impurities would have to be potent to account for all the effects of saccharins A, B, and C, and thus, saccharin per se or one or more of its metabolites might cause the effects.

The saccharins that humans consume in pharmaceutical products, beverages, and foods contain higher concentrations of impurities than the purified saccharin C used in our experiments, and thus could constitute a larger risk than pure sweetener. Moreover, since genetic and toxic effects of nutritional and other environmental agents are cumulative, the contributions of repeated exposures to single compounds are difficult to assess, particularly when a carcinogen is labile or when additive or synergistic consequences of multiple agents result.

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High Concentrations of Glutathione in Glandular Stomach: Possible Implications for Carcinogenesis

Abstract. *In laboratory rodents, concentrations of reduced glutathione (GSH) are exceedingly high (up to 7 to 8 millimolar) in the glandular gastric tissue compared to concentrations in other portions of the gastrointestinal tract or to those of most other organs. Gastric GSH varies diurnally, with the highest levels occurring in the late afternoon or early evening. Starvation, treatment with diethyl maleate, or cold-restraint stress all caused marked decreases in stomach GSH, whereas treatment with cobaltous chloride caused an increase in the GSH concentrations. The physiological significance of the high gastric GSH is unknown, but because this endogenous compound may strongly modulate (decrease or increase) the macromolecular binding of certain chemicals capable of inducing stomach tumors, the possible role of glutathione in the pathogenesis of chemically induced gastric cancer should be considered.*

Adenocarcinoma of the stomach is one of the most important and frequent of human cancers, and the demographic patterns of incidence suggest that its occurrence is carcinogen-related. Many early attempts to develop experimental models of carcinogen-induced gastric cancer in animals failed, or cancers developed only in the squamous portions (forestomachs) of rodent stomachs (*1*). For example, in rodents the forestomach is a major target tissue for carcinogenesis by polycyclic aromatic hydrocarbons (PAH), whereas the glandular portions of the stomachs (the part most analogous anatomically to the human stomach) are highly resistant to these compounds (*1*). In contrast, glandular stomach tissue is highly susceptible to carcinogenesis by certain other kinds of compounds, such as *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) (*2*). It is probable that the covalent interaction of the ultimate carcinogenic forms of these types of compounds with cellular macromolecules is a critical initiating event leading to the production of tumors. Reduced glutathione (GSH), an endogenous, nucleophilic tripeptide, may markedly alter the macromolecular binding of these two types of carcinogens in opposite ways: GSH can inhibit macromolecular binding of carcinogenic PAH metabolites (*3*, *4*), but on the other hand it may actually stimulate macromolecular alkylation by agents such as MNNG (*5*). Because of these differences, the relative concentrations of GSH in various portions of the gastrointestinal tract were of interest. We report here that the glandular gastric tissue of laboratory animals contains exceedingly high concentrations of GSH,

Table 1. Treatments causing changes in GSH concentrations in glandular stomachs and livers of rats. Care of animals before the experiment was the same as that described in Fig. 1 legend. All measurements were made at 4 to 6 p.m.; appropriate control groups were run separately for each treatment. All animals for the cobaltous chloride experiment (including controls) were fasted 24 hours prior to and during the experiment. The values shown were calculated from determinations [using the modified Ellman procedure (*7*)] on groups of four to seven rats each. All GSH concentrations represented in the table were significantly different ($P < .01$) from the corresponding control concentrations (analyses by Student's *t*-test on the respective mean absorbance values); S.E., standard error of the mean.

Treatment	Mean percentage of control GSH ± S.E.	
	Liver	Glandular stomach
Food deprivation (48 hours)	80 ± 4	70 ± 3
Diethyl maleate (0.9 ml/kg, subcutaneous, 1 hour prior)	23 ± 10	27 ± 4
Cold-restraint stress (4 hours)	76 ± 1	60 ± 1
Cobaltous chloride (45 mg/kg, subcutaneous, 15 hours prior)	190 ± 10	140 ± 9

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raising the possibility that GSH may be an important determinant of the organotropism of certain kinds of chemicals capable of causing gastric cancer. These results also raise the question of the physiological significance of GSH in normal gastric function (*6*).

