178–180°; mp 146–148°) by the rat uterotropic bioassay. The data shown in Table II indicate that at a total dose of 1 mg administered *per os* over a period of 5 days, the two isomers are relatively inactive.

In summary, comparison of the fragmentation pattern of zearalenone with F-5-3 and F-5-4 shows the latter to be stereoisomers differing from the parent compound (zearalenone) only by a hydroxyl group in the 8′ position. Formation of the 8′-chloro derivatives of both F-5-3 and F-5-4, followed by dehydrochlorination to 1′,7′-zearaldienone, and finally reduction of the latter to zearalanone, demonstrate the two isomers to be 8′-hydroxyzearalenone. Compounds F-5-3 and F-5-4 did not consume any periodate when reacted with H_5IO_6, demonstrating that both compounds are not α-hydroxy ketones.

LITERATURE CITED

Bolliger, G., Tamm, Ch., *Helv. Chim. Acta* **55**, 3030 (1972).
Christensen, C. M., Nelson, G. H., Mirocha, C. J., *Appl. Microbiol.* **13**, 653 (1965).
Jensen, N. P., Brown, R. D., Schmitt, S. M., Windholz, T. B., Patchett, A. A., *J. Org. Chem.* **37**, 1639 (1972).
Mirocha, C. J., Christensen, C. M., *Microbial Toxins* **7** (1971).
Mirocha, C. J., Christensen, C. M., Nelson, G. H., *Cancer Res.* **28**, 2319 (1968).
Scott, W. M., Wacks, M. E., Eckelson, C. D., Towne, J. C., Cazee, C., *Org. Mass Spectrom.* **1**, 847 (1968).
Stob, M., Baldwin, R. S., Tuite, J., Andrews, F. N., Gillette, K. G., *Nature (London)* **196**, 1318 (1962).
Umberger, E. J., Gass, G. H., Curtis, J. M., *Endocrinology* **63**, 806 (1958).
Urry, W. R., Wehrmeister, H. L., Hodge, E. B., Hidy, P. H., *Tetrahedron Lett.* **27**, 3109 (1966).
Weiss, F. T., "Determination of Organic Compounds, Methods and Procedures," Wiley-Interscience, New York, N.Y., 1970.

Received for review May 28, 1974. Accepted August 12, 1974. Scientific Journal Series Paper No. 8713, Minnesota Agricultural Experiment Station. This research was supported in part by U.S. Public Health Service Research Grant No. 2R01-F-D-00035-06.

Gas Chromatographic–Mass Spectrometric Determination of *N*-Nitrosodimethylamine Formed in Synthetic and Human Gastric Juice

Richard P. Lane,[1] Roy H. Rice,[2] and Milton E. Bailey*

An analytical procedure for determination of *N*-nitrosodimethylamine in human gastric juice is described. *N*-Nitrosodiethylamine is used as an internal standard to quantitate *N*-nitrosodimethylamine and high-resolution mass spectrometry is used to detect and confirm identity of the nitrosamine. This procedure could be useful for estimating levels of nitrosamines at 0.1 ppb. Glycine competed for nitrite in a reaction mixture containing nitrite, *N*-nitrosodimethylamine, and glycine in synthetic gastric juice. A mixture of amino acids retarded nitrosamine formation at pH 2.5, but not at other pH values studied. Meat extract from cooked pork also retarded nitrosamine formation at pH 2.5 and 4.5.

As a result of the extreme toxicity (Ender *et al.*, 1964) and carcinogenicity (Druckery *et al.*, 1969) of *N*-nitrosamines it is necessary to evaluate the possibility of the occurrence of these compounds in the human environment. It has been suggested that nitrite and secondary amines, though not abundant, may react in various stages of food processing (Ender and Ceh, 1970) or during gastric digestion of nitrite treated foods (Lijinsky and Epstein, 1970). Though results of several studies have demonstrated the formation of *N*-nitrosamines from uncommonly large amounts of both precursors during processing (Ender and Ceh, 1970; Sen *et al.*, 1970) and in gastric digestion (Sen *et al.*, 1969; Greenblatt *et al.*, 1971; Alam *et al.*, 1971) it has not been demonstrated that a significant health hazard exists as a result of nitrite in the various cured food products at levels currently permitted by law.

