six results[3]. From the data presented in this paper, a range of 35–41% of label claim is obtained from the use of water that contains air concentrations that do not exceed the saturation point of air in water at 37°. Thus, it should be possible to narrow the present acceptance range for these tablets, or at least to make the range more meaningful, if the air concentration in the water is controlled at the beginning of the test.

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Drug Interactions I: Detection of Inorganic Nitrite in Organic Nitrate Esters Under Acidic Conditions Simulating the Human Stomach

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Abstract □ Both unformulated (bulk) and formulated (drugs) organic nitrate esters (isosorbide dinitrate, nitroglycerin, and pentaerythritol tetranitrate) were studied in the presence and absence of hydrochloric acid to determine if they could be sources of nitrite (and therefore lead to nitrosamine formation) under acidic conditions similar to those found in the stomach. The presence and generation of nitrite ion was detected by a modification of the Griess reaction. Bulk isosorbide dinitrate and nitroglycerin were found to be contaminated with 13.8–121.4 μmoles of inorganic nitrite per mole of nitrate ester. In addition, in the presence of hydrochloric acid, these preparations generated 0.52–1.18 μmoles of inorganic nitrite/mole of nitrate ester/min. Unformulated nitroglycerin generated nitrite at a rate roughly twice that of isosorbide dinitrate. In contrast, no evidence for nitrite contamination or generation by pentaerythritol tetranitrate was found. Tablets and capsules of isosorbide dinitrate contained ~27–216 μmoles of nitrite/mole of nitrate ester and, in the presence of hydrochloric acid, generated an average of 0.55 μmole nitrite/min. For isosorbide dinitrate, this rate was similar for bulk and formulated drug. In comparison to isosorbide dinitrate, the amount of nitrite initially present in tablets and capsules of nitroglycerin varied more widely (~25–2290 μmoles nitrite/mole of nitrate ester), and in this case nitrite was generated at higher rates than unformulated drug averaging ~4.7 μmoles nitrite/mole of nitrate ester/min. Contrary to a literature report, we found that nitrate ion is not reduced to nitrite by hydrochloric acid (pH 1–3). These data suggest that the continuous production of nitrite ion from isosorbide dinitrate and nitroglycerin is due to the hydrolysis of nitrite ester impurities, a reaction known to be strongly catalyzed by the chloride ion. Although the generation of inorganic nitrite from organic nitrate esters is of interest, the low levels of nitrite produced are unlikely to lead to intragastric nitrosamine formation.

Keyphrases □ Isosorbide dinitrate—presence and generation of inorganic nitrite, simulated gastric conditions, Griess reaction □ Nitroglycerin—presence and generation of inorganic nitrite, simulated gastric conditions, Griess reaction □ Pentaerythritol tetranitrate—presence and generation of inorganic nitrite, simulated gastric conditions, Griess reaction □ Inorganic nitrite—presence in and generation from the nitrate esters isosorbide dinitrate, nitroglycerin, and pentaerythritol tetranitrate, simulated gastric conditions, Griess reaction

Organic nitrate esters such as nitroglycerin have been used for many years on an intermittent basis to relieve the symptoms of angina pectoris. Recently, organic nitrates have been used on a continuing basis to prevent anginal attacks; they are often ingested with other medications such as tranquilizers or are prescribed concomitantly with β-adrenergic blocking drugs such as propranolol hydrochloride for an additive pharmacological effect (1). Since these medications are taken for many years, often for the lifetime of the patient, it is important to evaluate the safety of simultaneous ingestion of such drugs (2).

There has been much discussion regarding the potential hazards of nitrosamines formed from therapeutic drugs during their passage through the GI tract (3, 4). In fact, in studies where animals are fed inorganic nitrite along with various drugs such as chlordiazepoxide, the formation of carcinogenic nitrosamines has been demonstrated (5). To form nitrosamines, an acidic milieu, the presence of amines, and a source of nitrite are required. The human stomach provides such an appropriate acidic environment (6), and most antihypertensive, β-adrenergic blocking, and tranquilizing drugs are secondary or tertiary amines. Nitrosamine formation can occur from a tertiary amine, but an oxidative cleavage to a secondary amine is required first (7). Nitrosation of secondary amines is a well-established reaction (7).

