Table II—Effect of Clofibrate, Phenobarbital, Halofenate, and Placebo Treatment on Plasma Half-Life of Antipyrine in Rhesus Monkeys[a]

Group	Period	Treatment	Half-Life, hr.	Antipyrine Concentration, mcg./ml. 0.5 hr.	1 hr.	2 hr.	3 hr.	4 hr.
I	Before	None	1.0 ± 0.1	80.4 ± 8.6	63.6 ± 4.2	27.2 ± 4.3	12.9 ± 3.5	7.3 ± 2.1
I	After	Clofibrate	1.3 ± 0.2	74.7 ± 3.8	53.4 ± 4.7	32.8 ± 3.1	18.8 ± 3.0	11.0 ± 3.9
II	Before	None	1.2 ± 0.2	80.2 ± 8.2	61.6 ± 8.0	31.8 ± 5.2	15.9 ± 4.7	13.1 ± 4.5
II	After	Phenobarbital	0.6 ± 0.2[b]	54.5 ± 7.0	31.4 ± 6.1	13.2 ± 3.6	6.7 ± 2.8	1.4 ± 1.6
III	Before	None	1.2 ± 0.2	90.9 ± 7.5	65.7 ± 5.7	37.7 ± 13.4	22.0 ± 9.2	12.4 ± 4.8
III	After	Halofenate	1.3 ± 0.4	88.8 ± 9.9	60.5 ± 4.4	37.4 ± 11.0	24.0 ± 9.7	13.5 ± 7.6
IV	Before	None	1.2 ± 0.5	90.3 ± 6.1	65.7 ± 7.7	38.2 ± 11.6	23.6 ± 11.7	11.0 ± 8.7
IV	After	Placebo	1.3 ± 0.2	88.8 ± 12.0	64.9 ± 13.6	38.6 ± 6.4	24.8 ± 7.6	12.5 ± 4.9

[a] The half-life was estimated by means of a GE Mark I FORTRAN computer program. All values given are the mean ± *SD*. For Group I, $n = 4$; for Group II, $n = 8$; for Group III, $n = 8$; and for Group IV, $n = 12$. [b] Difference was highly significant ($p < 0.001$). All other $t_{1/2}$ values after treatment were not significantly different from pretreatment values.

studies specifically designed to reveal possible clinical interactions between halofenate and the drugs used in this study are currently being planned.

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Rapid Gastric Absorption of Sodium Nitrite in Mice

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Abstract □ The concentration of available gastric sodium nitrite and the major pathways involved in its disappearance in mice following single administration were determined. At 10 min. after oral administration, 85% of the available sodium nitrite was lost from the mouse stomach. Ligation of the gastroduodenal junction had no effect on nitrite loss. Following 30 min. incubation *in vitro*, where loss of nitrite by absorption was prevented, there was 63% loss of sodium nitrite, of which 40% had been converted to sodium nitrate. The authors concluded that the major pathway of loss of available gastric nitrite is absorption directly from the stomach into the bloodstream.

Keyphrases □ Sodium nitrite—gastric absorption and metabolism, mice □ Nitrite/nitrate levels—sodium nitrite absorption, metabolic pathways, mice □ Absorption, gastric—sodium nitrite, mice

Technological advances both in agriculture and food preservation have led to increased concern over intake of nitrite and nitrate salts (1). The primary toxicological response to these salts is methemoglobinemia. Nitrate salts do not directly cause methemoglobinemia but rather are converted to nitrite salts by gastric flora (1).

Of perhaps greater significance than methemoglobinemia is the reaction of nitrite with secondary amines

under acidic conditions to produce carcinogenic nitrosamines. Although this reaction proceeds in the small intestine of the rat (2, 3) and at near neutral pH in the presence of enteric bacteria (4), it proceeds much faster at acidic pH's present in the stomach (2, 5). It is of consequent interest, therefore, to determine gastric nitrite concentration at various time intervals after administration in order to determine potential reactivity with secondary amines. The potential reactivity is a function of both nitrite concentration and duration of time the reaction can take place. Additionally, the metabolic fate of nitrite was studied to determine whether nitrite was chemically altered or whether nitrite passed into the duodenum.

Three major pathways may be involved in the disappearance of nitrite from the stomach. First, nitrite may leave the stomach through the gastroduodenal junction and be absorbed from the small intestine. Second, nitrite may react chemically with gastric components; nitrite is acid unstable and spontaneously decomposes to nitrate and nitrogen dioxide. Additionally, nitrite may be utilized in such reactions as diazotization and nitrosation or may serve as the substrate for bacterial nitroreductase (6, 7). Third, nitrite may be absorbed directly from the stomach into the bloodstream. Due to the high reactivity of nitrite with hemoglobin and the presence of methemoglobin reductase in erythrocytes, which rapidly degrades methemoglobin, there is no way to quantitate directly gastric absorption of nitrite salts by blood levels.

EXPERIMENTAL

Animals—Male Swiss ICR/Ha mice, weighing between 20 and 25 g., were used. The mice were maintained on food[1] and water *ad libitum*.