Recent advances in analytical methodology (Howard *et al.*, 1970; Fazio *et al.*, 1971b; Crosby *et al.*, 1972) have enabled detection and confirmation of various *N*-nitrosamines at trace levels in a number of different food products. At present there have been a number of positive identifications of these compounds in various nitrite and non-nitrite-treated foods at levels ranging from 4 to 130 ppb (Fazio *et al.*, 1971a,b; Crosby *et al.*, 1972; Wasserman *et al.*, 1972). Recently, Sen *et al.* (1972) confirmed the presence of 120–450 ppb of *N*-nitrosodimethylamine in samples of toxic fish meal though neither nitrite nor nitrate was used in processing.

Increased analytical capabilities in *N*-nitrosamine research have been largely a result of development of efficient extraction and clean-up procedures, and sensitive detection methods. This has enabled a practical detection limit, with positive confirmation, of 10 ppb for certain *N*-nitrosamines (Fazio *et al.*, 1971a; Wasserman *et al.*, 1972). Crosby *et al.* (1972) increased this detection limit to 1–4 ppb by increasing sample size and using high-resolution mass spectrometry.

Considering these recent advances in analytical methodology and the lack of information concerning gastric formation of *N*-nitrosamines from the occasional low amounts of nitrite in the human diet, it was desirable to develop an analytical method suitable for analysis of trace levels of *N*-nitrosodimethylamine isolated from human gastric juice.

Described here is an analytical method capable of detection and confirmation of *N*-nitrosodimethylamine at levels as low as 0.1 ppb in aqueous solutions of human gastric juice following the incubation of nitrite and dimethylamine. We found the "modified thermionic detector" (alkali flame detector) described by Howard *et al.* (1970) to be of great significance in the routine analysis of gastric juice extracts from DMNA due to the large number of interfering compounds. High-resolution mass spec-

Department of Food Science and Nutrition, University of Missouri, Columbia, Missouri 65201.
[1] Present address: Warner Jenkinson, St. Louis, Mo. 63106.
[2] Present address: Station Chemical Laboratories, University of Missouri, Columbia, Mo. 65201.

Table I. Glc-Mass Spectral Operating Conditions

Gas chromatograph	Varian Aerograph
Mass spectrometer	CEC 21–110C
Separator	Llewellyn
Ionizing volt	70 eV
Ion source pressure	1.4×10^{-5} Torr
Source temperature	250°
Trap current	80 μA
Accelerating current	8 kV
Multiplier volt	Min gain
Resolution	1/1500 and 1/12,000
Column: 10% w/w Carbowax 1540 + 3% w/w KOH on 100–120 mesh acid-washed Chromosorb P, 3.0 m × 2 mm i.d. glass	
Transfer line: 4 mm i.d. glass at 200° with two-way stainless steel valve (Nupro)	

trometry was used to confirm the presence of these low levels of DMNA.

EXPERIMENTAL SECTION

Materials. All reagents used in this study were analytical reagent grade or purified by the procedures of Howard *et al.* (1970). The modified concentrator tube was fabricated from Pyrex tube glass, with female standard taper 19/22 ground glass joint, to hold 7–8 ml of solution at a level well below the ground glass joint and having a smooth taper to a narrow neck region (5 mm i.d.) at the bottom of the tube. This design resulted in a smoother concentration and simplified the substitution of the micro-Snyder apparatus when the solution had concentrated to the 7-ml level. The micro-Snyder column was the two-stage type described by Burke *et al.* (1966) and made from 10 mm o.d. glass tubing with a male standard taper 19/22 ground glass joint at the base.

Extraction Procedure. A 100-ml sample of gastric juice reaction mixture was transferred to a 1000-ml round-bottomed flask following 1-hr reaction at 37°. The gastric juice was made alkaline by adding 150 ml of 16% KOH, with *ca.* 20 ml of this being used to wash the reaction vessel. Methanol (10 ml) was added and the solution refluxed under the Friedrich condenser. Following 4 hr of reflux, the solution was cooled, a distilling column (200 mm long) and condenser were inserted, and 100 ml of the sample was carefully distilled into a 500-ml separatory funnel (low actinic glass) with a Teflon stopcock. The distillate was acidified (pH 1.5) with 5 ml of 2.5 *N* H_2SO_4. A small amount of Na_2SO_4 was added and the acidic distillate extracted four times with 50-ml volumes of CH_2Cl_2 (3-min shake). The CH_2Cl_2 extract was extracted with 100 ml of 1 *N* NaOH and the lower CH_2Cl_2 layer slowly passed through 30 g of Na_2SO_4 in a 60-ml glass fritted funnel, into a 300-ml Kuderna-Danish evaporative concentrator with modified concentrator tube. The funnel was rinsed with 50 ml of CH_2Cl_2. Carborundum grains were added and a three-stage Snyder concentrating column inserted. This apparatus was lowered into a heated (75°) oil bath and the extract carefully concentrated to *ca.* 7 ml (requires 4 hr).

