

## SHORT PAPERS

# NITROSAMINE LEVELS IN HUMAN BLOOD, URINE AND GASTRIC ASPIRATE FOLLOWING INGESTION OF FOODS CONTAINING POTENTIAL NITROSAMINE PRECURSORS OR PREFORMED NITROSAMINES

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**Abstract**—In studies of the effect of diet on nitrosamine levels in selected human physiological fluids, volunteers were fed meals containing fish or beef (sources of precursor amines) or bacon (a source of preformed nitrosamines), in combination with spinach and vegetable juice to supply nitrite via possible reduction of nitrate. Blood, urine and gastric contents were sampled periodically for up to 4 hr after feeding. The results of the study indicated that traces of nitrosamines, usually *N*-nitrosodimethylamine, were present in many samples of blood, urine and gastric contents, even after an 8-hr fast. Eating the test meals led to a slight increase in nitrosamine levels in the blood and stomach contents in a few subjects. The data obtained from this study suggest that gastric formation of nitrosamine does not appear to be an important health factor in normal people, since the levels of nitrosamines found in physiological fluids are not markedly increased after eating.

### Introduction

Nitrite is present in saliva in fasting subjects, and salivary nitrite levels increase after ingestion of vegetables (Hajimu, Boriboon, Nakamura *et al.* 1975; Tannenbaum, Weisman & Fett, 1976). The nitrite is formed by oral bacterial reduction of nitrate and could serve to nitrosate ingested compounds in the stomach (Spiegelhalter, Eisenbrand & Preussmann, 1976) to produce potentially carcinogenic *N*-nitrosamines. Similarly other sites in the body could also favour nitrosamine formation, particularly under abnormal conditions. Sander & Seif (1969) demonstrated the feasibility of *in vivo* formation by feeding nitrate and diphenylamine to an achlorhydric subject and subsequently detecting *N*-nitrosodiphenylamine in the stomach contents. Volatile nitrosamines have been detected in human blood (Fine, Ross, Rounbehler *et al.* 1977; Lakritz, Simenhoff, Dunn & Fiddler, 1980), in urine (Brooks, Cherry, Thacker & Alley, 1972; Hicks, Gough & Walters, 1978) and in faeces (Wang, Kakizoe, Dion *et al.* 1978).

\*Retired.

†Reference to a brand or firm name does not constitute endorsement by the US Department of Agriculture over other products of a similar nature that are not mentioned.

‡Note: Precaution should be exercised in the handling of nitrosamines since they are potential carcinogens.

Abbreviations: DCM = Dichloromethane; NDEA = *N*-nitrosodiethylamine; NDMA = *N*-nitrosodimethylamine; NMEA = *N*-nitrosomethylethylamine; NPYR = *N*-nitrosopyrrolidine.

Several studies to determine the extent of *in vivo* nitrosamine formation have been conducted. Walters, Dyke, Saxby & Walker (1976) passed food slurries through an oral tube and detected up to 0.5 ppb (ng/ml) *N*-nitrosopiperidine in the stomach contents. Fine *et al.* (1977) reported *in vivo* formation of *N*-nitrosodimethylamine (NDMA) and *N*-nitrosodiethylamine (NDEA) in blood from a single subject who had eaten a meal containing bacon, beer, vegetables and bread. The effect of diet on blood-nitrosamine levels was also studied by Yamamoto, Yamada & Tanimura (1980) who fed Japanese diets rich in amines and nitrate to volunteers.

The study reported here was concerned with the effects of diet on levels of nitrosamines (arising either from ingestion of preformed nitrosamines or from endogenous formation) in the blood, urine and gastric contents of volunteers fed several different diets.

### Experimental‡

Twenty-one normal healthy male and female volunteers (aged 19–60 yr) participated in this study on one or more occasions. All physiological samples were obtained under the supervision of a gastro-enterologist at a hospital.

*Diets.* For study A (the preliminary study) and study B, the test meals consisted of 100 ml commercial vegetable juice (containing tomato, carrot, celery, beet, parsley, lettuce, watercress, spinach and ascorbic and citric acids) together with 100 g canned spinach and 125 g fresh cod. The fish fillets were baked at

300°F for 35 min in aluminium foil without condiments. For study C, 125 g pan-fried beef or 125 g fried bacon was substituted for the fish, rendered fat being removed and not ingested. Samples of all foods were assayed for nitrosamines. The volunteers fasted overnight for at least 8 hr before the study began.