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We have been concerned as to whether or not organic nitrate drugs could be a source of nitrite ion and therefore give rise to nitroso compounds during dissolution in the stomach. Although organic nitrate esters are known to be converted to nitrite by hepatic enzymes (8), the conversion of organic nitrate esters to inorganic nitrite in actual or simulated gastric contents has not been described. Nevertheless, Oishis (9) has reported, on the basis of polarographic studies, a mechanism by which nitrite could arise from nitrate in the presence of hydrochloric acid. He found that even in dilute solution, the interaction of nitric acid and hydrochloric acid (aqua regia reaction: $HCl + HNO_3 \rightarrow HNO_2 + HOCl$) gave rise to an equilibrium that favored the products over starting compounds in the ratio 99:1.

Earlier studies in our laboratory showed that nitrosamines could be recovered from incubations of isosorbide dinitrate with hydroxyzine hydrochloride, a tertiary amine (2), or propranolol hydrochloride, a secondary amine (10). In this report, we examined whether or not organic nitrate esters are a source of inorganic nitrite, and if not, other possible origins of inorganic nitrite. Various preparations of isosorbide dinitrate, nitroglycerin, and pentaerythritol tetranitrate were examined for the presence of nitrite under acidic conditions simulating those found in the human stomach.

EXPERIMENTAL

Materials—Unformulated samples, tablets, and capsules of isosorbide dinitrate, nitroglycerin, and pentaerythritol tetranitrate were gifts from various manufacturers. Because of their explosive properties, organic nitrates are routinely stored and handled as either lactose or mannitol mixtures. When formulated as drugs, the active ingredients are combined with inert materials and lubricants. In the studies described here, samples of the bulk (unformulated) materials were used as received, and in addition, a bulk sample of isosorbide dinitrate (25% in mannitol) was purified by sublimation and also by recrystallization from absolute ethanol.

Sulfanilic acid[1] and 1-aminonaphthalene[1] were analytical reagent grade. All reagents, water, and glassware used in these studies were free of contaminating nitrite and periodically checked using the Griess test (11).

Bulk Organic Nitrates—Samples were incubated in hydrochloric acid, pH 1.0, at 37° accompanied by gentle shaking to simulate conditions of acidity, temperature, and pH found in the human stomach. Incubations were conducted for 60–90 min to determine the time course of inorganic nitrite production during the period that a drug is likely to remain in the stomach. Under these conditions, the free nitrite ion (nitrous acid) was found to be unstable. Therefore, the Griess test (11, 12) was modified in that sulfanilic acid was added at the outset to trap nitrite released from the nitrate esters as the diazo derivative. Under these conditions, diazotization is complete in <1 min.

The assay procedure was as follows: Sulfanilic acid (12.9 m*M*) in 0.12 *M* HCl was added to an aqueous solution of organic nitrate ester in a final concentration of 10% dimethylformamide in water. Aliquots (1.5 ml) of this incubation mixture were removed at specific times and allowed to react with 1-aminonaphthalene (15.7 m*M*), forming a red dye which gave maximal absorbance at 520 nm within 2 min. Nitrite concentrations were calculated as a function of the absorption of the dyestuff using a molar extinction coefficient of 33,000 liter/mole-cm at 520 nm. The minimum detectable amount of nitrite in the assay, based on an absorbance reading of 0.010 is ~0.5 nmole of nitrite. It should be noted that the Griess test is specific for the nitrite ion, and its sensitivity for nitrite detection is considerably better than other published methods (12) including chemiluminescence (13) and a recently reported high-performance liquid chromatographic (HPLC) technique (14).

