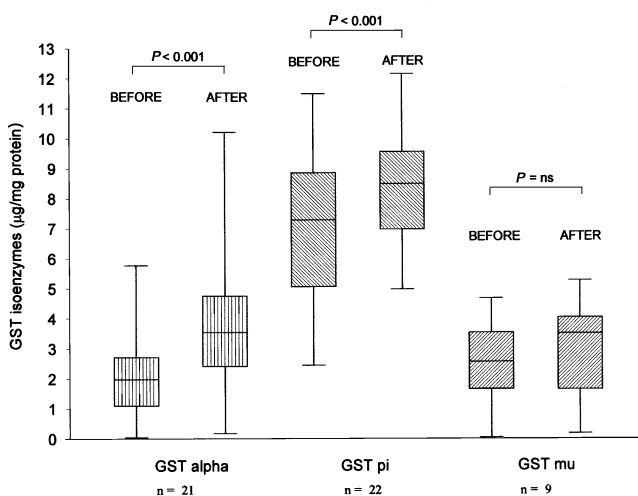


Fig. 2. Glutathione S-transferase isoenzyme levels in antral mucosa before and after successful eradication of *H. pylori*. Box-Whisker plots showing range, 25th and 75th percentile and median.

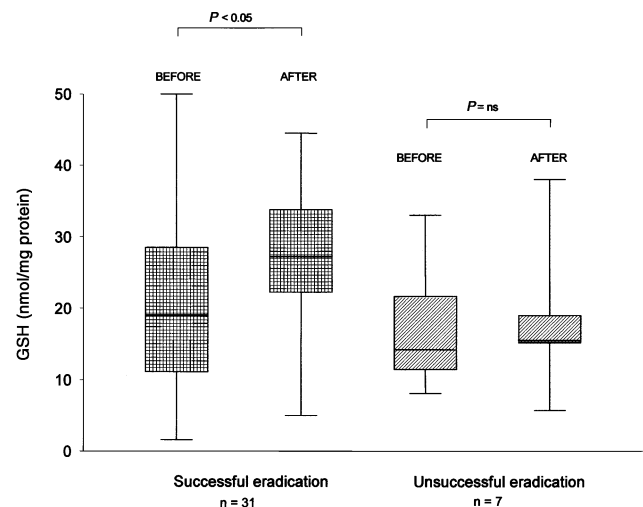


Fig. 3. Glutathione levels in antral mucosa before and after *H. pylori* eradication treatment. Box-Whisker plots showing range, 25th and 75th percentile and median.

findings of Farinati *et al.*, who found increased concentrations of GSSG in infected mucosa, signaling increased oxidation of GSH.⁵⁾ Secondly, local factors or changes in the micro-environment, either produced by the bacterium or generated in the mucosa as a consequence of infection, might impair GSH synthesis.

GSH plays an important role in gastric mucosal cytoprotection. This effect has mainly been attributed to its scavenging capabilities. GSH is a highly effective scavenger of free radicals, including the reactive oxygen species generated by neutrophils. These free radicals can cause severe cellular damage. For example, peroxidation of the cellular lipid membrane is caused by a chain reaction, which can be initiated by a single radical.⁸⁾ Also, extensive damage to DNA can be caused by free radicals, including point mutations, strand breaks and sister chromatid exchange.⁹⁾ This genetic damage might lead to activation of oncogenes, or inactivation of tumor suppressor genes.¹⁰⁾ So free radicals are able to induce not only direct tissue injury, but also genetic damage, which could lead to malignant transformation.

Production of these chemical species by neutrophils is greatly increased in *H. pylori* infection.^{6,7)} Interestingly, the increase of reactive oxygen species in the *H. pylori*-infected mucosa is dependent on the strain: CagA-positive strains, with a high ulcerogenic potential cause a greater increase than CagA-negative strains, implicating that free radicals might play a role in peptic ulceration.^{7,27,28)} CagA-positive strains have also been associated with an increased risk of gastric cancer,²⁹⁾ implicating a possible role in carcinogenesis.