The apparatus was removed from the oil bath and rinsed with CH_2Cl_2. Upon cooling, the concentrator tube was carefully removed, and additional carborundum added, along with 1 ml of *n*-hexane, and the micro-Snyder column (pre-wetted with CH_2Cl_2) was inserted. This micro apparatus was suspended above a gentle steam bath made from a 600-ml beaker covered with aluminum foil with a small 0.5-in. hole. The concentrator tube was lowered *ca.* 1 in. into this hole, and the extract was further concentrated to 1.0 ml.

Column Clean-Up. The 1.0-ml concentrate was transferred with a disposable pipet to an acidic Celete column described by Howard *et al.* (1970). After clean-up, the solubles were eluted from the column with 70 ml of CH_2Cl_2 and collected in a 250-ml Kuderna-Danish concentrator with modified concentrator tube. Five micrograms of *N*-nitrosodiethylamine (DENA) was added as internal standard and the cleaned extract concentrated to <1.0 ml using the method previously described. Following concentration, the extract was transferred to 4-ml storage vials with Teflon caps and stored in a refrigerator until analysis. Tightly capped vials could be stored for at least 2 weeks without loss of *N*-nitrosodimethylamine (DMNA).

Glc Analysis. An F&M Model 402 gas chromatograph equipped with an alkali flame detector (Howard *et al.*, 1970) was employed for routine analyses. Gas flows were: 44 ml of hydrogen, 40 ml of nitrogen, and 275 ml of air per minute. Column temperature was maintained at 140°, while injection port and detector temperatures were 185 and 230°, respectively. The ionization detector electrode was positioned at 1–2 mm above the alkali coil and hydrogen flow adjusted periodically to give a background current of 2.6×10^{-9} A. This enabled a maximum sensitivity for DMNA of 1.5×10^{-11} g/sec (1.5×10^{-9} C response/ng of DMNA) at an attenuation of 8×10^{-10} AFS. While a higher hydrogen flow resulted in an increase in background current and an increased response for nitrogen, this was not favorable for routine analyses as the higher flame caused an increase in noise and readily exhausted the alkali coil. An 8-μl sample was injected on 10 w/w % Carbowax 1540 + 3 w/w % KOH coated 100–120 mesh Chromosorb P packed in a 3.0 m × 4 mm i.d. glass column.

Glc-Mass Spectral Confirmation. A Varian Aerograph 1520 gas chromatograph-CEC 21-110C mass spectrometer combination equipped with peak matching facilities provided low- and high-resolution spectra. For operating conditions see Table I.

(a) *Sample Preparation.* Samples with an apparent DMNA content of 1.0 μg or more were further concentrated to <200 μl with a gentle stream of clean N_2 for low-resolution mass spectral analysis.

(b) *Low Resolution Mass Spectrometry.* Samples were scanned at a speed of 10 sec/decade at a retention time equal to that of the DMNA standard. At this time, the F.I.D. detector indicated that two or more peaks were eluted and identification of DMNA could not be confirmed by this method. Attempts were made to separate the various interfering solutes by using different chromatographic columns, programs, and flow rates, but these were not successful. The low-resolution spectra did indicate that DMNA was probably present in the mixture.

(c) *High-Resolution Mass Spectrometry.* Peak matching equipment of the CEC 21-110C was used to verify the presence of the parent ion peak of DMNA (*m/e* 74.0480) in the samples, at the same retention time as the standard. The mass spectrometer was tuned to a resolution of 1/12,000 (10% valley). Perfluorokerosene peaks of *m/e* 68.99520, 73.99680, and 75.00463 were used to check the accuracy of the peak matching equipment. With ion peak *m/e* 68.99520 as a reference peak, *m/e* 73.99680 was measured as 73.99688. Ion peak *m/e* 75.00463 was measured as 75.00463. During the experiment as a constant *m/e* 75.00463 was monitored to ensure high accuracy.