When measured in the late afternoon, the concentrations of GSH in glandular gastric tissue of the rat were similar to those found in liver and exceeded those in all other tissues (Fig. 1A) or portions of the gastrointestinal tract (Fig. 1B) that were examined. Similarly high concentrations were found in glandular stomach tissues of other species including the mouse, rabbit, guinea pig, and hamster. No significant differences were found in GSH concentrations in rat glandular stomachs divided arbitrarily into upper, middle, and lower thirds. The values shown in Fig. 1 are the relative absorbance values (at 412 nm) obtained by using an adaptation (*7*) of the procedure of Ellman (*8*) for the measurement of nonprotein sulfhydryl; all values shown fell within the linear range of the assay and therefore are directly proportional to the respective nonprotein sulfhydryl concentrations. In most tissues the nonprotein sulfhydryl content is comprised almost entirely of GSH, making the Ellman procedure a convenient assay for GSH. Using a more specific fluorometric assay for GSH (*9*), we found that most of the sulfhydryl we measured with the Ellman procedure represented GSH (95 percent in glandular stomach and 90 percent in liver). It was necessary to modify slightly the fluorescence method by addition of varying amounts of supplemental GSH to representative samples as an internal standard to correct for differences in fluorescence quenching (*10*) between the different tissues. The absorbance values for glandular stomach (Fig. 1, A and B) correspond to tissue concentrations of 7.23 and 8.26 m*M*, respectively. An attempt to measure oxidized glutathione by using an enzymatic assay (*7*) resulted in no significant increase in absorption at 412 nm after incubation of glandular stomach homogenates with glutathione reductase and reduced nicotinamide adenine dinucleotide phosphate for 20 minutes at 37°C, indicating that most of the glutathione present in rat glandular stomach was in the reduced form.

It is important to note that the GSH values shown in Fig. 1 were obtained in the late afternoon. Since hepatic GSH concentrations vary with the time of day (*11*), we compared the temporal pattern of rat glandular stomach and hepatic GSH concentrations. Gastric GSH varied diurnally (Fig. 2); concentrations were highest in the late afternoon and lowest at night and early morning. Diurnal variation of hepatic GSH followed an entirely different pattern; we found hepatic concentrations highest at night and early morning, but lowest in the late afternoon, similar to results reported by others (*11*). Thus measurements of GSH made in the morning consistently showed hepatic concentrations considerably higher than those of glandular stomachs, whereas measurements made in the late afternoon usually showed gastric GSH concentrations nearly equal to, and sometimes actually greater than, the hepatic concentrations. The ratios of glandular gastric GSH to hepatic GSH were highest in young rats (130 to 140 g) compared to weanling (40 to 50 g) or older rats (400 to 500 g).

Table 1 shows that food deprivation (48 hours) caused significant decreases in both gastric and hepatic GSH. Other studies (data not shown) indicated that the diurnal variations of both gastric and

