Determination of Total *N*-Nitroso Compounds and Their Precursors in Frankfurters, Fresh Meat, Dried Salted Fish, Sauces, Tobacco, and Tobacco Smoke Particulates

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Total *N*-nitroso compounds (NOC) and NOC precursors (NOCP) were determined in extracts of food and tobacco products. Following Walters' method, NOC were decomposed to NO with refluxing HBr/HCl/HOAc/EtOAc and NO was measured by chemiluminescence. NOC were determined after sulfamic acid treatment to destroy nitrite, and NOCP were determined after treatment with 110 mM nitrite and then sulfamic acid. Analysis without HBr gave results ≤20% of those with HBr. This NOC method was efficient for nitrosamines but not nitrosoureas. The standard nitrosation for determining NOCP gave high yields for readily nitrosated amines, including 1-deoxy-1-fructosylvaline, but not for simple amines, dipeptides, and alkylureas. Mean NOC and NOCP results were (respectively, in μmol/kg of product) 5.5 and 2700 for frankfurters, 0.5 and 660 for fresh meat, 5.8 and 5800 for salted, dried fish, and 660 and 2900 for chewing tobacco (all for aqueous extracts) and 220 and 20000 nmol/cigarette for MeCN extracts of cigarette smoke filter pads.

Keywords: *N-Nitroso compounds; nitrosation; thermal energy analysis; frankfurters; meat; salted, dried fish; sauces; tobacco; cigarette smoke*

INTRODUCTION

N-Nitroso compounds (NOC) comprise nitrosamines (R_1R_2NNO) and nitrosamides [$R_1CON(NO)R_2$], produced by the nitrosation of secondary amines and *N*-alkyl amides, respectively (*1*). Most NOC induce cancer in laboratory animals (*2*, *3*), and NOC may be involved in the etiology of several human cancers (*4*, *5*). People are exposed to NOC in foods, by smoking or chewing tobacco products, and by the in vivo formation of NOC, for example, in the stomach from the acid-catalyzed reaction with nitrite of dietary amines and amides that are NOC precursors (NOCP) (*4*). NOCP may be converted to NOC during food storage or cooking or in vivo. Total NOC in human gastric juice [mean level = 2.5 μmol/L (*6*)] and feces [mean level for subjects on a high-meat diet = 23 μmol/kg (*7*)] could arise from dietary NOC and from the in vivo nitrosation of NOCP.

NOC can be determined by Walters' method, involving treatment of NOC with HBr to yield NO. The NO is determined by thermal energy analysis (TEA, chemiluminescence), in which the NO reacts with ozone to give NO_2 in an excited state, which emits infrared light. This is measured with a detection limit of 100 pmol. The method is based on the finding (*8*) that HBr, but not HCl, reacts with NOC to produce nitrite, presumably via the reaction shown below. TEA can be used to determine total NOC by the Walters method or can be linked to gas chromatography (*9*) or HPLC (*10*) to determine individual NOC. Other methods for determining individual NOC include gas chromatography−mass spectrometry (*11*). The Walters method was described in 1976−1978 (*12*, *13*), was further developed (*14*−*19*), and has been reviewed (*15*, *20*, *21*). This method was used to determine NOC in nitrite-cured meat (*20*, *22*, *23*) and in human gastric juice (*17*, *19*), urine (*18*), and feces (*7*, *24*). In those materials studied in detail, <30% of the total NOC was identified as volatile nitrosamines and nonvolatile nitrosamino acids (*18*, *25*−*27*). The fact that >70% of these NOC have not been identified is of concern. Therefore, we have begun to purify and identify the NOCP (which are far more abundant than the NOC) in frankfurters (*28*) and wished to determine the levels at which NOC and NOCP occur in various foods. We define NOCP as compounds that yield NOC after nitrosation under our standard conditions. NOCP represent the potential for NOC formation under these relatively mild conditions.

$$R_1R_2NNO \xrightarrow{HBr} R_1R_2NH + NOBr \rightarrow$$

$$Br_2 + NO \xrightarrow{[O]} NO_2^-$$

The NOC method used here was modified from two studies on the analysis of human gastric juice. Pignatelli et al. (*17*) treated gastric juice with sulfamic acid (SA) and then analyzed the product by two TEA systems, one using HCl in an HOAc/EtOAc mixture (the "HCl mode") and the other using HBr in a similar mixture (the "HBr mode"). TEA-responsive compounds included (a) inorganic nitrite, which was determined in both the HCl and HBr modes and was destroyed by SA (*17*); (b) unidentified "TEA-responsive compounds" that were not decomposed by SA and produced NO in the HCl and (it was presumed) the HBr mode; and (c) NOC, which were also not destroyed by SA and produced NO in the HBr but not the HCl mode (*8*). The difference between results

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obtained by the HBr and HCl modes after treatment with SA was taken to give the NOC level. On this basis, about half the TEA response by the HBr mode for SA-treated human gastric juice was attributed to NOC and about half to other TEA-responsive compounds. Xu and Reed (*19*) also defined NOC in gastric juice as the difference between results obtained by the HBr and HCl modes, but they inserted additional traps downstream from the reaction flask and included both HCl and HBr in analyses by the HBr mode.

Sodium nitrite is added to meat and fish products to preserve them and to improve their taste and color (*29*). The hypothesis that these products were involved in the etiology of stomach cancer was introduced (*30*, *31*) to help explain the high incidence of stomach cancer (a) in developing countries and (b) in the United States and Europe before the 1940s, when stomach cancer was the principal type of cancer (*32*). In both of these situations, meat and fish are/were often preserved with excessive amounts of salt [a promoting factor for stomach cancer (*31*)] and nitrite and nitrate relative to the controlled conditions of modern manufacture. Processed meat (mainly sausages) was significantly linked with colon cancer in two of four prospective studies reported in 1990−1994 (*33*−*35*) and in a current prospective study on nearly 500000 subjects from nine European countries (preliminary report by E. Riboli, Conference on Diet and Nutrition, American Institute for Cancer Research, 2001). Most of nine case-control studies reported associations between the consumption of frankfurters (hot dogs, franks) and childhood brain cancer (*36*), and two studies reported associations between frankfurter use and childhood leukemia (*37*, *38*). These are two of the most common childhood cancers (*32*). Frankfurter consumption was estimated in the pregnant mothers, the fathers, and/or the affected children. The view that NOC were involved seemed plausible because alkylnitrosoureas injected into pregnant rats induce brain tumors and leukemia in their offspring (*2*, *3*). This association was disputed because of the problems of recalling the diet and confounding with other factors and because the nitrite level in frankfurters, but not the incidence of these cancers, has been falling (*39*). Accordingly, we concentrated initially on the analysis of frankfurters.

Salted, dried fish was also examined because this is a risk factor for stomach and nasopharyngeal cancer (*35*). These risks may be due to food components such as NOC and NOCP (*4*, *31*) and not only to salt. We analyzed sauces because fish sauce has been linked with gastric cancer in China and produced methylnitrosourea (MNU) when it was nitrosated under mild conditions (*40*, *41*) and because many sauces are stored for long periods at room temperature, which might permit NOC and NOCP to accumulate. Finally, cigarette smoke particulates and tobacco were analyzed because they contain nitrosamines that may be involved in the etiology of lung and other types of cancer in smokers and of oral cancer in tobacco chewers (*5*).