Reactions of Nitrite, Dimethylamine, and Glycine in Synthetic Gastric Juice. Nitrite and dimethylamine were incubated in synthetic gastric juice at 37°, pH 3.4, for 1 hr with and without glycine to study its effect on N-nitrosation. The synthetic gastric juice was prepared according to Spector (1956) relative to concentrations of $CaCl_2$, KCl, NaCl, pepsin, and HCl. The concentrations of sodium nitrite, dimethylamine, HCl, and glycine were, respectively, 20–30, 20–30, and 0–10 mg/100 ml of reaction mixture. Reactions were carried out at 37°.

Table II. Composition of Amino Acid Solutions

Solution ident	Amino acid	Concn, mg/ml
A	Glycine	0.51
	L-Leucine	0.28
	DL-Isoleucine	0.20
	DL-Valine	0.37
	L-Alanine	1.51
	L-Lysine[a]	0.64
	DL-Phenylalanine	0.20
	DL-Tyrosine	0.36
	L-Histidine[b]	0.47
	L-Aspartic acid	0.27
	L-Glutamic acid	0.33
	L-Methionine	0.12
	DL-Ornithine[b]	0.36
	Total	5.62
B	Taurine	3.85
	Carnosine	21.80
	Total	25.65

[a] Dihydrochloride. [b] Hydrochloride.

Sample Preparation and Chemical Reaction Mixtures in Human Gastric Juice. Gastric juice was collected by stomach tube from normal male and female students between 1 and 3 p.m. following fasting for 24 hr except toast for breakfast. The samples were filtered and compiled according to pH.

Two solutions (A and B) of amino acids were prepared at concentrations given in Table I. A 5-ml aliquot of A and a 1-ml aliquot of B were added to the appropriate gastric juice samples prior to incubation. This mixture was used to simulate free amino compounds of cooked pork.

A 200-ml aqueous meat extract was prepared by filtering a meat slurry of 144 g of comminuted broiled (76°) pork loin in 288 ml of distilled water. Aliquots used represented the soluble components of 30 g of cooked meat.

Fifty milliliter aliquots of gastric juice were used at four different pH values (1.7, 2.5, 3.5, and 4.5) in three different reaction mixtures: (A) 50 ml of gastric juice, 10 mg of dimethylamine (18.2 mg of dimethylamine hydrochloride), and 10 mg of nitrite (15 mg of sodium nitrite) diluted to 100 ml with water after pH adjustment with 1 *N* HCl or 1 *N* NaOH; (B) same as A except it contained 5 ml of amino acid mixture A (Table II) and 1 ml of amino acid mixture B (Table II); (C) same as A except it contained 40 ml of meat extract. The reaction mixtures were incubated with gentle shaking in 250 Teflon-sealed, brown glass bottles for 1 hr at 37°.

RESULTS AND DISCUSSION

The response of the alkali flame detector was calibrated with nanogram quantities of DMNA to ensure linear response. The relative molar response [RMR(DMNA/I.S.)] using *N*-nitrosodiethylamine (DENA) as the internal standard was 0.96. These results indicated that the detector was responding primarily to nitrogen. If the detector was responding solely to nitrogen the RMR(DMNA/I.S.) by calculation would be 1.00 as both DMNA and DENA contain the same number of nitrogen atoms. The RMR(DMNA/I.S.) then was used to indicate the selectivity of the detector as well as for the quantitation of DMNA in samples.

Since the internal standard was added prior to the final concentration, it was necessary to determine if this concentration step caused a change in RMR(DMNA/I.S.). After several concentrations from 100 to 0.5 ml it was concluded that the relative molar response remained unchanged.