Because the pH of human stomach contents varies, nitrite levels were measured at several hydrogen ion concentrations. The pH of the solution

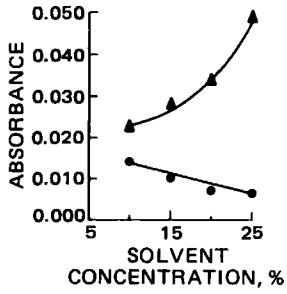

Figure 1—*Effect of organic solvents on absorbance. The Griess reaction was performed at pH 1.0 in solutions containing 8.47 mmoles/liter of isosorbide dinitrate; absorbance was measured after 90 min of incubation. Key: (▲) dimethylacetamide; (●) dimethylformamide. Points are mean values obtained from at least two experiments.*

was raised by adding sodium hydroxide to the sulfanilic acid reagent mixture such that the desired pH of 2.0 or 3.0 was achieved in the final incubation mixture. At pH 2.0 or 3.0 at 37°, diazotization and coupling times varied from 5 to 30 min. No studies were performed at pH >3.0 due to the insolubility of the components of the Griess reagents.

Organic nitrate esters were found to be only partially soluble in aqueous solutions as well as in the Griess reagent mixture. It was therefore necessary to use dimethylformamide as a cosolvent to ensure complete solubility and thus accurate measurement of nitrite under the experimental conditions described above. HPLC analysis was used to compare the quantity of organic nitrate ester added to the Griess reaction mixture and the amount of organic nitrate recovered from the incubation mixture. This was done by weighing organic nitrate esters mixed with sugars, extraction into 100% dimethylformamide, and injection onto a μ-Bondapak C-18 column. These samples were compared with companion samples that were added to the Griess reaction mixture and also with samples that were back-extracted from the Griess reaction incubation mixture into methylene chloride. Samples were separated by HPLC and detected by a UV detector at 254 and 280 nm. The retention time for isosorbide dinitrate separated by methanol–water (55:45), isocratic, flow rate 2 ml/min, was 3.3 min; for nitroglycerin in a 50–100% acetonitrile linear gradient, flow rate 1.5 ml/min, the retention time was 6 min. HPLC analysis of organic nitrate esters under the conditions described confirmed their complete solubility in the Griess reaction mixture at a final dimethylformamide concentration of 10%.

Formulated Organic Nitrates (Drugs)—Formulated tablets and the contents of capsules of isosorbide dinitrate, nitroglycerin, and pentaerythritol tetranitrate were ground using a mortar and pestle prior to testing. The resulting powder was dissolved in dimethylformamide and centrifuged at 2000×*g* for 10 min at 4° to separate the nitrate ester from insoluble components of the formulated preparations. Samples were diluted with water to contain 1.0, 1.5, and 2.0 mg of nitrate ester/ml in the final 10% dimethylformamide mixture. The concentration of organic nitrate ester actually present in the drug extract was confirmed by the aforementioned HPLC method. At time zero, sulfanilic acid and 1-aminonaphthalene in hydrochloric acid, pH 1.0, were simultaneously added to the drug extract and incubated at 37° with shaking. Aliquots were removed at various intervals up to 90 min, passed through a filter[2] to remove particles, and the absorbance was read at 520 nm. This procedure differs from that described above in that sulfanilic acid and 1-aminonaphthalene were added together instead of sequentially.

The effect of the sequence of addition of sulfanilic acid and 1-aminonaphthalene on detection of nitrite was evaluated as follows: In one case, sulfanilic acid was incubated with the reaction mixture and 1-aminonaphthalene was added at the end of the incubation period. In the second case, the sulfanilic acid and 1-aminonaphthalene were added together at the initiation of the incubation period. When scanned by spectrophotometry, absorption maxima (520 nm) were superimposable for sodium nitrite standards, sodium nitrite mixed with unformulated isosorbide dinitrate and nitroglycerin, and sodium nitrite mixed with isosorbide dinitrate and nitroglycerin extracted from tablets and capsules.

RESULTS

Effect of Organic Solvents—Bulk organic nitrate esters were found to be only partially soluble in aqueous solutions as well as in the Griess

[1] J. T. Baker Chemical Co., Phillipsburg, N.J.

[2] Millipore Corp.

