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Relationship of Rat Urinary Metabolites of *N*-Nitrosomethyl-*N*-alkylamine to Bladder Carcinogenesis[1]

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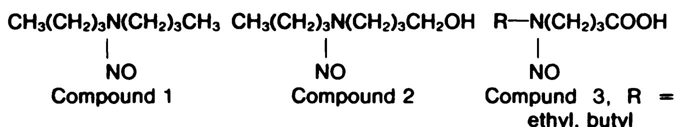
ABSTRACT

Nitrosomethylalkylamines with chain lengths from C_4 (*n*-butyl-) to C_{14} (*n*-tetradecyl-) were each administered to three rats at doses equimolar with 12 mg of the butyl compound. All of the compounds administered to rats at this dose, twice a week for 30 weeks, induced tumors in 100% of the animals. Some of the compounds with even-numbered alkyl chains induced bladder tumors, and a connection was sought with the metabolites of these excreted in urine. The pooled 24-hr urine was extracted with ethyl acetate before and after acidification to provide a neutral fraction and a fraction containing nitrosoamino acids. The fraction containing the acids was analyzed by capillary gas chromatography and by gas chromatography-mass spectrometry after esterification with diazomethane; the neutral fraction was analyzed similarly. The principal metabolite of the nitrosamines with odd-numbered chains was found in the acidic fraction and was identifed as nitrosomethyl-2-carboxyethylamine. There were several acids in the mixtures derived from the nitrosamines with even-numbered chains, nitrososarcosine and nitrosomethyl-3-carboxypropylamine being the major components. There was no trend in the yields of the nitrosamino acids that could be correlated with the differences in carcinogenic potency between the nitrosamines; the maximum yield of acids was more than 30% (from the tetradecyl compound). The principal component of the neutral fraction (≤1% of the nitrosomethylalkylamine administered) was nitrosomethyl-2-oxopropylamine. The yield of this compound increased with increasing length of the even-numbered chain nitrosamines.

INTRODUCTION

Of all the nitrosamines that have been tested for carcinogenicity, only a very few induce bladder tumors in rats. Druckrey *et al.* (3) observed bladder tumors after treatment with *N*-nitrosodi-*n*-butylamine (Compound 1; see below) and *N*-nitroso-*n*-butyl-4-hydroxybutylamine (Compund 2). Okada and Ishidate (10) showed that other *N*-nitrosoalkyl-4-hydroxybutylamines induced bladder tumors as did the principal urinary metabolite, the carboxypropyl derivative (Compound 3) of the ethyl and butyl analogs.



This group (10) and that of Blattmann *et al.* (2) also found β-hydroxylation products among the urinary metabolites and thus proposed (based on the Knoop mechanism of fatty acid metabolism) that only even-numbered alkyl chain compounds would give rise to bladder tumors. This hypothesis was supported by the observation that *N*-nitrosomethyldodecylamine induced bladder tumors (6), but liver tumors were produced by the undecyl homolog (7). Support was found more recently in a series of tests in which bladder tumors were induced by the corresponding *n*-octyl, *n*-dodecyl, and *n*-tetradecyl homologs but not by the homologous odd-numbered chain compounds (5).

We have now examined in detail the urinary metabolites of a series of methyl-*n*-alkylnitrosamines (C_4 to C_{14}) seeking a link with the induction of bladder tumors by 4 of the even-numbered alkyl chain compounds, the methyl-*n*-octyl-, methyl-*n*-decyl-, methyl-*n*-dodecyl-, and methyl-*n*-tetradecylnitrosamines.

MATERIALS AND METHODS

Metabolite Isolation

A solution of the nitrosamine in corn oil (0.52 mM; 0.2 ml) was administered by gavage to each of 2 or 3 adult male Fischer rats from the Frederick Cancer Research Center colony. This dose was identical with that given to each of the rats in the chronic toxicity tests as a twice weekly regimen (5). The rats were approximately 6 months old and generally weighed 250 to 340 g; 7 weighed less and 3 weighed more. The rats were housed in plastic metabolism cages (1 or 2/cage) and given water *ad libitum*.