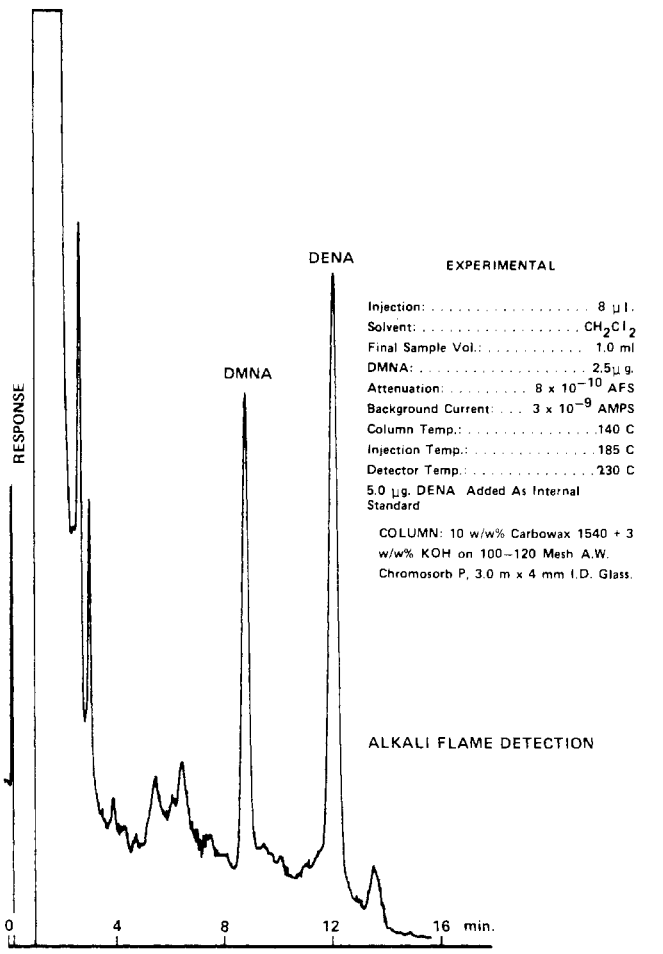

Figure 1. Typical gas–liquid chromatogram of DMNA extracted from human gastric juice reaction mixture (100 ppm of NO_2^- plus 100 ppm of dimethylamine) using DENA as internal standard.

The possibility of DMNA formation during the extraction procedure was checked by adding 100 ppm of NO_2 and 100 ppm of dimethylamine to two gastric juice samples after the KOH solution had been added. The solutions were extracted by the procedure described and DMNA was not found in either of the samples.

A typical gas chromatogram of DMNA extracted from a reaction in human gastric juice is shown in Figure 1. Although the retention times for this peak and DMNA in a standard solution were identical it was necessary to confirm identity of the nitrosamines by mass spectroscopy. The low-resolution (1/1500) mass spectra of a gastric juice reaction extract and 100 ng of DMNA are shown in Figure 2. The conditions shown in Table I were used for these determinations. The extract had been concentrated to <200 µl and contained 10 ng/µl of apparent DMNA as determined by preliminary glc analysis. A 10-µl sample was injected. The absolute intensities of the *m/e* 74 peak for the sample and the standard were approximately equal. The ion peaks, *m/e* 42, 43, and 30, were also present in both the extract and the standard. There were, however, several other prominent peaks (*m/e* 59, 57, 55, and 71) in the extract that were not in the background or the standard. Though these peaks were apparently due to extraneous material they could not all be eliminated and some of the peaks apparently were from material with the same retention time as DMNA.