*Sampling procedures and protocols.* For blood analyses, 10 ml of venous blood was drawn by venepuncture using a syringe and was transferred to and stored in a glass beaker. Commercially available evacuated blood-collection tubes were not used because of the possibility of nitrosamine contamination from the rubber stoppers, as reported by Lakritz & Kimoto (1980). Urine samples were collected in plastic specimen bottles. Samples of gastric contents were obtained by inserting a Levin tube through the nose (or in two cases through the mouth) into the stomach. Neither local anaesthetics nor any liquids were necessary to facilitate the placement of the Levin tube. Samples of 25 ml were aspirated from the stomach for the fasting specimen; the other samples varied in volume. Volunteers were usually in a prone position and were rotated so that sufficient sample could be obtained. All samples collected were immediately made alkaline and frozen.

The preliminary study was conducted to establish appropriate experimental conditions. Due to the paucity of information concerning the rate of formation, transport and metabolism of nitrosamines in humans, it was necessary to determine the optimum times for sample collection. For this study physiological samples were collected from ten subjects before ingestion and at various intervals ranging from 15 min to 4 hr after ingestion of the fish diet. To obtain samples of gastric contents, the Levin tubes were initially placed through the nose or mouth. The food was puréed, since it was assumed that it would be difficult to eat with a Levin tube in place. The presence of an intubation tube in the mouth caused excessive salivation, however, so this practice was discontinued and all samples were collected via nasal intubation. This enabled the subjects to ingest the food intact.

In study B, six subjects (nos 11–16) were all intubated nasally and were fed the diet of fish, spinach and vegetable juice. Samples of gastric aspirate, blood and urine were taken before and 1 hr after feeding and a second blood sample after a further 1 hr. In study C, five volunteers (nos 17–21) were fed the diet in which fried bacon replaced the cod, and in addition to the fasting samples, samples from the stomach were taken 1 hr after the meal and blood and urine samples 1 and 2 hr after feeding. These same subjects participated in a second session in which the sampling schedule was the same but the bacon in the test meal was replaced by a grilled hamburger.

*N-Nitrosamine analyses.* Samples of blood and gastric contents were analysed for volatile nitrosamines by a modification of a procedure described by Telling, Bryce & Althorpe (1971). *N*-Nitrosomethylethylamine (NMEA; 1.0 ml of a 0.05 µg/ml solution) was added to each sample as an internal standard. Samples were added to flasks containing NaOH, Ba(OH)<sub>2</sub> and water and the mixture was distilled. Sodium chloride was added to the distillate, which was then extracted three times with dichloromethane (DCM). The DCM layer was washed with 6 N-HCl and with 5 N-NaOH,

dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated to 1.0 ml in a Kuderna-Danish evaporator flask equipped with a concentrator tube. Urine samples were extracted in a liquid/liquid extractor described by Fazio, White, Dusold & Howard (1973). Sodium chloride and the internal standard (NMEA) were added to the urine sample, which was continuously extracted with DCM for 5 hr. The extracts were concentrated as described above. Food samples (fish, spinach, meats) were digested in alcoholic KOH and then subjected to liquid/liquid extraction with DCM followed by distillation from base.

The DCM extracts were concentrated and analysed with a gas chromatograph interfaced with a Thermal Energy Analyzer (Model 502, Thermo Electron Corp., Waltham, MA), a selective nitrosamine detector. The nitrosamines were separated on a Varian-Aerograph Model 1720 gas chromatograph (Palo Alto, CA) equipped with a nickel column (9 ft × 1/8 in. OD) packed with 15% Carbowax 20 M-TPA on 60–80 mesh Gas Chrom P. The injector port temperature was 220°C and the column temperature was programmed from 110 to 220°C at 4°C/min. With a helium flow rate of 42 ml/min, NDMA, NDEA and *N*-nitrosopyrrolidine (NPYR) eluted at 4.2, 5.6 and 14.5 min, respectively. The Thermal Energy Analyzer was operated under conditions similar to those used by Fine & Rounbehler (1975). Samples containing concentrations as low as 0.1 ppb could be detected readily. Presumptive confirmation was obtained by the ultraviolet photolysis procedure described by Doerr & Fiddler (1977). Samples containing sufficient amounts of nitrosamines were also subjected to gas chromatographic-mass spectrophotometric analysis for confirmation. The analyses were performed using a Varian-Aerograph Model 2700 gas chromatograph equipped with a glass column (6 ft × 1/4 in. OD) packed with 15% Carbowax 20 M-TPA on Gas Chrom P connected to a Varian MAT 311A mass spectrometer (Finnigan-MAT, Sunnyvale, CA). The helium flow rate was 15 ml/min; the temperatures of the detector, injector port and gas chromatograph-mass spectrometer interface systems were 200, 200 and 180°C, respectively. Column and operating conditions were similar to those described by Lakritz & Kimoto (1980).