The urine was collected from each cage at room temperature during 24 hr and combined. The neutral fraction was obtained by extracting the urine (pH 6.5) 3 times in polypropylene centrifuge tubes with an equal volume of ethyl acetate. The mixture was shaken on a Vortex mixer for 1 min and centrifuged at 10,000 rpm for 10 min. The organic phase was removed by pipet, dried ($CaCl_2$), and concentrated in a vacuum (40°; 30 mm) to 2.0 ml for GLC[3]-TEA analysis.

The extracted urine was acidified to pH 1.5 with 12 N hydrochloric acid, and the extraction procedure was repeated. The organic extract was treated with ethereal diazomethane to esterify the carboxylic acids. After standing at room temperature for at least 1 hr, the solution was treated with acetic acid to destroy excess diazomethane and then was concentrated in a vacuum to 10.0 ml for GLC-TEA analysis.

In 2 instances, the extracted urine was neutralized to pH 6, incubated with glucuronidase, and reextracted. No additional nitrosamines were found.

Quantitative GLC-TEA was carried out on several different GLC columns. The best resolution was obtained on 25-m vitreous silica WCOT Carbowax 20M (Hewlett-Packard) and OV-101 (SGE) capillary columns. The GLC furnace of the TEA was connected directly to the

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[3] The abbreviations used are: GLC, gas-liquid chromatography; TEA, Thermal Energy Analyzer; MS, mass spectrum or mass spectrometry; NMOP, nitroso-3-methylamino-2-propanone or *N*-nitroso-*N*-methyl-2-oxopropylamine; NMCP, nitrosomethyl-3-carboxypropylamine.

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outlet of the GLC column to minimize the interface distance. An internal standard, nitrosodi-*n*-butylamine, was added to each sample prior to the quantitative GLC analysis. The concentration was comparable to that of the components to be quantified. The TEA detector was directly interfaced to a Hewlett-Packard Model 3540 computer for measurement of peak areas, and the calculations were carried out by a package of programs written in LABASIC on this computer.

Components which had previously been identified by GLC-MS exact mass measurements (Table 3) and for which standards were available were identified in each sample by retention time relative to the internal standard. Concentrations were calculated using relative molar response factors.

To evaluate the reliability and efficiency of our analytical procedure, several nitrosamines were added to urine collected from rats which had not been given any nitrosamines. The urine was then analyzed as described above. Recoveries were as follows: *N*-nitrososarcosine, 70%; 4-(nitrosomethylamino)-butanoic acid, 86%; *N*-nitroso-4-methylamino-1-butanol, 70%. It is, therefore, reasonable to assume that we suffered only small losses during work-up. Moreover, when we added 1.2 mg sarcosine to urine of untreated rats, no nitrososarcosine was detectable in the acids extract, thus ensuring that there was no artifactual nitrosamine formation during work-up. (The minimum detectable amount of nitrososarcosine was 0.005% of the added sarcosine.)

High-resolution GLC-MS was performed on a 6-ft × 2-mm glass column packed with 4% OV-17 on Chromosorb W in a Perkin-Elmer Sigma 2 gas chromatograph interfaced to a VG ZAB-2F mass spectrometer operating in the EI mode under computer control; data acquisition was with a VG Model 2035 data system.

Methylalkylnitrosamines

These compounds were either available from previous studies or were prepared by standard procedures, or by improved procedures. Analysis by GLC established their purity at >98%. The trace impurities were the adjacent homologs.

Metabolites

***N*-Nitrosomethylsarcosine.** This was prepared by esterification of *N*-nitrososarcosine (kindly provided by Dr. S. Koepke).

4-(Methylnitrosamino)butanoic Acid. *N*-Methyl-2-pyrrolidinone (50 g; 0.43 m) was boiled under reflux overnight with 6 N hydrochloric acid (200 ml). The solution was evaporated, diluted with water (250 ml), and nitrosated in the usual way with sodium nitrite (147 g; 2.14 m) to give the nitrosamino acid: 34.5 g (56%). IR (film), 1700 cm^{-1}; UV (ethanol), 348 nm (94) [literature (11): UV (ethanol), 348 nm (92)].