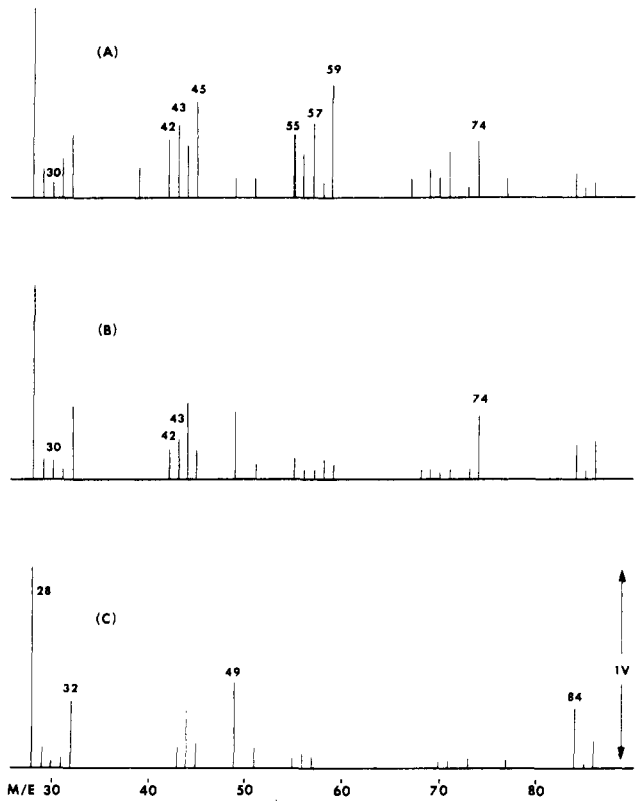

Figure 2. Mass spectra of extract from human gastric juice and *N*-nitrosodimethylamine (DMNA): (A) 100 ng of apparent DMNA extracted from human gastric juice reaction mixture (100 ppm of NO_2^- + 100 ppm of dimethylamine, pH 3.5); (B) 100 ng of DMNA standard; (C) background for B.

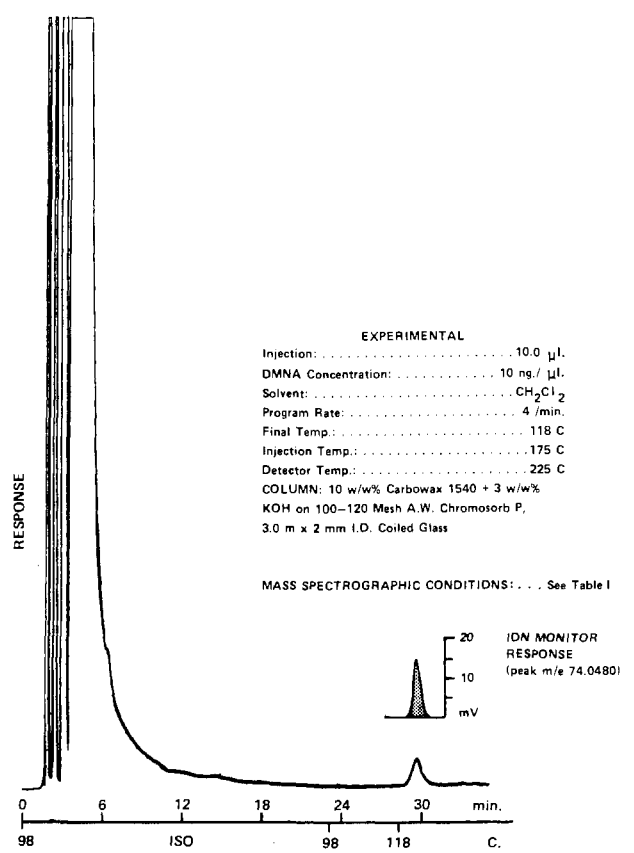

Figure 3. Gas–liquid chromatogram of DMNA standard showing high resolution mass spectral response (see inset, Ion Monitor Response) (peak *m/e* 74.0480) at 29.9-min retention time.