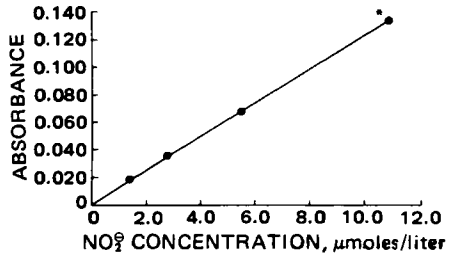

Figure 2—*Effect of 10% dimethylformamide on the measurement of inorganic nitrite by the Griess reaction. All points obtained for sodium nitrite in hydrochloric acid, pH 1.0, with and without 10% dimethylformamide, are completely superimposable. Points are mean values obtained from at least two experiments.*

reagent mixture. It was therefore necessary to use organic solvents to ensure complete solubility and thus accurate measurement of nitrite. Various cosolvents were tested and, surprisingly, were found to interfere with accurate measurement of the nitrite. For example, the addition of ethanol or acetone to a known quantity of inorganic nitrite lowered the apparent nitrite concentration as measured by the Griess test. Ethanol appeared to destroy nitrite itself while acetone appeared to destroy the diazonium ion intermediate. The aprotic solvents dimethylacetamide, dioxane, and dimethylformamide did not affect solutions of inorganic nitrite. However, when increasing concentrations of dimethylacetamide were added to solutions of isosorbide dinitrate of identical composition, the apparent nitrite concentration was increased. In contrast, increasing amounts of dimethylformamide slightly decreased absorbance readings (Fig. 1). Because of its negligible interaction with nitrites, nitrates, and the Griess reaction, dimethylformamide was used as the solvent in subsequent experiments (Fig. 2).

Presence of Nitrite in Bulk Organic Nitrate Esters—Two samples of isosorbide dinitrate were analyzed by the Griess reaction as previously described. Both preparations contained an initial level of nitrite that varied 10-fold between the two samples (Fig. 3A and B). In addition, in the presence of hydrochloric acid these preparations generated nitrite. Both the initial levels of nitrite as well as that generated in hydrochloric acid increased linearly with increasing concentrations of isosorbide dinitrate. The increase in the production of nitrite in the presence of hydrochloric acid was apparent for 6 hr. After this period, there was no further generation of nitrite. In contrast with the generation of nitrite in hydrochloric acid, initial levels of nitrite in phosphoric acid remained constant over time (Fig. 4).

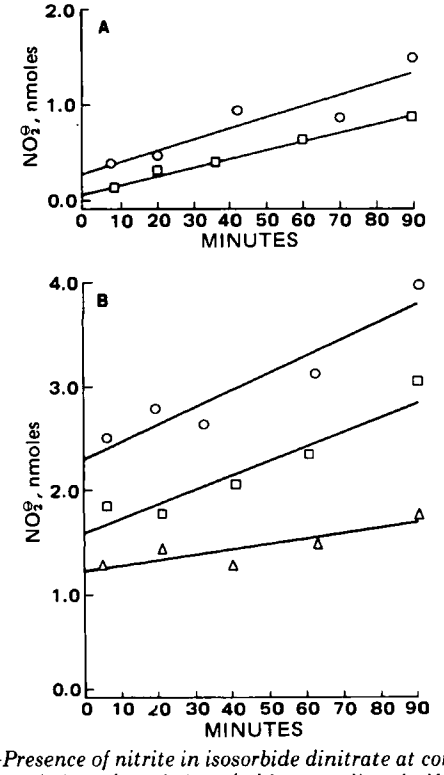

Figure 3—*Presence of nitrite in isosorbide dinitrate at concentrations of 2.0 (○), 1.5 (□), and 1.0 (△) mg/ml in 10% dimethylformamide in hydrochloric acid, pH 1.0, using formulations of 25% isosorbide dinitrate in lactose (A) or mannitol (B). Points are mean values obtained from at least two experiments.*