Methyl 4-(Methylnitrosamino)butanoate. *N*-Methyl-2-pyrrolidinone (10 ml; 0.1 m) was boiled under reflux in 6 N hydrochloric acid (100 ml) overnight. The solution was evaporated in a vacuum to dryness, and the residue was boiled under reflux overnight in methanol (100 ml). After evaporation to an oil, the residue was nitrosated in the usual way with sodium nitrite (30 g; 0.43 m) to give the nitroso ester: 3.64 g (25%). IR (film), 1722, 1430 cm^{-1}; UV (ethanol), 347 nm (85); MS (70 eV), 160 (M^+, 0.67), 130 (18.1), 99 (20.5), 98 (41.9), 73 (27.3), 70 (31.0), 59 (30.4), 42 (100).

Methyl 5-(Methylnitrosamino)pentanoate. This compound was prepared from 1-methyl-2-piperidinone by the procedure described above. b.p. 103–104° at 0.18 mm Hg; UV (ethanol), 347 nm (103).

$C_7H_{14}N_2O_3$

Calculated: C 48.26, H 8.10, N 16.08
Found: C 48.26, H 8.08, N 15.96

Methyl 6-(Methylnitrosamino)hexanoate. This compound was prepared from *N*-methylcaprolactam by the procedure described above. UV (ethanol), 346 nm (108).

$C_8H_{16}N_2O_3$

Calculated: C 51.05, H 8.57, N 14.88
Found: C 50.99, H 8.64, N 14.73

***N*-Nitroso-4-hydroxybutylmethylamine.** A solution of 4-chlorobutanol (2.14 g; 0.02 m) in methanol (100 ml) was stirred in an ice bath while methylamine was bubbled through for 1 hr. The solution was boiled under reflux for 72 hr and then evaporated in a vacuum to a semicrystalline solid. The crude product, dissolved in 30% acetic acid (50 ml), was nitrosated by stirring for 1 hr with sodium nitrite (7 g; 0.1 m). The reaction mixture was neutralized with K_2CO_3 and extracted with chloroform (3 × 50 ml) which was dried (K_2CO_3) and evaporated to a dark yellow oil: 1.25 g (47%). The nitrosamine was pure by gas chromatography and gas chromatography-TEA. IR (film), 3400 (OH, broad), 1420 (NO), 1330 cm^{-1}; UV (ethanol), 346 nm (85); MS, 132 (M^+, 0.88), 115 (24.6, M^+ − OH), 114 (1.1, $M^+ - H_2O$), 42 (100%).

5-(Methylnitrosamino)butyrolactone. This compound was prepared by a modification of the procedure used for the preparation of the butyl analog (8) and had the anticipated spectral properties.

***N*-Nitroso-*N*-methyl-2-hydroxypropylamine.** This compound was prepared by an alternate procedure to that published previously (13).

1-Amino-2-propanol was formylated by boiling with ethyl formate to give 1-(*N*-formyl)amino-2-propanol in 93% yield. The formamide was reduced with $LiAlH_4$ to *N*-methyl-2-hydroxypropylamine in 74% yield, and this was converted to the nitrosamine in 68% yield (after distillation, b.p. 76° at 0.03 mm Hg). The spectral properties were in accord with those published previously (13).

NMOP This compound was also prepared by an alternate procedure to that published previously (13).

N-Methyl-2-hydroxylpropylamine was oxidized by CrO_3/H_2SO_4, and the product nitrosated *in situ* to give NMOP in 42% yield (after distillation, b.p. 93–96° at 0.5 mm Hg). The spectral properties were in accord with those published previously.

RESULTS

Although we could recover 70% of *N*-nitroso-4-hydroxybutylmethylamine added to blank urine, we have not detected it as a metabolite from any nitrosomethylalkylamine. This compound was found by Blattmann *et al.* (2) as a metabolite from nitrosomethylbutylamine. We also did not find nitroso-4-methylaminobutyrolactone, the methyl homolog of a mutagenic putative metabolite of nitrosodibutylamine (8). Either of these compounds would have been detectable if formed to the extent of 0.01%.