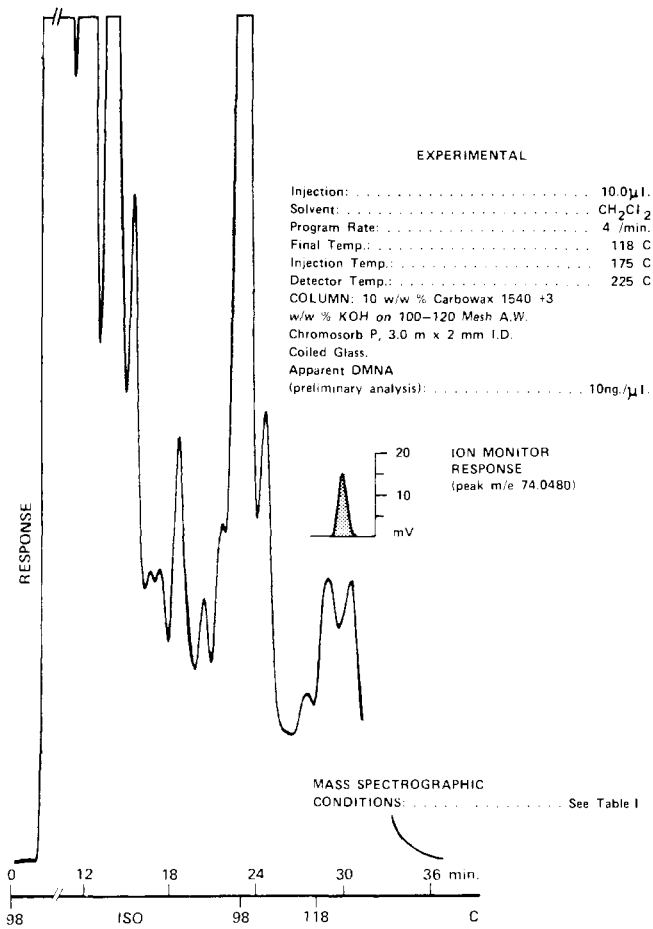

Figure 4. Gas–liquid chromatogram of DMNA in human gastric juice reaction mixture (100 ppm of NO_2^- plus 100 ppm of dimethylamine) showing high-resolution mass spectral response (see inset, Ion Monitor Response) (peak *m/e* 74.0480) at 29.9-min retention time.

The high-resolution mass spectral analyses offered more substantiating data than low-resolution mass spectra for verification of DMNA in the samples. Using the peak matching equipment of the CEC 21-110C, a 15-mV response was measured for a 100-ng DMNA standard at the molecular ion peak *m/e* 74.0480 (Figure 3). A 10-µl injection of a sample containing 10 ng/µl of apparent DMNA gave an equal response at ion peak *m/e* 74.0480 (Figure 4). It was only in the area of the 29.9-min retention time that this particular ion peak was present. In addition, there was no other ion peak between *m/e* 73.5000 and 74.5000 during the time the DMNA peak was being monitored.

From the above data, it was concluded that the apparent DMNA determined quantitatively by glc from human gastric juice was indeed *N*-nitrosodimethylamine; furthermore, the ion peak *m/e* 74 seen at low resolution was concluded to be DMNA. The conditions used in the high-resolution analyses were such that the sensitivity could readily be increased 100 times if necessary. This would make possible the confirmation of 1 ng of DMNA on the column or 100 pg/µl of DMNA in an extract of gastric juice using the procedure described. Thus, it is possible to confirm DMNA in gastric juice at a concentration of 0.1 ppb from 100 ml of gastric juice or 1 ppb from 10 ml of gastric juice. With this level of sensitivity, the quantitative aspects of the analysis are greatly enhanced. It should be possible to detect and confirm if DMNA formed *in vivo* after the digestion of cured meats or directly at very low levels in food materials.

The recovery of 2.5–10.0 µg of DMNA from ten 100-ml

Table III. Effect of Glycine on N-Nitrosation of Dimethylamine in Synthetic Gastric Juice[a,b]

$[NaNO_2]$, m*M*	[DMA-HCl], m*M*	[Glycine], m*M*	[DMNA], mg/l.	% inhib.
2.90	2.45	0.00	47	
2.90	2.45	1.33	8	83
4.35	3.68	1.33	35	43[c]

[a] Prepared according to Spector (1956). [b] 100-ml total volume; 1-hr reaction, 37°, pH 3.4. [c] Calculated from theoretical yield based on rate equation of Mirvish (1970).

aqueous solution samples averaged 87% (76–96%) and that from three 100-ml human gastric juice samples containing 5.0 μg of DMNA averaged 79% (76–82%).

Influence of Glycine on Nitrosamine Formation in Synthetic Gastric Juice. The effect of glycine on formation of DMNA in synthetic gastric juice is shown in Table III. Glycine apparently competed for nitrite as anticipated from the work of Taylor and Price (1929). The results indicated that 1.3 m*M* glycine inhibited nitrosamine formation approximately 80% in presence of the lower level of reactants and 87% with the higher level of reactants. The latter result was calculated using the theoretical yield from the rate equation of Mirvish (1970) assuming a rate constant of 0.198 mol^{-2} min^{-1} $l.^2$ at 37°, pH 3.4.

Influence of pH, Amino Acids, and Pork Muscle Extract on Nitrosamine Formation in Human Gastric Juice. Data in Table IV reveal that the pH for maximal nitrosamine formation in human gastric juice was 2.5 instead of 3.4 as predicted from nitrous anhydride reactions (Mirvish, 1970). Except at pH 2.5, the amino acid mixture did not impede nitrosamine formation and at pH 4.5, the nitrosamine formed in the presence of the amino acid mixture was unusually high. The meat extract did not appreciably effect nitrosamine formation except at pH 2.5 and 4.5 where the inhibition was approximately 30%.