We determined NOCP in addition to NOC because this should help in the evaluation of the ability of the products to generate NOC in vivo after the foods are consumed and there are few previous reports on such analyses. Our general approach was to analyze aqueous extracts of the products for (a) NOC by analysis after addition of SA to destroy nitrite and (b) NOCP by nitrosation under mild conditions, followed by the addition of SA and analysis for NOC.

MATERIALS AND METHODS

In view of the carcinogenicity and volatility of many NOC, they were worked with in a chemical hood and all necessary precautions were taken. Unused NOC were destroyed with aluminum/nickel alloy in alkali (*42*). Yellow lighting was used because NOC are photolabile (*43*). $NaNO_3$ was obtained from Mallinckrodt (Paris, KY). *N*-Nitrosoproline (NPRO) was synthesized (*44*). Other NOC were synthesized from the corresponding amines or amides and $NaNO_2$/HCl and characterized by their ultraviolet and ^1H nuclear magnetic resonance spectra. A sample of MNU was obtained from Sigma (St. Louis, MO). We purchased all other chemicals from Aldrich (Milwaukee, WI) and most glassware from Ace Glass (Vineland, NJ).

Analytical System for NOC. This was modified from previous procedures (*13*, *17*−*19*) and consists of an NOC detection system linked to a TEA (model 402, Thermedics, Waltham, MA) and an integrator (Hewlett-Packard, Avondale, PA). The reaction vessel is a 500-mL round-bottom flask with three 24/40 necks and a No. 7 "Ace-thred" neck and is placed in a heating mantle. The 24/40 necks are connected to (a) a glass gas inlet tube (Ace catalog no. 5295-12) extending 3.5 in. below its joint, a regulator and flow meter in the TEA, and an argon cylinder, (b) a 6-in. immersion thermometer fitted with a thermometer adapter, and (c) a 30-cm-long Graham condenser (Kontes, Vineland, NJ) cooled with an immersible pump (Little Giant 1 series, VWR, Batavia, IL), circulating iced water. The Ace-thred neck is fitted with a Teflon-lined septum (changed after each experiment), mounted on a nylon bushing (Ace catalog no. 5029), for injecting samples. The top of the condenser leads via a Claisen adapter to an air inlet stopcock and a gas outlet tube. The temperature can be changed by adjusting the heating mantle temperature.

Nalgene 180 clear plastic tubing [o.d. $^1/_2$ in., i.d. $^1/_4$ in.; and o.d. $^1/_4$ in., i.d. $^1/_8$ in.) and Teflon tubing (o.d. $^1/_8$ in. and i.d. $^1/_{16}$ in.), all from Nalgene (Milwaukee, WI)], fitting one inside the other, were used to connect seven 4 × 30 cm gas washbottles (Kimball Kontes, Vineland, NJ, catalog no. 15060-125) with each other, the reaction vessel, and the TEA. Washbottles 1−4 contain, respectively, 60 mL each of 1.5 N NaOH, 5 N NaOH, 99+% NaOH pellets, and anhydrous granular 99+% Na_2SO_4. Washbottles 5 (with 20 mL of EtOAc), 6 (with 20 mL of acetone), and 7 (left empty) are kept at −30 °C in a freezer (Cryofridge, Baxter, McGaw Park, IL). The inlet of washbottle 7 is fitted with a fritted disk (Kimball Kontes catalog no. 28220-1251). To minimize leaks, the rotary valve in the TEA is bypassed with a three-way stopcock, which can disconnect the NOC assembly or let in air. Joints are lubricated with Lubriseal (Thomas Scientific, Swedesboro, NJ). The washbottles are cleaned and their contents replaced every second experiment. The gas exit from the TEA is led into a chemical hood.

HBr Mode. EtOAc (160 mL) is added to the reaction vessel. Oxygen flow for the TEA (25 mL/min), the TEA ozonator, and the argon flow (40 mL/min at 3 psi) are turned on. The TEA attenuation is set to 128. When the TEA vacuum reaches 0.5 mm, the heating mantle is turned on to give a reflux rate of 1 drop/s and a boiling point of 28 °C for the EtOAc (into which the thermometer dips). Concentrated HCl/glacial HOAc (5:95, 15 mL) and, later, 33% HBr in glacial HOAc (7.5 mL, from Fluka Chemika, Milwaukee, WI) are injected with syringes (filled in a chemical hood), and 15−30 min is allowed for the TEA response to return to baseline after each addition. Test samples (usually 100 μL) are injected with syringes (Hamilton, Reno, NV) when the response drops close to baseline (every 7−10 min) and produce sharp spikes in the integrator response lasting 3−4 min. NPRO standards (0.1 nmol, 100 μL of 1.0 μM NPRO prepared daily from 5 mM stock solution) are injected at the beginning, middle, and end of each run; these produced similar responses for 3−4 h. Yields are based on the standards and expressed as nanomoles per milliliter of solution or nanomoles per gram of sample. Common problems are a raised pressure due to leakage at joints or the septum and blockage, which reduces the measured pressure and raises the reflux temperature.

HCl Mode. The HCl/HOAc reagent was added as in the HBr mode, but HBr/HOAc was omitted. Although Xu and Reed (*19*) found a stable response in this mode at a reflux temperature of 30−32 °C, the response to NPRO in our HCl system operated at 30 °C was very broad, took 15−30 min to return to baseline, and rapidly decreased on subsequent NPRO injections, probably because of loss of HCl. Hence we lowered the reflux temperature to 26 °C, used 160 instead of 120 mL of EtOAc, and raised the HCl level from 44 to 51 mM. Response then remained stable for >4 h.

Analyses of Foods and Tobacco Products for NOC and NOCP. Tabulated results are generally mean values for the complete analysis of two separate batches of each product, with duplicate analysis of the final extract in each case. Unless specified otherwise, each sample was taken from a different brand (for commercial products) or batch (for noncommercial products). Mixtures were shaken with a Vortex mixer. A Sorvall RC-5B centrifuge (Wilmington, DE) operated at 5 °C was used to sediment solids. Solutions were generally evaporated to 5−10 mL in a Buchi rotary evaporator operated at <40 °C and 20−25 mm Hg and then to dryness (or to an oily mixture in the case of meat products) with a stream of N_2 in test tubes kept in an unheated Reacti-Vap evaporating unit (Pierce, Rockford, IL).