## Results and Discussion

### *Preliminary study*

The levels of NDMA found in the analyses for volatile nitrosamines carried out on gastric aspirates from the ten subjects tested in the preliminary studies are presented in Fig. 1. Concentrations of NDMA were considerably higher in the first two subjects, possibly because in these two instances the meals were prepared and puréed several hours before they were consumed and were stored at room temperature, thus providing opportunity for the formation of nitrosamines. In addition to the NDMA detected in the gastric samples from subject no. 2, two of these samples contained 2 and 3 ppb NDEA although this nitrosamine was not detected in the food prior to

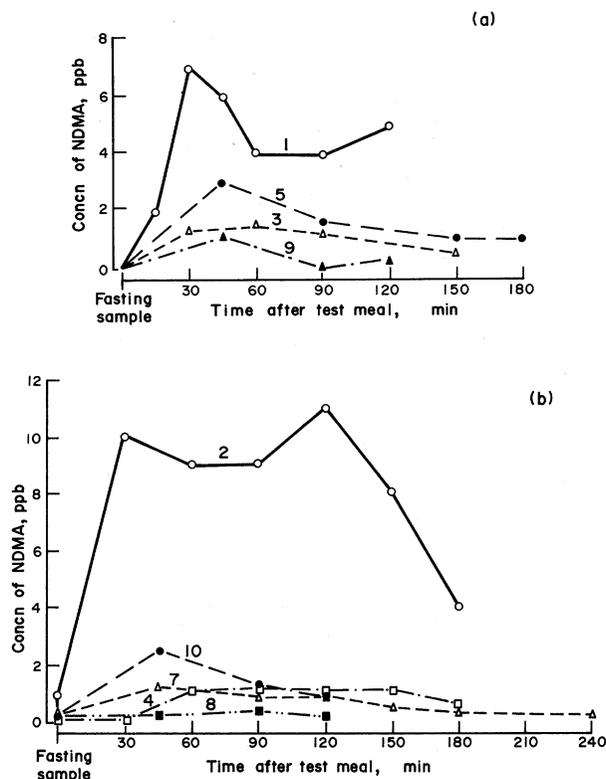


Fig. 1. Concentrations of *N*-nitrosodimethylamine (NDMA) in samples of gastric aspirate taken from test subjects after fasting and at intervals after ingestion of a meal consisting of fish, spinach and vegetable juice. Numbers on graphs (a) and (b) identify the different subjects. No samples could be taken from subject 6.

ingestion. Both NDMA and NDEA were confirmed by mass spectrometry.

Examination of data by linear regression demonstrated that nitrosamine concentrations in the gastric contents reached a maximum between 30 and 60 min after food consumption. Rapid absorption of a nitrosamine (NPYR) in the stomach was previously demonstrated by Mysliwy, Wick, Archer *et al.* (1974) when they introduced  $\text{NaNO}_2$  and pyrrolidine into the stomach of a fistulated dog. Therefore the subsequent decrease in nitrosamine content in our study was not unexpected.

Blood nitrosamine levels from some subjects participating in the preliminary study are presented in Fig. 2a. In four (subjects 6, 7, 8 and 10) there was an increase in the nitrosamine concentrations; however such increases were not statistically significant when all the data were analysed.