There is also more direct proof that increased concentration of reactive oxygen species as a consequence of *H. pylori* infection is indeed directly responsible for tissue damage. Subjects with *H. pylori* infection show significantly higher concentrations of malondialdehyde, a by-product of lipid peroxidation²⁾ and 8-hydroxyguanosine, a marker for free radical damage to DNA.³⁰⁾ Leakage of lactate dehydrogenase, a marker of cell damage, is greatly increased in experiments in which *H. pylori* is co-incubated with gastric cells. This leakage is sharply reduced by adding free radical scavengers to the experiment.³¹⁾ In accordance with this, treatment of patients with the radical scavengers does prevent *H. pylori*-induced tissue damage to a certain extent. A study by Salim showed that treatment of duodenal ulcer patients with the radical scavengers allopurinol or dimethyl sulfoxide was able to prevent ulcer recurrence at least as effectively as ranitidine.³²⁾

Vitamin C, a scavenger of free radicals has attracted considerable attention after the finding of a decreased concentration of the active ascorbate molecule in the gastric juice of *H. pylori*-infected patients.^{33,34)} This phenomenon is accounted for by infection of the antral mucosa with CagA-positive *H. pylori* strains.^{35,36)} Like *H. pylori* infec-

tion,^{37,38)} a diet deficient in vitamin C has been associated with an increased incidence of gastric malignancies.³⁹⁾ GSH also plays a key role in vitamin C metabolism, since it regenerates the biochemically inactive dehydroascorbate, formed by reaction of vitamin C with free radicals, into the active ascorbate.⁴⁰⁾

In addition, GSH has other important functions. Various substances present in our diet, which have strong mutagenic potential, can be conjugated with GSH. This reaction is catalyzed by GST enzymes.^{11,41)} Conjugation with GSH decreases their biological activity and increases their water solubility, thus allowing more rapid excretion. Earlier results from our group showed a highly significant inverse relationship between GST activity and incidence of malignancy in different parts of the digestive tract.¹³⁾ Decrease of tissue GST activity has also been associated with a variety of malignant tumors.¹⁴⁻²⁰⁾ This might be caused by a diminished clearance of mutagens or carcinogens. It is of interest that many such substances exert their effect by generating free radicals.⁴¹⁾ In a quantitative sense the most important GST in antral mucosa is GST-P1, followed by GST α and GST-M1.¹³⁾ GST-P1 is expressed mainly in mucous cells, incidentally in parietal cells and not at all in chief cells, whereas GST α is equally expressed in mucous and parietal cells and is hardly seen in chief cells.⁴²⁾ Restoration of GST α and GST-P1 levels after eradication of *H. pylori* infection may therefore result in better protection of the gastric cells that express these GST isoenzymes.

However, both Se- and non-Se-dependent antral GPO activity did not change after eradication of *H. pylori*. This is in accordance with *in vivo* experiments by Suzuki *et al.*, who inoculated Mongolian gerbils with *H. pylori* and only found an initial increase in gastric GPO activity.⁴³⁾ GPO levels were normalized after 12 weeks.

Besides transferase activity, GST enzymes may also have non-Se-dependent peroxidase activity, which has been associated with repair of damage to lipid and DNA structures caused by free radicals. This peroxidase activity is mainly confined to the α and θ isoenzyme subclasses.^{12,44)} Our results show the α subclass to be reduced in active *H. pylori* infection, though GPO activity was not lower.

In conclusion, we find that antral GSH concentration and GST activity are enhanced after eradication of *H. pylori* infection. The low levels of GSH and GST activity in *H. pylori*-infected individuals may result in a diminished scavenging capacity for free radicals, which are generated in abundance in active infection and can cause significant damage to cellular structures, including DNA. In addition, the ability of the tissue to inactivate potentially mutagenic or carcinogenic substances might be impaired. These effects might play an important role in tissue damage and carcinogenesis during *H. pylori* infec-

tion. Eradication of the infection restores GSH and GST levels.