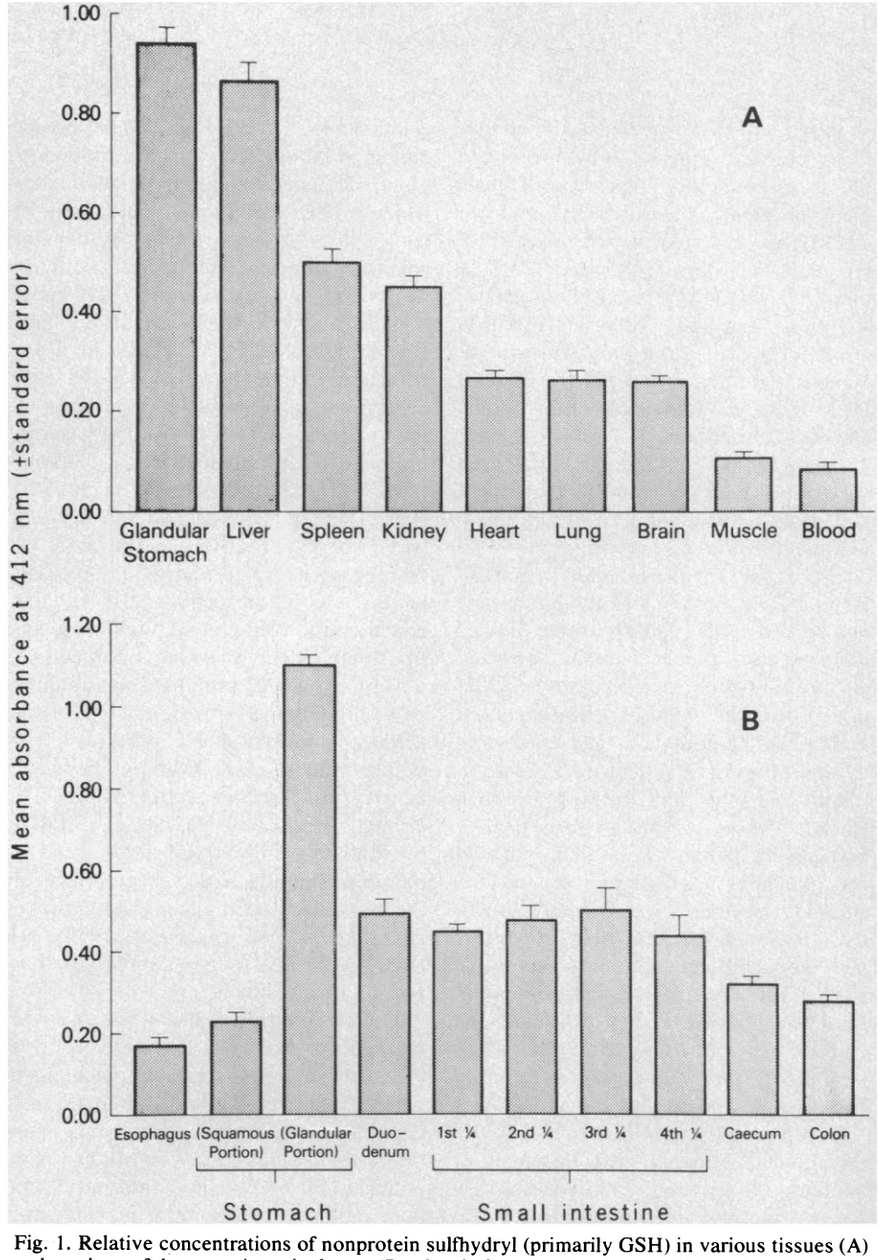

Fig. 1. Relative concentrations of nonprotein sulfhydryl (primarily GSH) in various tissues (A) and portions of the gastrointestinal tract (B) of male Sprague-Dawley rats (130 to 135 g). Values shown were obtained by using a modified Ellman procedure (*7*) and are means (± standard error) of determinations on five animals. Several replications of this experiment gave essentially the same result. Animals were not used earlier than 5 to 7 days after arrival from the supplier (Taconic Farms, Germantown, N.Y.); during the preexperiment period they were housed in groups of eight to ten animals each, were maintained on a 12 hour light/dark cycle, and were given food (NIH open formula diet) and water without restriction.