Figure 2b depicts the urinary NDMA levels. Concentrations greater than 1 ppb NDMA were detected only in volunteers 1 and 3. In both of these cases, no nitrosamines were detected in the fasting state, and a progressive increase after treatment suggested possible absorption followed by rapid excretion by the kidney. Urine specimens from the remaining subjects contained less than 0.7 ppb NDMA and were unaffected by the test diet. Statistical evaluation showed no correlation between ingestion and the formation and/or excretion of nitrosamines.

#### Studies B and C

Information obtained in the preliminary study was used as a guide to establish the parameters and techniques to be used in the subsequent investigations. In study B, six subjects (nos 11–16) were all intubated nasally and fed. The sampling schedule used for the six subjects fed fish, spinach and vegetable juice in study B and the results of the analyses of all the physiological fluids and the foods ingested are presented in Table 1.

Ingestion of the sample meal, which presumably contained high concentrations of amines and nitrate, had no apparent influence on *in vivo* nitrosamine formation in the stomach. No statistically significant increases in nitrosamines were detected in the stomach contents or blood. Urinary levels were unaffected, nitrosamines being essentially absent. Only a slight increase of 0.8 ppb NDMA was noted in a single set of blood samples (no. 15). NDMA increased in the gastric aspirate from one subject (no. 16) from a fasting level of 1.4 ppb to 3.7 ppb, but the meal fed in that particular case contained a higher concentration of existing NDMA than any other meal in this series. The decrease in NDEA from 3.8 ppb, in the fasting state, to 0.2 ppb may have been due to dilution in the stomach or to absorption.

To broaden the scope of the study, bacon, a cured food which often contains preformed nitrosamines,

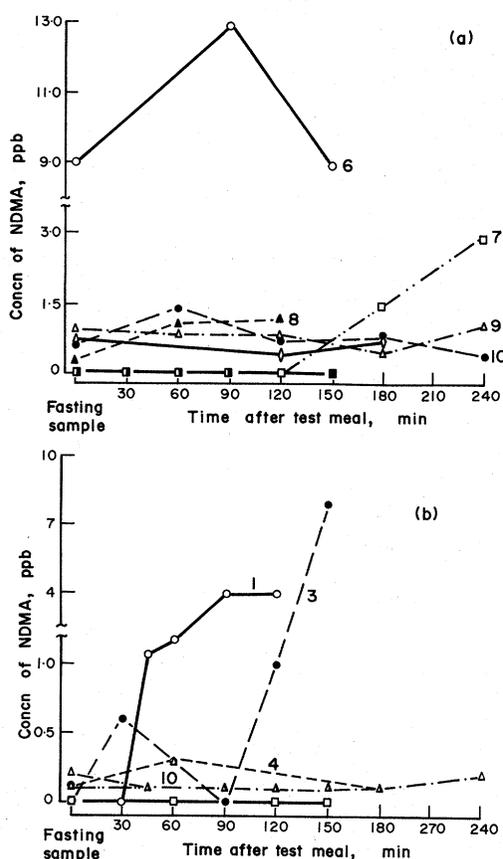


Fig. 2. Concentrations of *N*-nitrosodimethylamine (NDMA) in (a) blood samples and (b) urine samples taken from test subjects after fasting and at intervals after ingestion of a meal consisting of fish, spinach and vegetable juice. Numbers on the graphs identify the different subjects.

was substituted for fish in the sample diet (study C). In an effort to reduce variability, each volunteer participated twice. At the first session volunteers ingested vegetable juice, spinach and fried bacon, which at

times contained normally incurred preformed NPYR. A grilled hamburger replaced the bacon at the second session. The results of this study (Table 2) indicated that eating these foods did not significantly alter the levels of nitrosamines in any of the physiological specimens tested. In this study, the gastric contents was sampled 1 hr after the subject completed the meal, and blood and urine were collected at 1- and 2-hr intervals. The concentrations of nitrosamines, if any, in samples from the stomach remained unchanged or increased only slightly after consumption of meals containing nitrosamine or nitrosamine precursors. Whether the increases, when they occurred, were due to ingestion of preformed nitrosamine, or to *in vivo* gastric formation cannot be ascertained. However, the data suggest that the former possibility is more likely to be correct.