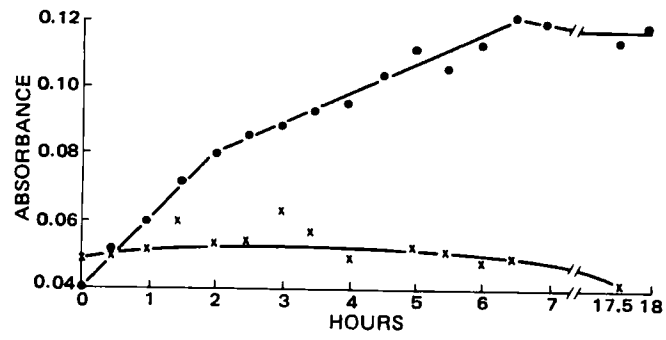

Figure 4—*Time course for generation of nitrite from isosorbide dinitrate, 25% in lactose. Points represent nitrite generated from isosorbide dinitrate (2.0 mg) in 10% dimethylformamide, pH 1.0, in hydrochloric acid (●) and phosphoric acid (×).*

Examination of samples of isosorbide dinitrate (25% in mannitol) purified by sublimation and by recrystallization, indicated the presence and generation of nitrite in amounts similar to the previously examined parent sample, except that initial levels of nitrite were lower (Table I). Thus, in hydrochloric acid, all preparations of isosorbide dinitrate generated nitrite at similar rates.

Samples of isosorbide dinitrate in lactose and mannitol were examined by the Griess reaction at pH 1, 2, and 3 under diazotization and coupling times ranging from 5 to 30 min. The amount of nitrite detected initially or generated within this range of pH was the same as that for isosorbide dinitrate samples described above (data not shown).

In comparison with isosorbide dinitrate, on a molar basis, nitroglycerin contained higher initial levels of nitrite, and nitrite was generated in hydrochloric acid at rates roughly twice that observed with isosorbide dinitrate (Table I, Fig. 5). Like isosorbide dinitrate, the production of nitrite is linear with time and the rates are essentially parallel for different concentrations of nitroglycerin. In hydrochloric acid, but not in phosphoric acid, nitrite was generated for 4 hr (Fig. 6). Because of its volatile and explosive properties, nitroglycerin was not purified further. Significantly, corresponding experiments performed on pentaerythritol tetranitrate failed to give absorbance readings above background levels; no evidence for the presence or generation of nitrite was obtained.

Finally, to eliminate the possibility that the nitrite ion could arise by reduction of the nitrate ion, we examined the action of hydrochloric acid (pH 1.0–3.0) on dilute solutions of sodium nitrate (40 m*M*) and nitric acid (40 m*M*) over 24 hr. Within the detection limits of the Griess test, no evidence of nitrite formation was obtained.

Presence of Nitrites in Formulated Organic Nitrate Vasodilators—The first studies on drugs containing isosorbide dinitrate and nitroglycerin using the sequential addition of Griess reagents (sulfanilic acid present during the incubation, followed by the addition of 1-aminonaphthalene) showed an initial level of nitrite present that appeared to decrease with time. This phenomenon may reflect destruction of the intermediate diazonium ion by an unidentified constituent(s) present in the formulated drugs. Therefore, the Griess reagents were added simultaneously so that immediately after its formation, the diazonium

Table I—Nitrite Detected in Bulk Organic Nitrate Ester Preparations

Compound	μmole of nitrite/mole of nitrate ester		
	After 2 min[a]	After 60 min[b]	Total[c]
Isosorbide dinitrate, sublimed	23.6	32.9	56.5
recrystallized	13.2	43.3	56.5
25% in lactose	13.8	30.9	44.7
25% in mannitol	114.6	44.9	159.5
Nitroglycerin, 10% in lactose	121.4	70.7	192.1
Pentaerythritol tetranitrate, 20% in lactose	—[d]	—	—

[a] The concentrations of nitrite initially detectable after 2 min in hydrochloric acid, pH 1.0. [b] The amount of nitrite generated in 60 min. [c] The sum of nitrite initially present plus nitrite generated over 60 min. [d] Not detected.