Meat Products. These were purchased at a supermarket and kept frozen at −15 °C for <2 months, except that fresh ground meat was analyzed after refrigeration for up to 2 days without freezing. The nine frankfurter brands analyzed were listed as containing beef as well as, in some cases, pork and/or chicken. Some brands were labeled as smoked. Fresh thigh muscle from adult male MRC−Wistar rats (killed with CO_2) were analyzed without freezing, immediately (one sample) or after storage for 6 days at 4−6 °C (one sample). All other samples were thawed at room temperature. About 35 g of each sample was cut into small pieces and blended for 2 min with 2.5 mL of distilled water/g of meat in an Osterizer blender (Oster, Milwaukee, WI) run at its top ("liquefy") speed. We centrifuged the homogenates (30 min, 11000 rpm, 4° C), filtered the supernatants, and recorded filtrate volumes. We evaporated 2 mL of filtrate, dissolved the residue in 850 μL of water with shaking, added 50 μL of 2 N HCl and 100 μL of SA reagent [a saturated solution of SA in water, ∼147 g/L (*45*)] to destroy nitrite, left the mixture for 15−120 min, and determined NOC in 100 μL samples. If the response was too high (when it could take 30−60 min for the response to return to baseline), suitable dilutions were injected. To determine NOCP, we evaporated 2 mL of filtrate, dissolved the residue in 750 μL of water, added 100 μL of 1 M $NaNO_2$ (100 μmol) and 50 μL of 2 N HCl (100 μmol, this brought the pH to 1.5−2.0 and nitrite to 111 mM), shook the mixture twice per second for 1 h at 37 °C, added 100 μL of SA reagent (∼150 μmol), left the mixture for 15−120 min at room temperature, diluted the sample 100 times in water containing 1% SA reagent, and analyzed 100 μL aliquots for NOC. In NOCP determinations, nitrosation and NOC analysis were performed on the same day. Calculations of NOC and NOCP levels were based on the filtrate volumes.

Salted, Dried Fish. Six types of this product were purchased in the Fujian province of China, on the coast ∼500 miles south of Shanghai. The fish were of various species and differed as to whether the scales were removed. One each of a similar product was obtained from Cape Town, South Africa, where dried fish ("boekoms") is consumed mainly by the indigenous population, and from Minnesota, where whole smoked herrings were preserved. These products were analyzed as described under Meat Products except that 5 mL of water/g of fish was used and the initial filtrates were analyzed without prior evaporation.

Sauces. Two soy sauces and the fish sauce were purchased in a market in the Fujian province of China. Other sauces were purchased at a supermarket or an Oriental store in Omaha, NE. The sauces were diluted if they were viscous and filtered, and the filtrates were analyzed as described under Salted, Dried Fish but using 2.5 mL of water/g of product.

Cigarette Smoke Filter Pads. Circular cigarette smoke filter pads (diameter = 45 mm) were prepared from reference cigarettes and contained known amounts of total particulate matter, tar, and nicotine. Five cigarettes/pad were smoked down to 30 mm butts with 11 puffs/cigarette. Blank pads exposed in the smoking machine without cigarettes and pads exposed to cigarette smoke weighed 03.5 and 3.8−5.2 g, respectively. Segments (60 mg) of each pad were cut into pieces, soaked overnight in 5 mL of MeCN, shaken for 2−3 min, and centrifuged. The supernatants were filtered. To determine NOC, extract samples (1.0 mL) were mixed with 100 μL of SA reagent, kept for 15−120 min, and analyzed. To determine NOCP, extract samples (0.4 mL) were mixed with 1.6 mL of water (no precipitate was seen), 100 μL of 2 N HCl, and 200 μL of 1 M $NaNO_2$, reacted for 1 h at 37 °C, mixed with 0.2 mL of SA reagent, and analyzed for NOC.

Cigarette and Chewing Tobacco. These were purchased in Omaha, NE. Tobacco (1.6 g) was removed from two cigarettes of each brand and suspended overnight in 15 mL of water. The mixtures were vortexed and centrifuged. Chewing tobacco (2 g) was soaked for 2 h in 8 mL of water and vigorously shaken, and the mixture was centrifuged. The supernatants were filtered, and the filtrate was analyzed as described under Sauces.

Statistics. Correlations were derived using Spearman correlation coefficients, which are based on the rank order of each measurement.

RESULTS

Analytical System. *Differences from Previous Studies.* The chief changes made were that we (i) determined NOCP in addition to NOC and (ii) generally used only the HBr mode, whereas previous studies used the difference between the HBr and HCl modes to determine NOC (*17*, *19*). Compared to the Xu and Reed method (*19*), we increased the EtOAc volume and the amount of HBr, cooled the condenser, and made other minor changes. Also, the reaction temperature was lowered to 28 °C because our system was stable for 3−4 h at this temperature and for only 1−2 h at 31−33 °C, the temperature used by Xu and Reed.

Ability of Nitrogen Compounds To Be Determined as NOC. Table 1 shows the relative molar response for 15 nitrogen compounds (Figure 1) with and without prior treatment with SA. The distilled water used as solvent showed a response that was attributed to nitrogen oxides or nitrite in the water and disappeared after SA treatment. This explains why the results for nitrosamine solutions after SA treatment were 5−10% less than those without SA treatment. In contrast, $NaNO_2$ and butyl nitrite showed responses without but not with SA treatment, indicating a quantitative reaction with SA. $NaNO_3$ showed little response. Three of the four nitrosamines and $NaNO_2$ gave similar molar responses. 1,4-Dinitrosopiperazine showed a much higher response, as expected. At our standard reflux temperature of 28 °C, NPRO, other nitrosamines, and the nitrosamide methylnitronitrosoguanidine (MNNG) showed narrow peaks (short elution times) of, typically, 3.5−4.0 min, but the nitrosamide MNU gave broad peaks 13−15 min wide, with integrator responses that were <20% of those for NPRO (Table 1). When the reaction temperature was raised from 28 to 33 and 36 °C, MNU showed peak widths of 8−9 min, with relative responses of 32−40%. The NPRO peaks remained sharp (peak width = 3.5−4.0 min) at all three temperatures. When aqueous solutions of MNU and MNNG were stored for 3 h at room temperature, the response for MNU dropped 21% without SA and was zero when SA was included, and that for MNNG dropped 50% without SA and 71% after adding SA. As in earlier studies (*12*, *15*), organic

Table 1. Relative Molar Responses of Various Nitrogen Compounds in the HBr Mode with and without Prior Treatment with SA[a]

chemical	amount injected[b] (nmol)	relative response/mol with SA	relative response/mol without SA
$NaNO_2$	0.085	0	96
$NaNO_3$	850	0.00004	0.0003
butyl nitrite	3.4	0	108
dimethylnitrosamine	0.085	102	96
NPRO	0.085	100	100
1,4-dinitrosopiperazine	0.083	156	165
N-nitrosomorpholine	0.088	81	89
MNU[c]	0.078	16	7
MNNG	0.091	77	92

[a] For tests without SA, 100 mL of aqueous solution was injected into the Walters system. For tests with SA, 0.5 mL of 2 N HCl and 1.0 mL of SA reagent were added to 8.5 mL of an aqueous solution of the test compound, and 100 μL of the mixture was analyzed. The result for distilled water without addition of SA (3.8 $\times$ 10^6 area units/0.1 mL) for the test compound without SA. Distilled water gave no response after SA was added. Results are expressed relative to these for NPRO, which showed responses of 24.8 (with SA) and 24.9 (without SA) $\times$ 10^7 area units/nmol. Nitrobenzene, nitrosobenzene, acetone oxine, and 1-nitroso-2-naphthyl-3,6-disulfonic acid (1 μmol each), 2-methyl-2-nitrosopropane (20 nmol) in 100 mL of acetone, and acetone (100 μL) gave responses of <2 $\times$ 10^6 area units without SA treatment. A creatinine solution in water (10 mg/mL) gave a negligible response. [b] Amount injected into the refluxing mixture. [c] These results are for a sample synthesized here. A sample of MNU purchased from Sigma gave similar results.