(Received June 1, 2001/Revised September 12, 2001/Accepted September 19, 2001)

REFERENCES

- 1) Correa, P. *Helicobacter pylori* and gastric carcinogenesis. *Am. J. Surg. Pathol.*, **19** (Suppl. 1), S37–S43 (1995).
- 2) Correa, P. The role of antioxidants in gastric carcinogenesis. *Crit. Rev. Food Sci. Nutr.*, **35**, 59–64 (1995).
- 3) Farinati, F., Cardin, R., Della Libera, G., Herszenyi, L., Marafin, C., Molari, A., Plebani, M., Rugge, M. and Naccarato, R. The role of anti-oxidants in the chemoprevention of gastric cancer. *Eur. J. Cancer Prev.*, **3** (Suppl. 2), 93–97 (1994).
- 4) Phull, P. S., Green, C. J. and Jacyna, M. R. A radical view of the stomach: the role of oxygen-derived free radicals and anti-oxidants in gastroduodenal disease. *Eur. J. Gastroenterol. Hepatol.*, **7**, 265–274 (1995).
- 5) Farinati, F., Cardin, R. and Della Libera, G. Lipid peroxidation and antioxidant defenses in human gastric mucosa: effect of *H. pylori*. *Eur. J. Gastroenterol. Hepatol.*, **5**, s9–s11 (1993).
- 6) Davies, G. R. and Rampton, D. S. *Helicobacter pylori*, free radicals and gastroduodenal disease. *Eur. J. Gastroenterol. Hepatol.*, **6**, 1–10 (1994).
- 7) Davies, G. R., Simmonds, N. J., Stevens, T. R., Grandison, A., Blake, D. R. and Rampton, D. S. Mucosal reactive oxygen metabolite production in duodenal ulcer disease. *Gut*, **33**, 1467–1472 (1992).
- 8) Halliwell, B. Tell me about free radicals, doctor: a review. *J. R. Soc. Med.*, **82**, 747–752 (1989).
- 9) Imlay, J. A. and Linn, S. DNA damage and oxygen radical toxicity. *Science*, **240**, 1302–1309 (1988).
- 10) Fritsche, M., Haessler, C. and Brandner, G. Induction of nuclear accumulation of the tumor suppressor protein p53 by DNA damaging agents. *Oncogene*, **8**, 307–318 (1993).
- 11) Jernström, B., Morgenstern, R. and Moldéus, P. Protective role of glutathione, thiols, and analogues in mutagenesis and carcinogenesis. *Basic Life Sci.*, **61**, 137–147 (1993).
- 12) Ketterer, B. Protective role of glutathione and glutathione transferases in mutagenesis and carcinogenesis. *Mutat. Res.*, **202**, 343–361 (1988).
- 13) Peters, W. H. M., Roelofs, H. M. J., Hectors, M. P. C., Nagengast, F. M. and Jansen, J. B. M. J. Glutathione and glutathione-S-transferases in Barrett's epithelium. *Br. J. Cancer*, **67**, 1413–1417 (1993).
- 14) Chen, H., Sandler, D. P., Taylor, J. A., Shore, D. L., Liu, E., Bloomfield, C. D. and Bell, D. A. Increased risk for myelodysplastic syndromes in individuals with glutathione transferase theta 1 (GSTT1) gene defect. *Lancet*, **347**, 295–297 (1996).
- 15) Lafuente, A., Pujol, F., Carretero, P., Villa, J. P. and Cuchi, A. Human glutathione S-transferase mu (GST mu) deficiency as a marker for the susceptibility to bladder and larynx cancer among smokers. *Cancer Lett.*, **68**, 49–54 (1993).