Time Course of *In Vivo* Disappearance of Sodium Nitrite from Mouse Stomach—Sodium nitrite, 150 mcg., was administered to each mouse by gavage in 0.1 ml. aqueous solution. Animals, in groups of 13–18, were then killed by cervical dislocation within a minute and at 10, 20, and 30 min. after sodium nitrite administration. Stomachs, together with attached 5-mm. segments of the esophagus and duodenum, were removed and assayed individually for sodium nitrite.

Effect of Ligation of Gastroduodenal Junction on Rate of *In Vivo* Disappearance of Sodium Nitrite from Mouse Stomach—Groups of 5–8 mice were anesthetized by intraperitoneal injection of 150 mg./kg. sodium hexobarbital. The gastroduodenal junction was ligated in some groups, while in controls the ligature was left loose. The stomachs were then injected intraluminally with 150 mcg. sodium nitrite in 0.1 ml. of water; the abdominal wall was then sutured. Mice were killed by cervical dislocation 10 or 30 min. later, and stomachs were removed and assayed individually for sodium nitrite.

Disappearance of Sodium Nitrite from Stomachs *In Vitro*—Each isolated stomach was injected intraluminally with 150 mcg. sodium nitrite in 0.1 ml. aqueous solution. Stomachs, in groups of 8–31, were incubated in empty flasks at 37° for 10 or 30 min.; the flasks were then placed on ice and the stomachs were assayed individually. Empty flasks were used in these studies to prevent movement of sodium nitrite out of the stomach by osmotic gradients.

***In Vitro* Oxidation of Sodium Nitrite to Sodium Nitrate**—Isolated stomachs from 16 mice were each injected directly with 150 mcg. sodium nitrite in 0.1 ml. aqueous solution and incubated individually for 30 min. in empty flasks at 37°; control stomachs from groups of 20 mice were incubated under identical conditions.

[1] Purina laboratory chow.

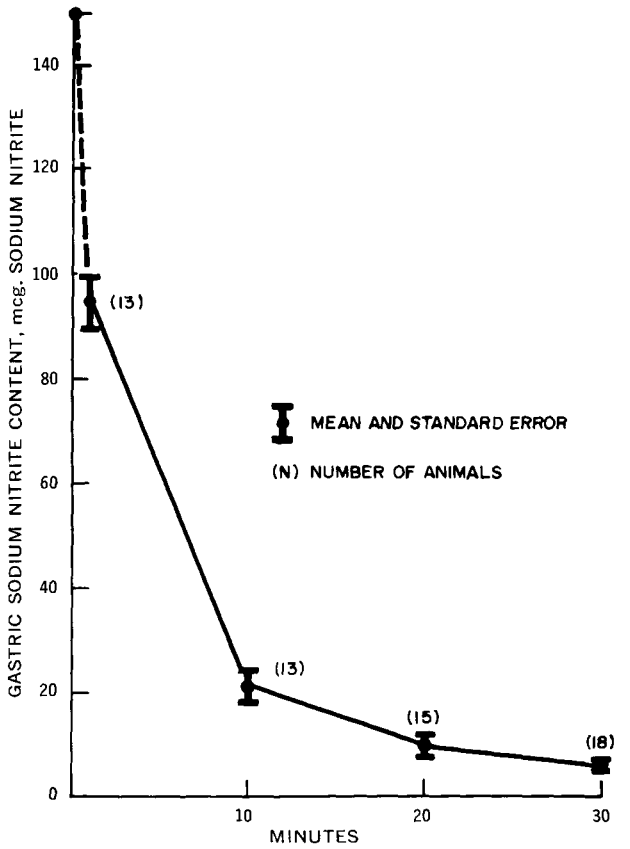

Figure 1—In vivo *disappearance of sodium nitrite from mouse stomach.*

The flasks were placed on ice, and individual stomachs were assayed for sodium nitrite and sodium nitrate.

Nitrite Determination—Individual stomachs from *in vivo* and *in vitro* experiments were placed in 20 ml. distilled water, buffered with 0.25 ml. 0.67 *M* NH_4Cl/NH_4OH at pH 9.6–9.7, and sliced open. Activated charcoal was added, and the flasks were agitated for 15 min. at room temperature. Then 0.2 ml. of 1.04 *M* $ZnSO_4$ was added, and the flasks were agitated for a further 5 min. Contents of each flask were centrifuged at 2000 r.p.m. for 15 min. Aliquots were taken for colorimetric determination of sodium nitrite. For sodium nitrate assays, similar aliquots were taken and treated with an excess of finely divided cadmium (9); following agitation for 2 hr., these were assayed for sodium nitrite. The differences between the sodium nitrite levels in the presence and absence of cadmium represent the amount of sodium nitrite oxidized to sodium nitrate in the isolated stomachs *in vitro*.