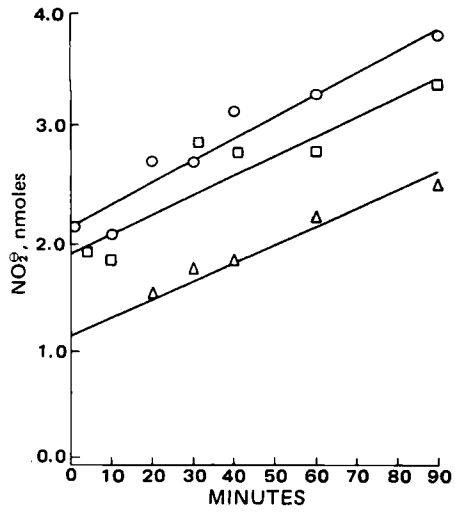

Figure 5—*Presence of nitrite in nitroglycerin, 10% in lactose, at concentrations of 2.0 (○), 1.5 (□), and 1.0 (△) mg/ml in hydrochloric acid, pH 1.0, in 10% dimethylformamide. Points are mean values obtained from at least two experiments.*

reagent could react with the coupling reagent, 1-aminonaphthalene. By this procedure, the production of inorganic nitrite was followed for 90 min, the longest time that a drug is likely to remain in the stomach. In contrast to isosorbide dinitrate and nitroglycerin, the green color of commercially formulated drugs that contained pentaerythritol tetranitrate obscured the absorbance of the dyestuff at 520 nm. Thus pentaerythritol tetranitrate-containing drugs were not analyzed for nitrite content.

At concentrations of nitrite <1 μmole/liter, the sequential and simultaneous addition of Griess reagents gave similar absorbance values. At higher concentrations of nitrite, in comparison with the sequential addition of reagents, the simultaneous addition of Griess reagents gave a decrease of 15–20% in absorbance readings for identical nitrite concentrations (Fig. 7).

Tablets and capsules of isosorbide dinitrate were found to contain varying amounts of nitrite initially (~27–216 μmoles of nitrite/mole of nitrate ester) and to generate an average of 0.55 μmole of nitrite/mole of nitrate ester/min (Fig. 8, Table II).

Tablets and capsules of nitroglycerin also varied widely in their nitrite content. Nitrite was present in initial concentrations of ~25–2290 μmoles of nitrite/mole of nitrate ester and was generated at an average rate of ~4.7 μmoles of nitrite/mole of nitrate ester/min (Fig. 8, Table II).

DISCUSSION

It is evident that most of the preparations that were examined contained small quantities of inorganic nitrite. In addition, it also appears that apart from pentaerythritol tetranitrate, these preparations probably

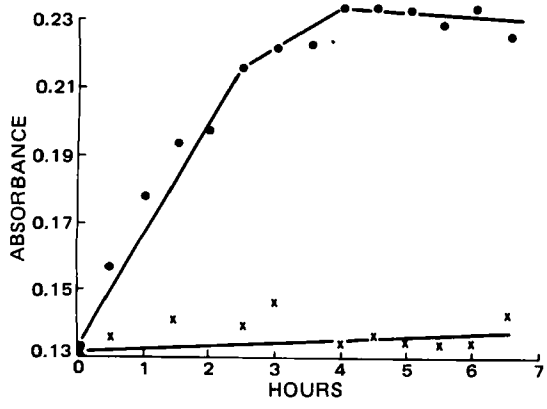

Figure 6—*Time course of generation of nitrite from nitroglycerin, 10% in lactose. Points represent nitrite generated from nitroglycerin (2.0 mg) in 10% dimethylformamide, pH 1.0, in hydrochloric acid (●) and phosphoric acid (×).*

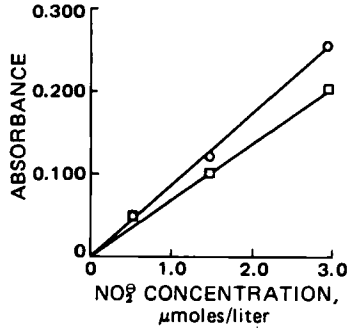

Figure 7—*Comparison of dye absorbance with sequential and simultaneous addition of Griess reagents using solutions with identical concentrations of nitrite in hydrochloric acid, pH 1.0, in 10% dimethylformamide. Key: (○) values obtained when only sulfanilic acid is present at time zero; (□) values obtained when sulfanilic acid and 1-aminonaphthalene are added together at time zero. Absorbance readings were taken 60 min later; points represent mean values from at least three experiments.*

contain small quantities of nitrite esters as impurities. These serve as the source of the continuous production of nitrite ion when the mixture is incubated with hydrochloric acid. Evidently a slow HCl-catalyzed hydrolysis is occurring; the sensitivity of nitrite esters to acidic hydrolysis is well documented (15).