- 16) Trizna, Z., Clayman, G. L., Spitz, M. R., Briggs, K. L. and Goepfert, H. Glutathione S-transferase genotypes as risk factors for head and neck cancer. *Am. J. Surg.*, **170**, 499–501 (1995).
- 17) Szarka, C. E., Pfeiffer, G. R., Hum, S. T., Everley, L. C., Balschem, A. M., Moore, D. F., Litwin, S., Goosenberg, E. B., Frucht, H., Engstrom, P. F. and Clapper, M. L. Glutathione S-transferase activity and glutathione S-transferase mu expression in subjects with risk for colorectal cancer. *Cancer Res.*, **55**, 2789–2793 (1995).
- 18) Heagerty, A. H., Fitzgerald, D., Smith, A., Bowers, B., Jones, P., Fryer, A. A., Zhao, L., Alldersea, J. and Strange, R. C. Glutathione S-transferase GSTM1 phenotypes and protection against cutaneous tumours. *Lancet*, **343**, 266–268 (1994).
- 19) Bell, D. A., Taylor, J. A., Paulson, D. F., Robertson, C. N., Mohler, J. L. and Lucier, G. W. Genetic risk and carcinogen exposure: a common inherited defect of the carcinogen-metabolism gene glutathione S-transferase M1 (GSTM1) that increases susceptibility to bladder cancer. *J. Natl. Cancer Inst.*, **85**, 1159–1164 (1993).
- 20) Seidegard, J., Pero, R. W., Markowitz, M. M., Roush, G., Miller, D. G. and Beattie, E. J. Isoenzyme(s) of glutathione transferase (class Mu) as a marker for the susceptibility to lung cancer: a follow up study. *Carcinogenesis*, **11**, 33–36 (1990).
- 21) Wiencke, J. K., Kelsey, K. T., Lamela, R. A. and Toscano, W. A., Jr. Human glutathione S-transferase deficiency as a marker of susceptibility to epoxide-induced cytogenetic damage. *Cancer Res.*, **50**, 1585–1590 (1990).
- 22) van Poppel, G., de Vogel, N., van Bladeren, P. J. and Kok, F. J. Increased cytogenetic damage in smokers deficient in glutathione S-transferase isozyme mu. *Carcinogenesis*, **13**, 303–305 (1992).
- 23) Verhulst, M. L., van Oijen, A. H. A. M., Roelofs, H. M., Peters, W. H. M. and Jansen, J. B. M. J. Antral glutathione concentration and glutathione S-transferase activity in patients with and without *Helicobacter pylori*. *Dig. Dis. Sci.*, **45**, 629–632 (1999).
- 24) Howie, A. F., Douglas, J. G., Fergusson, R. J. and Beckett, G. J. Measurement of glutathione S-transferase pi isoenzyme in plasma, a possible marker for adenocarcinoma of the lung. *Clin. Chem.*, **36**, 453–456 (1990).
- 25) Nijhoff, W. A., Groen, G. M. and Peters, W. H. M. Induction of rat hepatic and intestinal glutathione S-transferases and glutathione by dietary naturally occurring anticarcinogens. *Int. J. Oncol.*, **3**, 1131–1139 (1993).
- 26) Shaw, S., Herbert, V., Colman, N. and Jayatilleke, E. Effect of ethanol-generated free radicals on gastric intrinsic factor and glutathione. *Alcohol*, **7**, 153–157 (1997).
- 27) Rautelin, H., Blomberg, B., Fredlund, H., Jarnerot, G. and