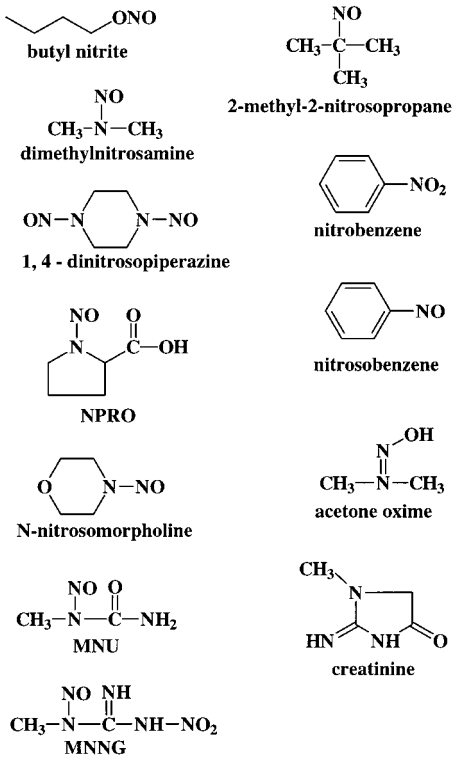

Figure 1. Nitrogen compounds tested for their ability to react as NOC in the HBr mode (see Table 1).

nitrogen compounds lacking the *N*-nitroso group gave low responses.

Ability of Amines and Amides To Produce NOC under Standard Conditions. We wished to determine which compounds in food extracts were most likely to produce NOC under our standard nitrosation conditions, that is, could constitute the NOCP. Hence, various amines and amides (Figure 2) were nitrosated with 50 and 400 mM $NaNO_2$ under otherwise standard conditions, and

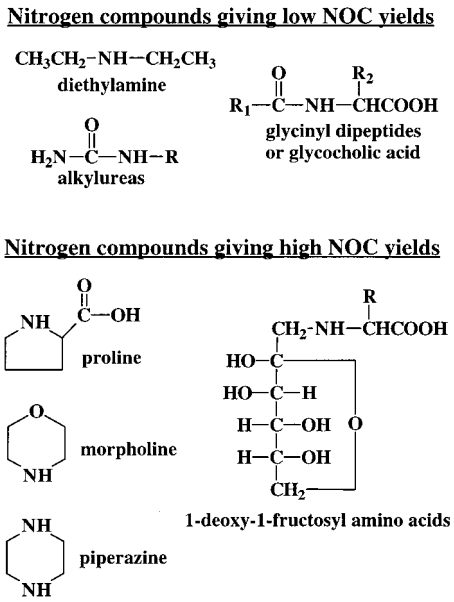

Figure 2. Nitrogen compounds tested for their ability to produce NOC on treatment with nitrite (see Table 2).

Table 2. Ability of Different Nitrogen Compounds To Produce NOC on Treatment with Nitrite[a]

nitrogen compd	NOC yield from nitrogen compd (%) with 50 mM nitrite	NOC yield from nitrogen compd (%) with 400 mM nitrite
histidine		
tryptophan		
glycylglycine	0.7	0.8
glycylphenylalanine	0.5	0.8
phenylalanylleucine	0.14	0.05
leucinylalanine	0.30	0.29
diethylamine	0.3	3.1
methylurea	6	12
ethylurea	12	23
n-butylurea	11	10
1-deoxy-1-fructosylvaline	67	66
proline	45	85
morpholine	87	91
piperazine	97[b]	98[b]

[a] Nitrosation of 0.9−1.3 mM nitrogen compound was performed under the standard conditions (incubation for 1 h at 37 °C), except that we used 50 mM $NaNO_2$ + 50 mM HCl (pH ~2.0) or 400 mM NaNO2 $NaNO_2$ + 400 mM HCl (pH ~1.0) instead of the standard 110 mM nitrite (pH ~2.0). NOC were determined after addition of SA reagent (0.3 mL for 50 mM nitrite, 1.2 mL for 400 mM nitrite). [b] Calculated on the basis of conversion to dinitrosopiperazine.

NOC yields were determined (Table 2). As nitrosation of glycine and its ethyl ester produces unusual alkylating agents (*46*, *47*), two of the four tested dipeptides contained glycine. NOC yields were <2% for four dipeptides, ≤23% for diethylamine and three *N*-alkylureas, and 45−100% for proline, morpholine, piperazine, and the Amadori compound, 1-deoxy-1-fructosylvaline. The peaks obtained on nitrosation of the three *N*-alkylureas were broad, similar to those for MNU.

Response in the HCl Mode. In this mode, 1.0 nmol of $NaNO_2$ gave a response of (1.1−1.6) $\times$ 10^8 area units when SA was not added. Butyl nitrite showed 81% of this response on a molar basis. When 0.1 nmol of NPRO was analyzed after addition of SA, it showed no response in the HCl mode and (1.4−1.6) $\times$ 10^7 area units in the HBr mode. Water extracts of three foods were analyzed by the HCl and HBr modes, in both cases after the addition of SA. For both NOC and NOCP, responses in

Table 3. Analysis of Three Foods by HCl and HBr Modes

food	no. of foods analyzed[a]	results (μmol/kg of product, mean ± SD or individual results)					
		NOC			NOCP[b]		
		by HCl mode	by HBr mode	HCl/HBr ratio[c]	by HCl mode	by HBr mode	HCl/HBr ratio[c]
frankfurter	4	0.36 ± 0.19	6.1 ± 1.9	0.06	38 ± 6	880 ± 650	0.04
dried, salted fish	2	0.3, 0.3	3.2, 3.4	0.09	460, 830	8000, 11000	0.07
soy sauce	3	0.02, 0.09, 0.21	0.18, 0.49, 1.01	0.20	1400, 1500, 7800	21000, 28000, 28000	0.14

[a] Each analysis was performed on a different brand (frankfurters and soy sauce) or source (dried, salted fish) of product. We used 0.3 mL of SA reagent in these tests. [b] NOC after nitrosation under the standard conditions. [c] Mean by HCl mode/mean by HBr mode.

Table 4. NOC and NOCP Analyses after Extraction of a Dried Frankfurter Homogenate with Various Solvents[a]

solvent	results (μmol/kg of meat)	
	NOC	NOCP
water	7.4	3690
MeOH	7.9	4040
MeCN	0.7	93
acetone	0.8	180
CH_2Cl_2	1.9	960

[a] A frankfurter sample (71.2 g) was homogenized in 150 mL of water. The homogenate was freeze-dried to give 38.8 g of solid residue, which was powdered. Of the powder, 2 g samples were extracted with 10 mL of solvent by vortexing the mixture three times over 6 h, storing the sample at room temperature for another 20 h, and (for the water extract) centrifugation. Two samples (2 mL) of each extract were evaporated. The residue was redissolved in a mixture containing 1.0 mL of acetone, 0.6 mL of water, 0.1 mL of 2 N HCl, and 0.3 mL of SA reagent and analyzed for NOC or redissoved in a mixture of 1.0 mL of acetone, 0.3 mL of water, 0.2 mL of 1 N NaO_2, and 0.1 mL of 2 N HC1, incubated for 1 h at 37 °C, treated with 0.1 mL of 2 N HCl and 0.3 mL of SA reagent, and analyzed for NOCP.