Data on the carboxylic acids detected are detailed in Table 1. The amounts of recovered nitrosamine and of the neutral metabolites are in Table 2. No nitrosamino ester with more than 7 carbons was detected.

The carboxylic acid esters were identified by high-resolution GLC-high-resolution MS. Methyl nitrososarcosine, methyl 3-(nitrosomethylamino)-propionic, methyl 4-(nitrosomethylamino)butyric, 5-(nitrosomethylamino)-pentanoic, and 6-(nitrosomethylamino)hexanoic acids were confirmed by comparison of their mass spectra and retention times with those of authentic samples and particularly by exact mass measurement of the molecular ion and/or principal fragment ions (Table 3).

The major extractable neutral metabolite was identified as NMOP (Compound 4; see below) from the high-resolution GLC-MS and accurate mass measurement of the molecular ion (m/z = 116.0592) and first major fragment (m/z = 73.0380), $C_4H_8N_2O_2$ and $C_2H_5N_2O$, respectively, as well as by comparison with the spectrum of an authentic sample. The retention time was also identical with that of an authentic standard on 2 different GLC columns, a nonpolar packed column, 10% OV1 on Chromosorb W, and a polar vitreous silica capillary column

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Table 1

Metabolite yields of nitrosomethylaminoalkylcarboxylic acids in urine of rats given nitrosomethylaminoalkanes[a]

Precursor	No. of animals	Yield (%[b]) Products: $CH_3N(NO)(CH_2)_nCOOH$ where *n* is 1	2	3	4	5	6
With even-numbered chainlengths							
$CH_3N(NO)(CH_2)_3CH_3$	3	0.34	Tr[c]	1.81			
$CH_3N(NO)(CH_2)_3COOH$	2	17.5	ND	22.1			
$CH_3N(NO)(CH_2)_5CH_3$	3	5.4	ND	13.1	ND	6.2	
$CH_3N(NO)(CH_2)_7CH_3$	2	9.8	1.0	10.6	ND	3.0	
$CH_3N(NO)(CH_2)_9CH_3$	2	9.3	3.5	15.0	Tr	0.52	Tr
$CH_3N(NO)(CH_2)_{11}CH_3$	2	10.1	1.2	11.4	Tr	0.27	0.20
$CH_3N(NO)(CH_2)_{13}CH_3$	3	17.0	0.75	19.31	ND	0.29	
With odd-numbered chainlengths							
$CH_3N(NO)(CH_2)_4CH_3$	3	0.04	0.83	Tr	0.25		
$CH_3N(NO)(CH_2)_6CH_3$	3	0.66	6.0	0.43	2.1	0.34	0.38
$CH_3N(NO)(CH_2)_8CH_3$	3	1.1	12.0	0.70	0.60	0.06	
$CH_3N(NO)(CH_2)_{10}CH_3$	3	1.0	11.4	1.1	0.63		
$CH_3N(NO)(CH_2)_{12}CH_3$	3	0.66	21.0	0.83	0.79	Tr	Tr

[a] Results from pooled urine of one group of rats for each nitrosamine.
[b] Based on mmol of dose administered.
[c] Tr, trace; ND, not detected.

Table 2

Yields of neutral metabolites in urine of rats treated with nitrosomethylaminoalkanes

Precursor	Yield (%[a]) Recovered precursor	NMOP	NMHP[b]
With even-numbered chainlengths			
$CH_3N(NO)(CH_2)_3CH_3$	0.06	0.01	Tr
$CH_3N(NO)(CH_2)_3COOH$	29.4	0.25	ND
$CH_3N(NO)(CH_2)_5CH_3$	0.01		Tr
$CH_3N(NO)(CH_2)_7CH_3$	0.02	0.20	ND
$CH_3N(NO)(CH_2)_9CH_3$	0.01	0.22	
$CH_3N(NO)(CH_2)_{11}CH_3$	ND	0.29, 0.28[c]	
$CH_3N(NO)(CH_2)_{13}CH_3$	ND	0.53	
With odd-numbered chainlengths			
$CH_3N(NO)(CH_2)_4CH_3$	0.004	ND	
$CH_3N(NO)(CH_2)_6CH_3$	0.05	0.02	
$CH_3N(NO)(CH_2)_8CH_3$	0.01	0.03	0.01
$CH_3N(NO)(CH_2)_{10}CH_3$	ND	0.04	0.01
$CH_3N(NO)(CH_2)_{12}CH_3$	ND	0.04	0.03