In addition, acidic hydrolysis of nitrite esters is catalyzed by the presence of halide ions. Companion experiments were performed on all nitrate esters using phosphoric acid instead of hydrochloric acid to provide an acidic medium of identical pH. In all samples of isosorbide dinitrate and nitroglycerin, but not pentaerythritol tetranitrate, an initial level of nitrite was detected which corresponded to that seen in hydrochloric acid, but no further generation of nitrite was observed. These results are consistent with the findings of Allen, who showed that the rates of acid hydrolysis of propyl nitrite were increased by at least two orders of magnitude when equivalent concentrations of chloride ion were present (16).

The possibility exists, however, that the nitrite that is continually produced could come from the reduction of nitrate ion or nitrate ester by hydrochloric acid. If the claim by Oishis (9) that nitric acid is reduced to nitrous acid in dilute solution by hydrochloric acid is correct (the aqua regia mechanism), then it is difficult to explain the failure of pentaerythritol tetranitrate to yield nitrite. We have now examined dilute mixtures of both $NaNO_3$–HCl (1:4) and HNO_3–HCl (1:4) in water, over a time course of 24 hr and at pH values of 1 and 3. No evidence of nitrite (or nitrous acid) formation could be detected within the sensitivity range of the Griess reagent. These results are consistent with literature reports of hydrolysis of nitrate esters. Boschan (15) states that hydrolysis of nitrate esters predominantly yields nitrate ion, and that no subsequent reduction to nitrite occurs. However, nitrite esters containing an α-hydrogen can undergo elimination to directly form nitrite. Of course, nitric

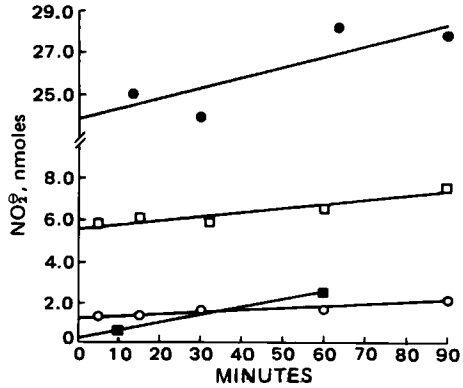

Figure 8—*Time course of nitrite production by some organic nitrate ester drug dosage forms. Drugs were incubated in hydrochloric acid, pH 1.0; points represent the mean values obtained from at least three experiments. Key: (●) 0.6-mg nitroglycerin tablet; (■) 6.5-mg sustained-action nitroglycerin capsule; (○) 40-mg sustained-action isosorbide dinitrate tablet; (□) 40-mg sustained-action isosorbide dinitrate capsule.*

Table II—Nitrite Detected in Organic Nitrate Ester Drug Formulations

Drug	μmole of nitrite/mole of nitrate ester		
	After 2 min[a]	After 60 min	Total[b]
Isosorbide dinitrate 40-mg tablet, SA[c]	63.6	28.0	91.6
40-mg capsule, SA	26.5	26.9	53.4
40-mg capsule, SA	216.0	44.0	260.0
Nitroglycerin, 6.5-mg capsule, SA	2290.0	299.0	2589.0
2.5-mg capsule, SA	1014.0	328.0	686.0
0.6-mg tablet	24.7	222.0	247.0

[a] Drugs were analyzed as described in *Experimental*. The 2-min time reflects absorbance values (520 nm) obtained 2 min after the simultaneous addition of Griess reagents and is assumed to reflect nitrite initially present. [b] Total of the nitrite initially present and the nitrite generated over 60 min. [c] SA = sustained-action or time-released preparation.