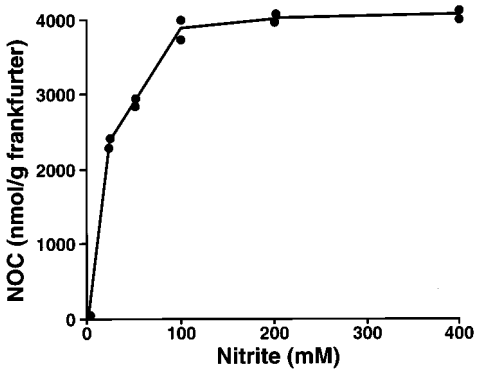

Figure 3. Effect of varied nitrite concentration on NOC yield from a frankfurter extract. In two separate experiments on the same brand of frankfurters, duplicate samples of aqueous extracts were nitrosated with 0−400 mM nitrite. Nitrosation was carried out with 25−400 mM nitrite under otherwise standard conditions (incubation for 1 h at 37 °C after addition of HCl to pH 1.5−2.0). The final volume was 2 mL. Up to 1.2 mL of SA reagent (for the test with 400 mM nitrite) was used.

the HCl mode were 4−6% (for frankfurters), 7−9% (for salted, dried fish), and 14−20% (for soy sauce) of those in the HBr mode (Table 3). The results for soy sauce in the HCl mode remained similar after the extract was evaporated and redissolved in water, indicating that the TEA-reactive substances were not volatile nitrite esters.

Use of Varied Amounts of SA. We normally added 0.1 mL of SA reagent (~150 μmol of SA) to food extracts before measuring NOC and, after nitrosation with 100 μmol of nitrite, NOCP (Materials and Methods, Meat Products). As 1 mol of SA decomposes 1 mol of nitrite (see equation below), the SA should have been sufficient

$$HNO_2 + NH_2SO_2OH \rightarrow N_2 + H_2SO4 + H_2O$$

to decompose all of the nitrite. To check this point, water extracts were analyzed for NOC and NOCP by the HBr mode using 0.1 or 0.3 mL of SA reagent. On analysis of four frankfurter brands for NOCP, the results with 0.3 mL of SA were 74, 94, 95, and 97% (mean = 90%) of those with 0.1 mL of SA.

Some Properties of NOC and NOCP in Frankfurters. *Extraction Procedures.* Whole homogenates of frankfurters in water were evaporated by freeze-drying, the residues were extracted with various solvents, and the extracts were analyzed (Table 4). The highest NOC and NOCP yields were obtained by extraction with water and MeOH, which were about equally effective. CH_2Cl_2 appeared to extract a second NOCP fraction amounting to one-fourth of that in the water and MeOH extracts. For all materials except cigarette smoke pads, subsequent tests were done on water extracts. The standard method for extracting frankfurters was to homogenize the food in 2.5 times its weight of water, centrifuge the mixture, and analyze the supernatant. To test this method, the standard extraction was carried out on a batch of frankfurters. The sediment from the centrifugation was re-extracted with the same volume of water, and this procedure was repeated a third time. Duplicate analyses of the three extractions gave mean yields of 57, 7.5, and 4.4 nmol of NOC and 3360, 770, and 70 μmol of NOCP/kg of frankfurter, respectively. Thus, the standard single treatment with water extracted 83% of the NOC and 80% of the NOCP.

Stability of NOCP in Frankfurters. The NOCP level in aqueous extracts of frankfurters was stable or even increased to 30% when the extracts were heated at 50 °C for up to 1 h and decreased 20% on heating at 100 °C for 15 min. When similar extracts (pH 6.3) were adjusted to pH 2 and kept for 18 h at 25 or 60 °C, the NOCP level dropped by 60−70%. When the extracts were adjusted to pH 10 and then stored similarly, their NOCP level dropped by 20−25%. Hence, the NOCP were more unstable in acid than in base.

Use of Different Nitrite Concentrations. To determine NOCP, we nitrosated extracts with 110 mM nitrite. Ideally, this process should convert all readily nitrosated NOCP to NOC, so that the NOC results accurately reflect the amount of these *N*-nitroso compound precursors. When frankfurter extracts were treated with various nitrite concentrations, close to maximum yields were obtained with 100 mM nitrite (Figure 3). The NOC yield with 400 mM nitrite was only 7% more than that with 100 mM nitrite.

Sharpness of Peaks. All analyses of frankfurters and the other products gave sharp TEA responses with peak widths of 3.5−4.0 min, similar to those shown by the NPRO standards.

Results for Foods and Tobacco Products. *Frankfurters.* Analysis of nine frankfurter brands gave mean results of 5.5 and 2700 μmol/kg of NOC and NOCP, respectively, with ranges of 240-fold for NOC and 8-fold

Table 5. NOC and NOCP Levels in Nine Brands of Frankfurter

	mean results (μmol/kg of product)	
brand	NOC	NOCP
1	2.0	3300
2	4.1	910
3	0.7	2300
4	9.2	1600
5	0.05	3400
6	29.2	6900
7	0.6	2500
8	3.1	2400
9	0.12	860

for NOCP between the products (Tables 5 and 6). Brand 6 had the highest and brand 9 the lowest levels of both NOC and NOCP (Table 5). These results did not show significant correlations between NOC and NOCP levels. The mean NOCP level was 490 times that for NOC (Table 6). Even the results for a single frankfurter brand varied with the time of purchase. Thus, samples of a single brand purchased from August to December 1998 contained (in the order of purchase) 1.0, 5.6, and 3.0 μmol of NOC/kg and 550, 1740, and 390 μmol of NOCP/kg of product.

Canned Corned Beef, Summer Sausage, and Fresh Meat. Canned corned beef and summer sausage (a nonrefrigerated product with a pH of 4.8−4.9 for aqueous extracts) showed NOC results similar to or somewhat lower than those for frankfurters and NOCP levels that were 40−47% of those for frankfurters (Table 6). Fresh ground meat gave mean results that were one-tenth (for NOC) and one-fourth (for NOCP) of those for frankfurters. Fresh beef, pork, and chicken gave similar results. Freshly dissected rat muscle contained little NOC but had a mean NOCP level that was 59% of that for frankfurters. These levels did not change much after rat muscle was stored for 6 days at 4−6 °C.

Salted, Dried Fish. Eight samples of this product, including six from China, had similar mean NOC and 2.1 times higher mean NOCP levels than those for frankfurters (Table 6), although NOCP levels per gram of dry weight were similar in the two products. A sample of smoked, dried herring from the United States had 3.6 times more NOC than the mean level for all dried fish.

Soy and Other Sauces. For 19 sauces, including 12 soy sauces, the mean results (Table 6) were about half of (for NOC) and 2.4 times (for NOCP) those for frankfurters. A ground bean sauce from China contained 29.6 nmol of NOC/g, the highest level seen in any food. Four other sauces from the United States and Canada showed low NOC levels and a mean NOCP level that was 63% of that for frankfurters.