The quantity of *N*-nitrosodimethylamine formed in human gastric juice by treating dimethylamine with sodium nitrite at pH 3.5, 37°, for 1 hr was approximately twice that predicted from kinetic data of Mirvish (1970). These results indicate that additional reaction mechanisms involving nitrosyl chloride and nitrosyl thiocyanate intermediates catalyze nitrosation of dimethylamine in human gastric juice. Nitrosyl thiocyanate catalysis could also account for the shift in pH optimum as found by Fan and Tannenbaum (1973) working with a model system.

Table IV. Influence of Free Amino Acids, Porcine Muscle Aqueous Extract, and pH on *N*-Nitrosodimethylamine Formation in Human Gastric Juice

	N-Nitrosodimethylamine formed, ppb		
pH	(GG)[a]	(GG + AA)[b]	(GG + ME)[c]
1.7	17.6	24.6	24.8
2.5	33.8	28.8	24.3
3.5	20.4	21.7	21.4
4.5	9.0	22.8	6.1

[a] 50 ml of gastric juice, 10 mg of dimethylamine, 10 mg of nitrite; diluted to 100 ml with H_2O following pH adjustment; 1-hr reaction; 37°. [b] Same as footnote *a* except reaction mixture contained 5 ml of amino acid mixture A and 1 ml of amino acid mixture B, Table II. [c] Same as footnote *a* except reaction mixture contained 41 ml of meat extract.

LITERATURE CITED

Alam, B.S., Saporoschetz, I.B., Epstein, S.S., *Nature (London)* **232,** 116 (1971).
Burke, J.A., Mills, P.A., Bostwick, D.C., *J. Ass. Offic. Anal. Chem.* **49,** 999 (1966).
Crosby, N.T., Foreman, J.K., Palframan, J.F.R., *Nature (London)* **238,** 342 (1972).
Druckery, H., Preussmann, H., Ivankovic, S., *Ann. N. Y. Acad. Sci.* **163,** 676 (1969).
Ender, F., Ceh, L., *Z. Lebensm.-Unters. Forsch.* **145,** 133 (1970).
Ender, F., Havre, G., Helgebostad, A., Koppang, N., Madsen, R., Ceh, L., *Naturwissenschaften* **51,** 637 (1964).
Fan, T.Y., Tannenbaum, S.R., *J. Agr. Food Chem.* **21,** 237 (1973).
Fazio, T., Damico, J.N., Howard, J.W., White, R.H., Watts, J.O., *J. Agr. Food Chem.* **19,** 250 (1971a).
Fazio, T., White, R.H., Howard, J.W., *J. Ass. Offic. Anal. Chem.* **54,** 1157 (1971b).
Greenblatt, M., Mirvish, S., So, B., *J. Nat. Cancer Inst.* **46,** 1029 (1971).
Howard, J.W., Fazio, T., Watts, J.O., *J. Ass. Offic. Anal. Chem.* **53,** 269 (1970).
Lijinsky, W., Epstein, S.S., *Nature (London)* **225,** 21 (1970).
Mirvish, S.S., *J. Nat. Cancer Inst.* **44,** 633 (1970).
Sen, N.P., Schwinghamer, L.A., Donaldson, B.A., Miles, W.F., *J. Agr. Food Chem.* **20,** 1280 (1972).
Sen, N.P., Smith, D.C., Schwinghamer, L., *Food Cosmet. Toxicol.* **7,** 301 (1969).
Sen, N.P., Smith, D.C., Schwinghamer, L., Howsam, B., *Can. Inst. Food Technol.* **3,** 66 (1970).
Spector, W.S., "Handbook of Biological Data," Wright Air Development Center, Air Research and Development Command, USAF, Wright-Patterson Air Force Base, Ohio, 1956, p 61.
Taylor, T.W.J., Price, L.S., *J. Chem. Soc.*, 2052 (1929).
Wasserman, A.E., Fiddler, W., Doerr, R.C., Osman, S.F., Dooley, C.J., *Food Cosmet. Toxicol.* **10,** 681 (1972).

Received for review May 24, 1973. Accepted July 22, 1974. Contribution from the Missouri Agricultural Experiment Station. Journal series no. 6745.