RESULTS AND DISCUSSION

Following oral administration, sodium nitrite rapidly disappears from the mouse stomach (Fig. 1); 85 and 95% losses were seen at 10 and 30 min., respectively. As can be seen from the small standard errors, gastric food content from animal to animal has very little correlation with gastric nitrite content. Stomachs from untreated controls contained less than 2 mcg. sodium nitrite. Data presented in Fig. 1 were tested for zero-, first-, and second-order kinetics[2]. These data only fit second-order kinetics, with a rate constant of 0.30 (hr. × mg. sodium nitrite per stomach)$^{-1}$. In acid conditions, nitrite forms an uncharged dimer (N_2O_3) (10). Since uncharged molecules generally pass through membranes more rapidly than charged

[2] Tests for order of kinetics were performed using the following equations:

0 order $[NaNO_2] = kt$
1st order $\log [NaNO_2] = kt$
2nd order $[NaNO_2]^{-1} = kt$

The second-order rate constants were calculated by averaging the independent determinations of k.

Table I—Effects of Ligation of Gastroduodenal Junction on *In Vivo* Disappearance of Sodium Nitrite from the Mouse Stomach

Time following Gastric Injection, min.	Number of Mice	Gastric Sodium Nitrite— Mean ± *SE*, mcg./Stomach	Gastric Sodium Nitrite— Percent Administered Dose
10	8—ligated	29.5 ± 2.7	20
10	5—not ligated	23.6 ± 6.1	16
30	8—ligated	13.8 ± 3.3	9
30	5—not ligated	10.3 ± 2.2	7

Table II—*In Vitro* Loss of Sodium Nitrite from Mouse Stomach

Duration of Incubation, min.	Number of Stomachs	Gastric Sodium Nitrite— Mean ± *SE*, mcg./Stomach	Gastric Sodium Nitrite— Percent Administered Dose
10	8	97.3 ± 4.4	65
30	31	55.3 ± 3.3	37

molecules, the gastric absorption of nitrite might have been expected to be second order if dimer formation was rate limiting.

The rate of sodium nitrite disappearance from the mouse stomach *in vivo* was not significantly reduced by ligature at the gastroduodenal junction (Table I). Although there was consistently more gastric sodium nitrite lost in mice not having the gastroduodenal junction ligated, this difference was not statistically significant and did not appear to represent a major pathway of nitrite loss. The corresponding second-order rate constants for the disappearance of nitrite from ligated and nonligated animals were 0.13 and 0.17 (hr. × mg. sodium nitrite per stomach)$^{-1}$, respectively.

The loss of sodium nitrite from the mouse stomach *in vitro*, where absorption of sodium nitrite was prevented, is shown in Table II. At 30 min. after incubation, 63% of gastric sodium nitrite was lost. The second-order rate constant for nitrite disappearance *in vitro* was 0.023 (hr. × mg. sodium nitrite per stomach)$^{-1}$. Additionally, within 30 min., 26% of administered sodium nitrite was converted to sodium nitrate (Table III). That is, 40% of the sodium nitrite lost *in vitro* was converted to sodium nitrate.

In vivo disappearance of sodium nitrite from the mouse stomach is very rapid, essentially reaching completion within 30 min. This appears largely due to gastric absorption since ligature of the gastroduodenal junction does not significantly reduce the rate of sodium nitrite loss. Although 35% of the sodium nitrite in the stomach *in vitro* was lost within 10 min. (Table II), this process was much slower than absorption. Even after accounting for *in vivo* nitrite loss by degradative processes, the rate of absorption was still 4.5 times greater than the rate of chemical degradation. About 60% of the sodium nitrite lost *in vitro* by nonabsorptive processes was either enzymatically reduced or bound to insoluble material (Tables II and III); 40% of gastric sodium nitrite was converted to nitrate. The mechanism through which sodium nitrate was formed in the mouse stomach probably involves acid hydrolysis. On the other hand, it is not possible to differentiate enzymatically reduced nitrite from nitrite bound to zinc sulfate-insoluble material.

The results presented here are supported by several studies dealing with the rapid direct and indirect effects of nitrite following oral administration. Maximum levels of methemoglobin in rats following oral administration of sodium nitrite occurred within 60 min. of ingestion (1); plasma nitrite concentrations in sheep injected intraluminally with potassium nitrite peaked at 2 hr. (11); and mortality in swine was maximal between 90 and 150 min. after

Table III—*In Vitro* Conversion of Sodium Nitrite to Sodium Nitrate in Mouse Stomach

Added Nitrite	Number of Stomachs	Gastric Sodium Nitrite, Mean ± *SE*, mcg. Sodium Nitrate/ Stomach	Percent Administered Sodium Nitrite Converted to Sodium Nitrate
—	20	19.3 ± 2.1	—
+	43	62.2 ± 12.0	25.7

single oral doses of sodium nitrite (12). Additionally, synergistic toxicity—mortality, hepatic necrosis, inhibition of liver protein synthesis, and inhibition of liver nuclear RNA synthesis (13, 14)—from endogenous nitrosamine formation following oral administration of sodium nitrite and dimethylamine was markedly dependent on the time between successive administration of these compounds. Synergistic toxicity was maximal when nitrite and dimethylamine were administered simultaneously and when dimethylamine was administered 60 min. prior to nitrite; however, no synergistic toxicity was observed when nitrite was administered 30 min. prior to dimethylamine. These data suggest that the rapid absorption of nitrite may well be a limiting factor in the *in vivo* biosynthesis of nitrosamines.

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