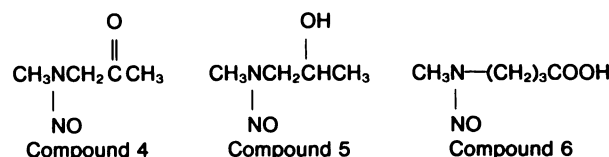
[a] Based on mmol of dose administered.
[b] NMHP, nitroso-3-methylamino-2-hydroxypropane; Tr, trace; ND, not detected.
[c] Results from 2 analyses.

(Carbowax 20M) and on an intermediate-polarity high-performance liquid chromatography column, bonded propionitrile, used in normal phase.

The proportion of this nitrosaminoketone in the urine increased with the length of the even-numbered alkyl chains; 0.01% was found from the C_4 homolog and amounts of 0.20% from C_8 to 0.53% from C_{14}. Much lower levels were seen from the odd-numbered alkyl homologs, typically 0.02 to 0.04% (Chart 1).

When NMOP was given to rats by gavage (1.2 mg/rat), only 0.8% was excreted unchanged. The major metabolite (3%) was the corresponding alcohol, nitrosomethyl-2-hydroxypropylamine (Compound 5), and approximately 0.1% was excreted as nitrososarcosine. The alcohol was identified by comparison of its retention time with a coinjected authentic sample. A comparable dose of the alcohol (1.2 mg/rat) led to only a trace of the ketone in the neutral fraction. The alcohol itself was reexcreted in 8.5% yield.

NMOP was also the major neutral urinary metabolite (0.25%) of NMCP (Compound 6), when 15 mg was given to each of 2 rats by gavage; 22% was excreted unchanged and 16.5% as nitrososarcosine.



Compound 4 Compound 5 Compound 6

To examine the effect of deuterium substitution on the proportion of urinary metabolites, nitrosomethyl-*n*-dodecylamine and nitrosomethyl-d_3-*n*-dodecylamine were administered in parallel experiments. As shown in Table 4, there was no significant change in the proportions of the various carboxylic acids excreted, but more than twice as much of the ketone (NMOP; Compound 4) was obtained from the deuterated compound.

The data in Table 4 also show the reproducibility of our data. Replicate experiments with nitrosomethyl-*n*-dodecylamine and nitrosomethyl-d_3-*n*-dodecylamine give reasonably close agreement for the metabolite yields of the acids (except for sarcosine from the d_3 compound) and remarkably reproducible yields for the NMOP.

DISCUSSION

The concept that nitrosamines which are bladder carcinogens are metabolized to an active form in the liver and that this active form is then transported through the kidneys to the bladder and there converted to a proximate carcinogen was suggested by Druckrey *et al.* (3) and refined by Okada (9) and Okada and Ishidate (10). Okada and Suzuki (11) further suggested that nitrosodi-*n*-butylamine was oxidized to nitrosobutyl-3-carboxypropylamine (Compound 6) which they found as the principal metabolite in the urine of rats treated with nitrosodi-*n*-butylamine and which they suggested was the proximate carcinogen.

Following the report that nitrosomethyl-*n*-dodecylamine was a bladder carcinogen in rats (6), as well as in hamsters (1), Okada *et al.* (13) examined the urinary metabolites of that compound and found a substantial amount of NMCP. They suggested that this was the proximate bladder carcinogen of nitrosomethyldodecylamine (13), even though it was not the principal metabolite in this case.