- Danielsson, D. Incidence of *Helicobacter pylori* strains activating neutrophils in patients with peptic ulcer disease. *Gut*, **34**, 599–603 (1993).
- 28) Nielsen, H. and Andersen, L. P. Activation of phagocytes by *Helicobacter pylori* correlates with the clinical presentation of the gastric infection. *Scand. J. Infect. Dis.*, **27**, 347–350 (1995).
- 29) Blaser, M. J., Perez Perez, G. I., Kleanthous, H., Cover, T. L., Peek, R. M., Chyou, P. H., Stemmermann, G. N. and Nomura, A. Infection with *Helicobacter pylori* strains possessing *cagA* is associated with an increased risk of developing adenocarcinoma of the stomach. *Cancer Res.*, **55**, 2111–2115 (1995).
- 30) Baik, S. C., Youn, H. S., Chung, M. H., Lee, W. K., Cho, M. J., Ko, G. H., Park, C. K., Kasai, H. and Rhee, K. H. Increased oxidative DNA damage in *Helicobacter pylori*-infected human gastric mucosa. *Cancer Res.*, **56**, 1279–1282 (1996).
- 31) Bagchi, D., Bhattacharya, G. and Stohs, S. J. Production of reactive oxygen species by gastric cells in association with *Helicobacter pylori*. *Free Radic. Res.*, **24**, 439–450 (1996).
- 32) Salim, A. S. The relationship between *Helicobacter pylori* and oxygen-derived free radicals in the mechanism of duodenal ulceration. *Intern. Med.*, **32**, 359–364 (1993).
- 33) Rathbone, B., Johnson, A. W. and Wyatt, J. I. Ascorbic acid: a factor concentrated in human gastric juice. *Clin. Sci.*, **76**, 241–247 (1989).
- 34) Sobala, G. M., Pignatelli, B., Schorah, C. J., Bartsch, H., Sanderson, M., Dixon, M. F., Shires, S., King, R. F. and Axon, A. T. Levels of nitrite, nitrate, N-nitroso compounds, ascorbic acid and total bile acids in gastric juice of patients with and without precancerous conditions of the stomach. *Carcinogenesis*, **12**, 193–198 (1991).
- 35) Rokkas, T., Liatsos, C. and Petridou, E. Relationship of *Helicobacter pylori* Cag A⁺ status to gastric juice vitamin C levels. *Eur. J. Gastroenterol. Hepatol.*, **29**, 56–62 (1999).
- 36) Sobala, G. M., Schorah, C. J., Shires, S., Lynch, D. A., Gallacher, B., Dixon, M. F. and Axon, A. T. Effect of eradication of *Helicobacter pylori* on gastric juice ascorbic acid concentrations. *Gut*, **34**, 1038–1041 (1993).
- 37) Parsonnet, J., Friedman, G. D., Vandersteen, D. P., Chang, Y., Vogelman, J. H., Orentreich, N. and Sibley, R. K. *Helicobacter pylori* infection and the risk of gastric carcinoma. *N. Engl. J. Med.*, **325**, 1127–1131 (1991).
- 38) The EUROGAST Study Group. An international association between *Helicobacter pylori* infection and gastric cancer. *Lancet*, **341**, 1359–1362 (1993).
- 39) O'Toole, P. and Lombard, M. Vitamin C and gastric cancer: supplements for some or fruit for all? *Gut*, **39**, 345–347 (1996).
- 40) Meister, A. Glutathione, ascorbate, and cellular protection. *Cancer Res.*, **54**, 1969s–1975s (1994).
- 41) Ames, B. N. Dietary carcinogens and anticarcinogens. *Science*, **221**, 1256–1264 (1983).
- 42) Schipper, D. L., Wagenmans, M. J., Van Haelst, U., Peters, W. H., Wobbes, T., Verhofstad, A. A., Lange, W. P. and Wagener, D. J. Immunohistochemical determination of glutathione S-transferases in gastric carcinomas and in adjacent normal gastric epithelium. *Anticancer Res.*, **16**, 565–571 (1996).
- 43) Suzuki, H., Mori, M., Seto, K., Kai, A., Kawaguchi, C., Suzuki, M., Suematsu, M., Yoneta, T., Miura, S. and Ishii, H. *Helicobacter pylori*-associated gastric pro- and antioxidant formation in Mongolian gerbils. *Free Radic. Biol. Med.*, **26**, 679–684 (1999).
- 44) Ketterer, B., Fraser, G. and Meyer, D. J. Nuclear glutathione transferases which detoxify irradiated DNA. *Adv. Exp. Med. Biol.*, **264**, 301–310 (1990).