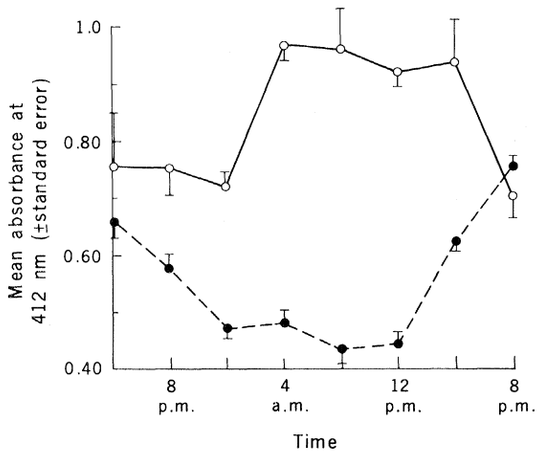

Fig. 2. Diurnal variations of rat glandular gastric (●) and hepatic (○) GSH concentrations. Values were obtained by using a modified Ellman procedure (*7*) and are means (± standard error) of determinations on groups of five animals each. Three replications of this experiment gave essentially the same result. Care of animals before the experiment was the same as described in Fig. 1 legend. Tissues from all the groups were collected, frozen, and stored on Dry Ice prior to the GSH assay.

hepatic GSH were decreased in amplitude, but not abolished, by prolonged (24- to 48-hour) food deprivation. Treatment of animals with diethyl maleate (DEM), an agent known to covalently complex and deplete hepatic GSH in vivo (*12*), strikingly decreased rat gastric as well as liver GSH (Table 1). Additional experiments (data not shown) indicated that the gastric GSH response to DEM was rapid (maximal within 1 hour) and dose-dependent (0.3 to 0.9 ml per kilogram of body weight, administered subcutaneously, giving 40 to 75 percent depletion, respectively). Stress by physical restraint for 4 hours in a cold (4°C) environment (*13*) also resulted in marked decreases in both rat glandular gastric and hepatic GSH (Table 1); other studies (data not shown) indicated that the stressor caused significant decreases in GSH in both fed and food-deprived rats and with restraint periods at any of several different times of day (8 p.m. to 12 a.m., 9 a.m. to 1 p.m., and 2 p.m. to 6 p.m.). Rats subjected to this stressor usually had severe hemorrhagic ulcerations in the glandular gastric mucosa, as described previously (*13*). Interestingly, rats treated with DEM also frequently had this same type of acute ulcerative gastric lesion, suggesting a possible relation between GSH depletion and the pathogenesis of acute gastric ulceration, although further studies will be required to assess this possibility.

In contrast to the decreases in GSH by the above treatments, prior treatment of animals with cobaltous chloride caused a marked increase in gastric and hepatic GSH concentrations (Table 1). Sasame and Boyd (*7*) previously have reported that concentrations of hepatic GSH were elevated by prior treatments with cobaltous chloride or salts of several other divalent metals.

The possibility that the high levels of glandular gastric GSH may be an important modulatory factor in the induction of gastric cancer by highly reactive electrophilic chemicals or metabolites should be explored. The resistance of the glandular stomachs of laboratory rodents to carcinogens, such as the PAH compounds, might be due in part to the high concentrations of glandular gastric GSH compared to the relatively low GSH concentrations in the squamous portions of the stomachs, which are highly susceptible to PAH compounds. Reduced glutathione is capable of deactivating carcinogenic PAH metabolites through the formation of less reactive conjugates (*3*, *4*), thereby decreasing their ability to covalently bind to macromolecular cellular constituents. Glutathione transferase activity, which may promote the deactivation of certain electrophilic metabolites by facilitating the formation of GSH conjugates, is present (albeit at low levels) in gastric mucosa (*14*). On the other hand, Lawley and Thatcher (*5*) found that GSH strongly stimulated alkylation of DNA by MNNG in vitro, and therefore it is possible that the high susceptibility of the glandular gastric mucosa to carcinogens like MNNG might be directly related to the high GSH content of that portion of the stomach.

The observation that a stressor could markedly alter GSH concentrations (Table 1) suggests a testable mechanism whereby stressors could modulate susceptibility to chemical carcinogens. Fox (*15*) recently has reviewed studies that indicate the possibility that stressors may modulate cancer risk in man, but the possible direct interaction of stressors on metabolic factors directly relating to chemical carcinogenesis has not heretofore been explored.

Factors associated with alterations in GSH concentrations (for example, diurnal variations, starvation, chemical treatments, and stressor exposure) may serve as useful experimental variables to aid the further elucidation of the physiological role or roles) for gastric GSH, as well as its possible role in gastric carcinogenesis.

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