Other investigators have conducted studies, but only in blood, to determine the effect of diet on the *in vivo* formation of nitrosamines. Yamamoto *et al.* (1980) observed no effect after feeding eight individuals a Japanese diet rich in nitrate and amines. Kowalski, Miller & Sen (1980) repeated the study by Fine *et al.* (1977), using eight subjects rather than one, and noted on occasion a slight increase in nitrosamines in some volunteers after they had eaten the same test meal, but the difference was less marked than that reported by Fine's group. The data obtained in this study on blood levels of nitrosamines are comparable to these investigations. The results illustrate that there are fluctuations in nitrosamine levels in blood, and point to the need for sufficient sampling, particularly in studies of a biological nature, before definitive conclusions are drawn.

The results compiled in this study indicate that nitrosamine levels in gastric contents, blood and urine from normal people are not significantly affected by ingestion of an ordinary diet containing preformed nitrosamine or potential nitrosamine precursors. The possibility that nitrosamines may be formed and rapidly metabolized or absorbed cannot be completely ruled out, and was not within the scope of this study.

Table 1. *N*-Nitrosodimethylamine in physiological samples and in the components of a test meal

Subject no.	Concn of <i>N</i> -nitrosodimethylamine (ppb*)									
	In gastric contents		In blood			In urine		In food†		
	Fasting‡	1 hr§	Fasting‡	1 hr§	2 hr§	Fasting‡	1 hr§	Cod	Spinach	Juice
11	0.4	0.2	0.6	ND	0.4	ND	ND	0.6	ND	0.1
12	0.2	0.3	0.4	0.6	0.4	ND	ND	1.5	ND	0.2
13	0.3	0.2	0.8	0.4	ND	0.2	ND	1.3	0.3	0.2
14	0.1	0.3	—	—	—	ND	ND	ND	0.2	0.1
15	0.3	0.6	0.4	1.2	1.0	ND	ND	0.8	0.3	0.2
16	1.4	3.7	0.6	0.7	0.7	0.2	0.1	3.0	0.6	0.2
	(3.8)	(0.2)								

ND = None detected

\*ng/ml or ng/g, as appropriate.

†As consumed by the subject indicated.

‡Sample taken at the end of an (at least) 8-hr fast, prior to ingestion of the test meal.

§Interval between ingestion of test meal and sampling.

||Concentration of *N*-nitrosodiethylamine, found only in these samples from subject 16.

Table 2. Influence of beef or bacon ingestion on the N-nitrosodimethylamine content of physiological fluids

Subject no.	Type of diet	Concn of N-nitrosodimethylamine (ppb*)										
		In gastric contents		In blood			In urine			In food†		
		Fasting‡	1 hr§	Fasting‡	1 hr§	2 hr§	Fasting‡	1 hr§	2 hr§	Meat	Spinach	Juice
17	Beef	0.2	0.2	ND	0.6	0.4	0.1	0.1	0.3	0.3	0.4	0.3
	Bacon	0.3	0.1	0.6	0.4	0.5	0.1	0.1	0.1	1.0	0.3	0.2
18	Beef	0.1	0.1	ND	ND	ND	0.1	0.1	0.1	0.3	ND	0.2
	Bacon	0.4	0.1	0.3	0.3	0.4	ND	0.1	0.1	1.4	0.5	0.2
19	Beef	ND	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.9	0.1	0.2
	Bacon	0.2	0.3	0.5	0.6	0.3	0.2	ND	0.3	2.1	0.2	0.2
20	Beef	ND	ND	0.5	0.5	0.4	0.7	ND	ND	0.4	0.2	ND
	Bacon	0.2	0.2	0.5	0.6	0.5	0.4	ND	0.2	1.3	0.2	0.2
21	Beef	0.7	0.2	0.4	0.4	0.4	ND	ND	ND	0.4	0.2	0.2
	Bacon	0.3	0.2	0.5	0.7	0.4	ND	ND	ND	0.9	0.2	0.3

ND = None detected

\*ng/ml or ng/g, as appropriate.

†As consumed by the subject indicated.

‡Sample taken at end of an (at least) 8-hr fast, prior to ingestion of the test meal.

§Interval between ingestion of test meal and sampling.

||The bacon samples in the diets fed to subjects 17, 18 and 21 also contained N-nitrosopyrrolidine, at levels of 2.2, 3.5 and 2.2 ppb, respectively.

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