The results of our present studies do support the idea that the metabolism of the nitrosomethylalkylamines with even-numbered chains is qualitatively similar, correlating with the fact that those which have 8 carbons or more in the alkyl chain are bladder carcinogens in rats (5). Moreover, the metabolism of those is quantitatively very different from that of the nitrosomethylalkylamines with odd-numbered chains, of which those with C_7 and above induce liver tumors and those with C_3 and C_5 induce esophageal tumors.

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Table 3

High-resolution MS data for identification of methyl nitrosocarboxyalkylmethylamines from urine of rats treated with nitrosomethyl-n-octylamine

Component	Observed mass	Empirical formula	Defect (mmu)[a]	Fragment	Compound
1	132.0492	$C_4H_8N_2O_3$	4.3	M^+	Nitrosomethylsarcosine
	102.0516	$C_4H_8NO_2$	3.9	$M^+ - NO$	
2	146.0680	$C_5H_{10}N_2O_3$	1.1	M^+	$CH_3N(NO)(CH_2)_2COOCH_3$
	116.0723	$C_5H_{10}NO_2$	1.2	$M^+ - NO$	
3	160.0806	$C_6H_{12}N_2O_3$	4.2	M^+	$CH_3N(NO)(CH_2)_3COOCH_3$
	145.0679	$C_5H_9N_2O_3$	6.6	$M^+ - CH_3$	
	130.0784	$C_6H_{12}NO_2$	8.4	$M^+ - NO$	
4	144.1024	$C_7H_{14}NO_2$	0.1	$M^+ - NO$	$CH_3N(NO)(CH_2)_4COOCH_3$
	113.0758	$C_6H_{11}NO$	8.1	$M^+ - NO - OCH_3$	
5	158.1197	$C_8H_{16}NO_2$	1.6	$M^+ - NO$	$CH_3(NO)(CH_2)_5COOCH_3$
	127.0967	$C_7H_{13}NO$	3.0	$M^+ - NO - OCH_3$	
6	172.1398	$C_9H_{18}NO_2$	6.3	$M^+ - NO$	$CH_3N(NO)(CH_2)_6COOCH_3$
	141.1111	$C_8H_{15}NO$	8.5	$M^+ - NO - OCH_3$	

[a] mmu, millimass unit.

Table 4

Metabolite yields from nitrosomethyl-n-dodecylamine and nitrosomethyl-d_3-n-dodecylamine[a]

		Yield (%[b])					
Compound	No. of animals	Sarcosine	NMC_3[c]	NMCP (Compound 6)	NMC_5	NMC_6	NMOP (Compound 4)
$CH_3N(NO)C_{12}H_{25}$	3	9.1	1.3	8.6	Tr	0.8	0.28
	2	10.1	1.2	11.4	Tr	0.3	0.29
$CD_3N(NO)C_{12}H_{25}$	3	9.8	1.6	12.1	ND	0.3	0.63
	2	20.2	0.9	14.2	ND	Tr	0.61

[a] Results from pooled urine of the number of rats indicated.
[b] Based on dose administered.
[c] NMC_3, nitroso-3-methylaminopropionic acid; NMC_5, nitroso-5-methylaminopentanoic acid; NMC_6, nitroso-6-methylamino hexanoic acid; Tr, trace; ND, not detected.