Cigarette Smoke Pads. Filter pads were obtained containing particulate matter from the smoke of reference cigarettes. Water, MeCN, and CH_2Cl_2 extracts of these pads gave NOC yields in ratios of about 1:10:3, respectively (Table 7). Hence, smoke pads were routinely extracted with MeCN (Materials and Methods, Cigarette Smoke Filter Pads). Table 8 shows the results for MeCN extracts of smoke pads prepared from 11 types of reference cigarette and for total particulate matter, tar, and nicotine in the smoke pads. The mean NOC level was 6% of that for NOCP, in contrast to the corresponding ratio of 0.2% for frankfurters. Tar and total particulate matter levels were closely correlated (correlation coefficient $r = 0.99$; $p < 0.0001$). Nicotine content was correlated with particulate matter ($r = 0.75$; $p < 0.007$) and tar ($r = 0.72$; $p < 0.01$). NOC level was correlated with particulates ($r = 0.58$; $p < 0.06$) and tar ($r = 0.65$; $p < 0.03$). NOCP level was closely correlated with particulates ($r = 0.80$; $p < 0.003$) and tar ($r = 0.79$; $p < 0.004$).

Cigarette and Chewing Tobacco. Commercial products were analyzed here. Water extracts were used (Materials and Methods, Cigarette and Chewing Tobacco) because they contained ≥11 times more NOC than did MeCN or CH_2Cl_2 extracts (Table 7). Mean NOC and NOCP levels in cigarette tobacco were 0.35 and 4.1 times, respectively, those in chewing tobacco. The NOC showed a mean level of 60 μmol/kg for tobacco from seven brands of cigarette and was 1450 μmol/kg for the eighth brand, a "basic" unfiltered cigarette (Table 9). Apart from the basic cigarette, the NOC and NOCP results did not vary much and were not correlated with

Table 6. Analysis of Water Extracts of Food Products for NOC and NOCP

			results (μmol/kg or μmol/L of food)			
			NOC		NOCP	
product[a]	origin	no. of samples[a]	mean ± SD[b]	range	mean ± SD[b]	range
frankfurter[c]	U.S.A.	9	5.5 ± 9.3	0.1−20.2	2700 ± 1800	900−6900
canned corned beef	Argentina and Brazil	2	6.0 ± 0.9	5.3−6.6	1100 ± 900	500−1800
summer sausage[c]	U.S.A.	3	3.7 ± 0.4	3.5−4.2	1300 ± 400	850−1600
fresh meat[d]	U.S.A.	5	0.5 ± 0.2	0.3−0.7	660 ± 210	800−900
fresh rat muscle	−[e]	2	0.3 ± 0	0.26−0.32	1600 ± 400	1300−1800
salted, dried fish	China	6	3.9 ± 2.0	1.8−6.0	6000 ± 3200	4200−12300
salted, dried fish	South Africa	1	2.0	−	3800	−
smoked, dried herring	U.S.A.	1	20.8	−	6900	−
all salted, dried fish	various	8	5.8 ± 6.2	1.8−20.8	5800 ± 2800	3800−12300
fish sauce[f]	China	1	0.6	−	430	−
soy sauce[f]	China	6	0.7 ± 0.8	0.1−1.0	7100 ± 3200	400−11000
soy sauce[f]	Japan	1	0.42	−	13000	−
soy sauce[f]	U.S.A.	5	1.4 ± 1.5	0.1−3.7	7900 ± 1100	6800−9800
ground bean sauce[f]	China	1	29.6	−	7800	−
other sauces[f,g]	U.S.A. and Canada	4	0.4 ± 0.3	0.1−0.5	3800 ± 2000	1700−5900
ketchup[f]	U.S.A.	1	0.7	−	950	−
all sauces[f]	−	19	2.7 ± 7.1	0.1−29.6	6600 ± 3400	430−13000

[a] Each sample was taken from a different brand or type of product. [b] Or individual result. [c] These extracts showed pH values of 6.1−6.5 (frankfurters) and 4.8−4.9 (summer sausage). [d] Three samples of ground beef and one each of ground chicken and pork. [e] −, not relevant or not determined. [f] Results are presented as μmol/L. All other results are presented as μmol/kg. [g] Steak and burger, Worcestershire, teriyaki marinade, and stir-fry sauces.

Table 7. Extraction of Tobacco Products with Water, MeCN, or CH_2Cl_2[a]

product	no. of samples	NOC (mean ± SE) water extract	MeCN extract	CH_2Cl_2 extract
cigarette smoke pads (μmol/kg of tar)[b]	4	3 ± 1	29 ± 4	10 ± 2
cigarette tobacco (μmol/kg of tobacco)	4	600 ± 540	5 ± 3	3 ± 2
chewing tobacco (μmol/kg of tobacco)	4	1100 ± 600	60 ± 20	100 ± 50

[a] All extractions were carried out as described under Methods for the extraction of filter pads with MeCN and of cigarette and chewing tobacco with water. Separate batches of material were extracted with each solvent. [b] From 1R1, 2R1, 1R3, and 4A1 cigarettes. Each pad contained 195−210 mg of tar.

each other. NOC levels in chewing tobacco varied from 10 to 2480 μmol/kg in different brands (Table 9). The three chewing tobacco samples packed in aluminum foil (row 7 of Table 9) showed a mean NOC level that was only 3% of that for the chewing tobacco listed in row 4 of Table 9. NOCP levels varied only 1.7-fold among the different brands of chewing tobacco. The mean NOC/mean NOCP ratios were 0.02 for all cigarette tobaccos and 0.23 for all chewing tobaccos.

Agreement between Duplicate Analyses. Variations between duplicate analyses were expressed as percentages of the mean values (difference between individual results × 100/mean results). Mean values for these results were as follows: for two analyses of the same frankfurter extract, measured on the same day, 6% for NOCP (8 samples); for full duplicate analyses starting on different days with different samples of the same product, for frankfurters, 16% for NOC (4 samples) and 21% for NOCP (4 samples), for cigarette smoke particulates, 14% for NOC and 30% for NOCP (11 samples), for cigarette tobacco, 26% for NOC and 6% for NOCP (8 samples), and for chewing tobacco, 16% for NOC and 19% for NOCP (13 samples).

DISCUSSION

The finding that results by the HCl mode were positive for $NaNO_2$ and butyl nitrite and negative for NPRO (Results, Analytical System, Response in the HCl Mode), agrees with a report that the HCl mode gave negative results for several NOC (Table 2 of ref *19*). NOC and NOCP concentrations in foods measured in the HCl mode were ≤20% of those measured in the HBr mode (Table 3). Hence, our food analyses, most of which used only the HBr mode, may have overestimated the NOC content by up to 20%, especially in the case of soy sauces. In previous studies, subtraction of the response in the HCl mode from that in the HBr mode was used to determine NOC (*17*, *19*, 26). For human gastric juice at pH 1−7, response in the HCl mode was 50−70% of that in the HBr mode (Table 3 of ref *17*). This difference from our results may be due to the difference in the materials examined. The identity of the compounds responding in the HCl mode after addition of SA [previously named "TEA-responsive elements" (*17*, *19*)] remains unknown.