Table III—Calculated Maximal Nitrite Concentrations in Gastric Juice Arising from Dissolved Organic Nitrate Ester Drugs

Drug	μmole of nitrite/liter of gastric juice[a]	
	After 2 min	Total, After 60 min
Isosorbide dinitrate, 40-mg tablet, SA[b]	1.08	1.55
40-mg capsule, SA	0.45	0.91
40-mg capsule, SA	3.66	4.40
Nitroglycerin, 6.5-mg capsule, SA	13.10	14.80
2.5-mg capsule, SA	5.80	3.90
0.6-mg tablet	0.14	1.41

[a] Nitrite concentrations are calculated on the basis of actual levels of nitrite found (Table II). Calculations are based on the ingestion of 60 mg of isosorbide dinitrate or 19.5 mg of nitroglycerin dissolved in a 15-ml volume of gastric juice. [b] SA = sustained-action or time-released preparation.

acid and sodium nitrate could not form nitrites according to this scheme.

In comparison with unformulated isosorbide dinitrate and nitroglycerin, tablets and capsules of these drugs generally contained higher levels of nitrite initially. For isosorbide dinitrate, levels were twofold higher while two of three nitroglycerin drugs tested contained ten to twenty times more nitrite than comparable amounts of unformulated nitroglycerin. In addition, as discussed above, the values obtained for nitrite probably reflect a 15–20% underestimation of the nitrite actually present.

For isosorbide dinitrate, the amount of nitrite generated with time, 0.55 μmole of nitrite/mole of nitrate ester/min was comparable for both unformulated and formulated drug. In contrast, drugs containing nitroglycerin generated nitrite at faster rates than unformulated drug, averaging 4.7 μmoles of nitrite/mole of nitrate ester/min for formulated drug, a figure somewhat higher than that of 1.2 μmoles of nitrite/mole of nitrate ester/min given for nitroglycerin formulated with lactose alone. Again, as is discussed above, the nitrite initially present is assumed to be there as inorganic nitrite contamination, whereas nitrite which is generated with time in hydrochloric acid is believed to be due to the acid- and chloride-catalyzed hydrolysis of nitrite esters (15, 16), which are believed to be present as impurities in these drugs.

Because many nitrosamines pose a carcinogenic risk one might reasonably ask if the levels of nitrite in these drugs are sufficient to pose a risk of nitrosamine formation from other amine drugs while dissolution in the stomach is taking place. In Table III, nitrite levels actually detected in six different organic nitrate ester drug formulations (Table II) were used to calculate the nitrite concentration that would be present in gastric juice if all of the drug were completely soluble in 15.0 ml of gastric juice. It should be noted however, that these compounds are known to have limited solubility in aqueous solutions. The dosage used for these calculations are the upper limit of currently recommended single therapeutic doses of these compounds—60 mg of isosorbide dinitrate and 19.5 mg of nitroglycerin (17).

On the basis of these data, only the 6.5-mg sustained-action capsules of nitroglycerin provided more than the 10 μmoles of nitrite necessary, on a theoretical basis, to nitrosate 80 mg of propranolol hydrochloride, a secondary amine (18), or 100 mg of hydroxyzine hydrochloride, a tertiary amine[3]. Despite this, when these nitroglycerin capsules were actually incubated with propranolol hydrochloride, a nitrosamine product could not be recovered (19).

In conclusion, the organic nitrate ester vasodilator drugs, isosorbide dinitrate and nitroglycerin, contain varying amounts of inorganic nitrite and nitrite esters present as impurities. Although these nitrate esters may contain adventitious nitrite or nitrite ester, no hazard appears to be posed by the possibility that they themselves can give rise to nitrites *via* reduction with hydrochloric acid. These impurities may arise during the manufacture of the raw material, formulation into therapeutic dosage forms, and/or from decomposition during storage. In terms of the amount of nitrite provided daily by the average American diet (13 mg, 2.3 mmoles), the amount of nitrite provided by these drugs in absolute terms is quite small (20). Moreover, the risk of nitrosating secondary or tertiary amine drugs simultaneously ingested with organic nitrate esters appears to be negligible.

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