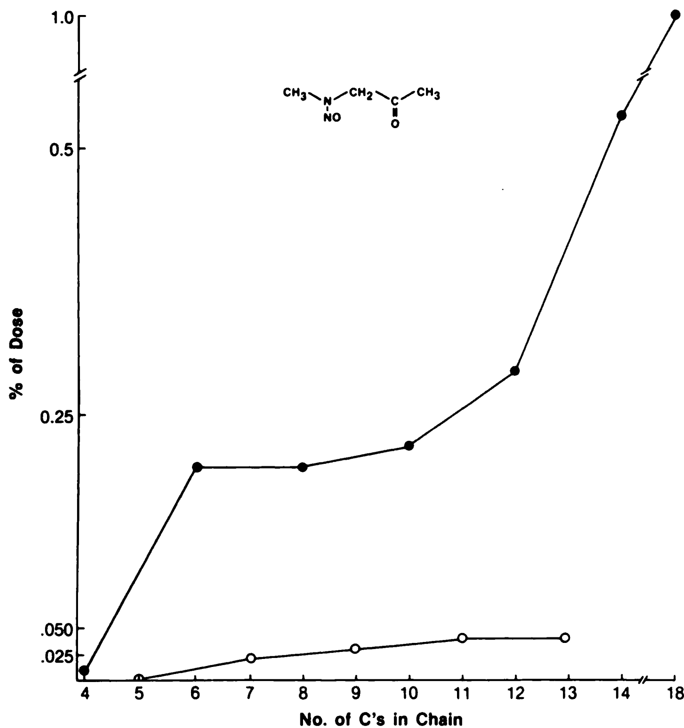

Chart 1. Excretion of NMOP as a percentage of administered dose of the nitrosomethylaminoalkanes from even-numbered chains (●) and from odd-numbered chains (○).

In our experiments, the nitrosomethylaminoalkanes with an even number of carbon atoms were metabolized to approximately equal amounts of nitrososarcosine and NMCP and serially increasing amounts of NMOP. Those with an odd number of carbons, on the other hand, were metabolized primarily to nitroso-3-methylaminopropionic acid. Only very small amounts of the other acids or of NMOP were found.

The absence in this study of the alcohol and of glucuronides which have been found by others (2, 10) is probably a reflection of the different doses used. We used a dose equal to that used as a twice weekly dose in the chronic carcinogenicity assay (5), ranging from 12 mg/rat for nitrosomethylethylamine to 26 mg/rat for nitrosomethylbutylamine. In other studies, doses of 140 to 500 mg/rat were used.

While NMCP is a common prominent metabolite of the even-numbered nitrosomethylalkylamines, it is by no means certain that it is the proximate carcinogen for the bladder. More of this nitrosamino acid is formed from nitrosomethyltetradecylamine (C_{14}) than from the C_{12}, C_{10}, or C_8 homologs, although the C_{14} homolog is no more potent a carcinogen than the lower homologs and appears to be less potent than the C_8 compound (5). The C_8 compound, nitrosomethyloctylamine, induces bladder tumors (together with liver tumors) in a much shorter time than the larger homologs, and this time to tumor can be considered an index of carcinogenic potency. It is also significant that nitrososarcosine is a common urinary metabolite of the nitrosomethylalkylamines with even-numbered chains and is produced to very similar extents from all of them and in comparable amounts with Compound 6, yet it is highly unlikely that this compound is a proximate bladder carcinogen. Indeed, nitrososarcosine at relatively high doses gives rise to esophageal tumors in rats, yet none of the larger nitrosomethylalkylamines has induced esophageal tumors in our experiments, although the smaller molecules have induced esophageal tumors (3, 5).

The presence of NMOP as a urinary metabolite of the even-numbered nitrosomethylalkylamines might be significant even though the quantities found are small (Chart 1). In fact, when

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NMOP (Compound 4) was administered to rats, it was extensively metabolized, as shown by the findings that only 0.8% is excreted unchanged and that it is converted to Compound 5 (3%). [These results are in qualitative agreement with the findings of Pour *et al.* (14) on the metabolism of this compound by Syrian golden hamsters.] This ketone could be easily formed by β-oxidation of NMCP (Compound 6) followed by decarboxylation and is, in fact, excreted (0.25%) when Compound 6 is fed to rats. The finding that the amount of the ketone increases when the *N*-methyl group of nitrosomethyldodecylamine is labeled with deuterium (Table 4), whereas the formation of the small nitrosamino acids is relatively unchanged, indicates that competition for oxidation occurs between the 2 aliphatic chains (4).

The significance of the metabolites of nitrosomethylalkylamines identified in the urine to bladder carcinogenesis remains to be determined. Chronic toxicity tests of NMCP, NMOP, and Compound 5 in rats are in progress.

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Relationship of Rat Urinary Metabolites of *N*-Nitrosomethyl-*N*-alkylamine to Bladder Carcinogenesis

George M. Singer, William Lijinsky, Leonard Buettner, et al.

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