Various nitrogen compounds were tested to explore under our conditions the extent to which they reacted as NOC in the presence and absence of SA (Table 1 and Figure 1). Our negative result for nitrobenzene contrasts with a report that it gave 1.4% of the response for nitrosamines (*12*). The almost complete absence of a response for $NaNO_3$ indicated that it was not necessary to remove nitrate before the NOC analysis and should be compared with reports that KNO_3 gave 0.002% (*12*) and 0.07% (*16*) of the response for nitrosamines and that

Table 8. Analysis of MeCN Extracts of Cigarette Smoke Pads[a]

cigarette type	no. of samples	results (individual values or mean ± SD) total particulate matter (mg/cigarette)	tar (mg/cigarette)	nicotine (mg/cigarette)	NOC (nmol/cigarette)	NOCP (nmol/cigarette)
R series[b]	5	29 ± 8	24 ± 7	1.66 ± 0.45	160 ± 40	20000 ± 7000
A series[c]	3	33 ± 3	29 ± 2	1.70 ± 0.53	240 ± 200	29000 ± 9000
low nicotine (1R4F)	1	11	9	0.80	650	8700
ultralow nicotine (4A1)	1	30	27	0.14	130	26000
ultralow nicotine (1R5F)	1	2	2	0.16	200	4400
all types (mean ± SE)	11	26 ± 3	22 ± 3	1.36 ± 0.20	220 ± 170	20000 ± 10000
blank pad[d]	1	−[e]	−	−	4	9

[a] The analyses for total particulate matter, tar, and nicotine were supplied by the Tobacco and Health Research Institute, University of Kentucky. NOC and NOCP were analyzed in MeCN extracts of the smoke pads. Cigarettes with "F" at the end of their names were filter cigarettes. [b] 1R1, 2R1, 2R1F, 1R3, and 1R3F cigarettes. [c] 1A2, 1A3, and 1A4 cigarettes. [d] Subjected to 55 puffs in the absence of cigarettes. Results are calculated as if five cigarettes had been smoked. [e] −, not analyzed.

Table 9. Analysis of Water Extracts of Cigarette and Chewing Tobacco

origin of tobacco	brands	results as μmol/kg of tobacco NOC mean ± SD	NOC range	NOCP mean ± SD	NOCP range
cigarettes[a]	1−7	60 ± 40	30−140	11700 ± 1700	9600−14700
cigarettes[b]	8	1450	−	12500	−
all cigarettes	1−8	230 ± 480	30−1500	11800 ± 1600	9600−14700
chewing tobacco[c]	1	2480	−	3000	−
chewing tobacco[c]	2−5	240 ± 50	200−300	2900 ± 760	2200−3800
chewing tobacco[d]	6−10	1000 ± 500	700−1700	2700 ± 400	2200−3100
chewing tobacco[e]	11−13	70 ± 80	10−160	2900 ± 0	−
all chewing tobacco	1−13	660 ± 760	10−1700	2900 ± 600	2200−3800

[a] Three of these brands were filter cigarettes. [b] A "basic" nonfilter cigarette. [c] Different finely ground moist products packed in small cans. [d] Variants of a single finely ground product, packaged in small cans. [e] Different brands of coarsely cut tobacco in aluminum foil packets.

only >1 mM nitrate in urine affected NOC measurements (*18*).

MNU showed poor responses and broad peaks at our standard reflux temperature of 28 °C, with somewhat better responses at 32−36 °C (Table 1 and Results, Analytical Systems, Ability of Nitrogen Compounds To Be Determined as NOC). Xu and Reed (*19*) reported that MNU gave broad peaks when HBr alone was included in the reflux mixture and that these peaks became narrow when HCl was added. In contrast, we found broad peaks for MNU, even though HCl was included in the reflux mixture. It appears that at 28 °C MNU reacts more slowly with HBr to give NO than do nitrosamines and that this accounts for its poor response under our conditions. In contrast, nearly quantitative MNU recoveries were reported from various foods (*41*, *48*) using denitrosation by HI at 70 °C and from gastric juice using HBr denitrosation at an unstated temperature (*17*). Our finding that MNU and MNNG decomposed extensively on storage in the presence of SA is attributed to acid-catalyzed denitrosation (*15*, *49*) at the pH of ∼2 reached after SA was added. Similarly, Pignatelli et al. (*17*) obtained recoveries of 25% for MNU and 9% for MNNG after storage for 1 month at −20 °C and pH 1.2−2.1. Taken together, our tests indicate that most of the compounds in foods and tobacco products denoted NOC are indeed NOC [hence we need not call them "apparent NOC" (*23*)] and that our method is suitable for nitrosamines and at least one nitrosamide but not for nitrosoureas. The stability of most of the frankfurter NOC in alkali (Results, Stability of NOCP in Frankfurters) and the sharp peaks observed in all of the product analyses (Results, Sharpness of Peaks) indicate that the NOC that occur and are produced from NOCP in these products are mostly nitrosamines and not nitrosoureas, which are rapidly destroyed by alkali (*49*) and gave broad peaks.

We studied the ability of various amines and amides to produce NOC under the standard nitrosation conditions used to measure NOCP (Table 2 and Figure 2). The results indicate that the NOCP in foods are unlikely to be peptides or simple secondary amines, for example, diethylamine and the muscle components spermidine and spermine (*50*), and are more likely to be readily nitrosatable amines, including glycosyl amino acids such as 1-deoxy-1-fructosylvaline. Readily nitrosated amines possess oxygen or nitrogen atoms attached to carbon atoms in α or β positions relative to the nitrosatable nitrogen (Figure 2) and, hence, should be relatively weak bases with pK_a values of <9 (*1*). Therefore, more of the nonionized species (R_1R_2NH) are available for reaction with the nitrosating agents N_2O_3, $H_2NO_2^+$, and NOCl derived from nitrous acid (HNO_2). The apparently low NOC yields of ≤23% from three *N*-alkylureas (Table 2) is attributed to the poor efficiency of our system for determining nitrosoureas.

The products were generally extracted only once with water, which removed 80−83% of the NOC and NOCP obtained by performing three such extractions (Results, Extraction Procedures). Hence, the true results were ∼20% higher than those reported here. SA was used to destroy nitrite, which would otherwise be determined as NOC (Table 1). The same method was used in two previous studies (*17*, *26*) but was not consistently adopted by Xu and Reed (*19*). Most of our results were obtained using 0.1 mL of SA reagent. This amount of SA should have destroyed all of the nitrite, even when nitrite was added during the assay of NOCP. Later, the NOCP yield for 0.3 mL of SA was found to be a mean of 90% of that for 0.1 mL of SA (Results, Use of Varied Amounts of SA). Hence, the tabulated results are believed to be reliable. The observation that nitrosation with 400 mM nitrite produced only 7% more NOC than nitrosation with 100 mM nitrite (Figure 3) indicated that all of the readily nitrosated NOCP was measured in the standard procedure for NOCP using 110 mM nitrite. Our procedures for determining NOC in cured meats (but not in the other products examined) involved evaporation to dryness, which would reduce the levels of volatile nitrosamines [and also of the slightly volatile MNU (N. P. Sen, personal communication)]. However, volatile nitrosamines constitute <10% of the total NOC in cured meat (*25*, *27*).

A comparison of the mean results for frankfurters and fresh meat (Table 6) indicates that ∼90% of the NOC and ∼75% of the NOCP in frankfurters were not present in fresh meat. Presumably, in frankfurters most of the NOCP arises during preparation or storage of this product, and most of the NOC arises by reaction of the added nitrite with the NOCP. Our mean results of 5.5 μmol/kg for NOC in frankfurters are comparable with values for cured meat of 14−32 μmol/kg found by Massey et al. [(*22*) and reviewed in ref *20*] and 1.9 μmol/kg found by Fiddler et al. (*23*). Fiddler et al. added SA to MeCN extracts of cured meat, prepared with 15 mL of MeCN/10 g of meat. The upper MeCN phase was analyzed for NOC by a TEA method. The relatively low results observed by these investigators might be explained by our finding (Table 4) that MeCN extracted only 3% of the NOC in dried frankfurters compared to that extracted by water, although Fiddler's group used MeCN saturated with water and not pure MeCN as the extractant. However, these workers also reported that corned beef contained mean NOC levels of 55 μmol/kg, much higher than the 6 μmol/kg listed in Table 6. Other reports recorded mean NOC values of 61 μmol/kg for raw and smoked bacon (*22*) and 0.5−12 μmol/L for beer (*51*). Chen et al. (*26*) analyzed six samples of Chinese fish sauce for NOC before and after nitrosation with 60 mM nitrite and treatment with SA, that is, for NOC and NOCP. They found 0.2−16.5 μM NOC and 2400−3500 μM NOCP in fish sauce, compared to 0.6 nM NOC and 430 μM NOCP in the fish sauce sample examined here (Table 6). Nitrosated fish sauce contains up to 1.4 μM MNU (*40*, *41*), which would have been poorly detected by our method but is a negligible fraction of the total NOCP level found here.

The mean of 220 nmol of NOC/cigarette found in cigarette smoke pads (Table 8) far exceeds the published values of 4.7 nmol/filter cigarette and 15.4 nmol/nonfilter cigarette for the sum of the tobacco-specific nitrosamines, including *N*′-nitrosonornicotine and 4-methylnitrosamino-1-(3-pyridyl)-1-butanone (NNK) (*5*). Hence, most of the NOC in cigarette smoke pads does not consist of these nitrosamines. The mean NOC and NOCP levels in cigarette and chewing tobacco were up to 120 and 4.4 times, respectively, those in frankfurters (Tables 6 and 9). We suspect that these compounds mostly arise during the long curing process for tobacco. In chewing tobacco, the mean NOC level of 660 μmol/kg was 8.9 times the maximum level of 74 μmol/kg for the tobacco-specific nitrosamines in this product (*5*). Hence, the NOC in chewing tobacco is unlikely to consist chiefly of these nitrosamines.

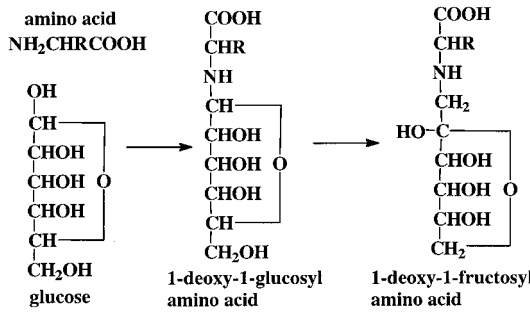

Figure 4. Formation of two types of *N*-glycosyl amino acids produced in initial stages of the Maillard reaction between glucose and amino acids.

The mean NOC/mean NOCP ratio in each product may indicate the extent of NOCP nitrosation. This ratio varied from 1/490 for frankfurters to 1/4.4 for chewing tobacco. However, the ability of the NOCP to form NOC in vivo may be at least as important as their ability to form NOC in the products themselves.

The wide variations in the NOC and NOCP levels in foods (Tables 5 and 6, e.g., compare frankfurter brands 6 and 9) and even in the same product purchased at different times (Results, Frankfurters) suggest that, if deemed necessary, one could discover what causes this variation and thereby control the manufacture of frankfurters and other foods to achieve lower NOC and NOCP levels. If the NOC and NOCP in frankfurters are identified and the NOC (occurring as such or derived by in vivo nitrosation of NOCP) are carcinogenic in laboratory animals, this may help to explain how cured meat may produce tumors in the colon and in children (see Introduction). The NOC in cigarette smoke particulates might contribute to the etiology of cancers caused by cigarette smoking (*5*). Most NOC and NOCP in chewing tobacco may be chemically similar to those in foods [although NOC concentration was >100 times higher in chewing tobacco than in frankfurters (Tables 6 and 9)] and might contribute to the etiology of oral cancer associated with this product (*5*). Some of the NOCP in fresh and preserved meat were probably present before slaughter because NOCP occurred in freshly dissected rat muscle and cold storage of this muscle for 6 days did not much affect its NOCP content (Table 6 and Results, Canned Corned Beef, Summer Sausage, and Fresh Meat).

The NOCP in the analyzed products are unlikely to be simple secondary amines, for example, spermidine and spermine [which occur in muscle (*50*)], because we expect such amines to be nitrosated about as slowly as diethylamine (Table 2). Our preliminary results on the purification and identification of NOCP in frankfurters (*28*) and the ready nitrosation of an *N*-glycosyl amino acid under the conditions used to measure NOCP (Table 2) suggest that the principal NOCP are 1-deoxy-1-glucosyl amino acids and/or the products of their Amadori rearrangement, that is, 1-deoxy-1-fructosyl amino acids (Figure 4). These compounds are formed in the early stages of the Maillard reaction by the reaction of glucose with amino acids (*52*) and some of them are mutagenic in the Ames test in the absence of microsomes, that is, are direct mutagens (*53*, *54*). We plan to describe the purification of the frankfurter NOCP in a later publication. The NOCP in other foods could be similar to those in frankfurters. In cigarette smoke particulates, the close correlations between NOCP, particulates, and tar (Results, Cigarett Smoke Pads) suggest that the NOCP, which might also consist mainly of nonvolatile glycosyl amino acids, were transported in the particulate matter of the smoke. Other preliminary experiments indicate that tryptophan and histidine, which can readily be nitrosated to produce mutagenic NOC (*55*, *56*), are not major components of the NOCP, at least in frankfurters, because free tryptophan and histidine concentrations in aqueous extracts of frankfurters are far lower than those of the NOCP (F. Perini, L. Zhou, and S. S. Mirvish, unpublished data). The hazard presented by these NOCP depend on the ease of formation of the corresponding NOC (Table 2) and on whether the derived NOC are carcinogens.

ABBREVIATIONS USED

MNNG, methylnitronitrosoguanidine; MNU, methylnitrosourea; NOC, *N*-nitroso compounds; NOCP, NOC precursors; NPRO, *N*-nitrosoproline; SA, sulfamic acid; TEA, thermal energy analysis/analyzer.

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