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# The Induction of Bladder Tumors in F344 Rats by Intravesicular Administration of Some Nitrosamines*[1]

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**Three nitrosamines, metabolically related and formed *in vivo* from the bladder carcinogen nitrosomethyl-*n*-octylamine, were administered to groups of 12 female F344 rats by intraurethral instillation twice a week for 30 weeks. All three compounds induced tumors in the urinary bladder. Nitrosomethyl-2-oxopropylamine at 10 mg/ml was the most potent, causing death of half of the animals with tumors at 43 weeks, following a total dose of 1.0 mmol; most of the rats also had tumors of the nasal mucosa, and there were some tumors of the kidney and kidney pelvis. Nitrosomethyl-2-hydroxypropylamine at 10 mg/ml (total dose 1.0 mmol) was much less effective, the median week of death being 83 weeks. In addition to bladder tumors, this group had tumors of the nasal mucosa, esophagus, and kidney. Nitrosomethyl-3-carboxypropylamine at 75 mg/ml and a total dose of 6.2 mmol per rat induced a high incidence of bladder tumors and tumors of the kidney pelvis, but not tumors of the nasal mucosa; the median week of death for this group was 55 weeks. It is concluded that nitrosomethyl-*n*-alkylamines that induce bladder tumors by oral administration to rats are metabolized to nitrosomethyl-3-carboxypropylamine, which is excreted in the urine and further metabolized to nitrosomethyl-2-oxopropylamine, the proximate bladder carcinogen.**

Key words: Bladder tumors — Nitrosamines — Rats

Although nitrosamines are a group of carcinogens that induce a broad spectrum of tumors, few have induced tumors of the urinary bladder. It might be expected that metabolites excreted into the urine could cause such tumors through direct exposure of the bladder epithelium. The first nitrosamine found to induce bladder tumors was nitrosodi-*n*-butylamine given orally to rats.[1] Several metabolic products of this nitrosamine were also shown to induce bladder tumors in rats, including nitroso-*n*-butyl-4-hydroxybutylamine and nitroso-*n*-butyl-3-carboxypropylamine, both of which are believed to be intermediates in the activation of nitrosodibutylamine.[2] More recently, several nitrosomethyl-*n*-alkylamines with long aliphatic chains have been shown to induce only bladder tumors in rats[3]; nitrosodibutylamine induces tumors in other organs in addition to the bladder.[1,4] Only those nitrosamines with alkyl chains containing an even number of carbon atoms gave rise to bladder tumors,[3] and Okada *et al.*[5] postulated that oxidative metabolism of the alkyl chain to a 3-carboxypropyl residue was responsible for the appearance of bladder tumors. The presence of nitrosomethyl-3-carboxypropylamine in the urine of rats given the bladder carcinogen nitrosomethyl-*n*-dodecylamine prompted this conclusion. Later, it was shown that the same nitrosamino acid was a common and major metabolite found in the urine of rats given a variety of nitrosomethyl-*n*-alkylamines that induce bladder tumors. Also present in those urines were two minor nitrosamine components,[6] nitrosomethyl-2-oxopropylamine (NMOP), which can be considered a product of the further $\beta$-oxidation of nitrosomethyl-3-carboxypropylamine (NMCOP) followed by decar-

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boxylation,[7] and nitrosomethyl-2-hydroxypropylamine (NMHP), a reduction product of NMOP.

Because NMOP was an end-product of the metabolism of the nitrosomethyl-*n*-alkylamines which induce bladder tumors in rats, and NMCOP induced bladder tumors by oral administration to rats,[8] it seemed probable that NMOP was the proximate bladder carcinogen from these nitrosamines. However, oral administration of NMOP to rats at several different dose rates failed to produce bladder tumors, although the compound was a potent carcinogen. At high doses (100 mg/liter) in drinking water NMOP induced a high incidence of liver tumors in female rats (lower in males), together with tumors of the esophagus.[8] When given by gavage at doses ranging from 1 mg–12.5 mg twice a week, only tumors of the esophagus were produced, in each case leading to early death of the animals; a single rat had a tumor of the bladder. At several different dose rates in drinking water, NMHP also induced only tumors of the esophagus in rats,[8,9] but no bladder tumors.

It was possible that the competing risk of esophageal tumors leading to early death of the rats, preceded the development of bladder tumors. Hashimoto *et al.*[10] reported the induction of bladder tumors in rats by repeated intravesicular instillation of nitroso-*n*-butyl-3-carboxypropylamine into the bladder. This technique offered a chance to circumvent the effect, possibly direct, of NMOP in the rat esophagus when it is given orally. We undertook, therefore, to administer NMOP, NMHP and NMCOP by instillation into the bladder of female rats, to observe the direct effects of these compounds on rat bladder epithelium.

## Materials and Methods

**Chemicals** NMOP, NMHP and NMCOP were prepared and purified as described previously.[6] They were dissolved, at the concentrations shown in Table I, in neutral deionized water that had been sterilized by boiling for 1 hr. The solutions were stored at 4° in the dark.

**Animal Treatments** Groups of 12 female F344 rats of the colony of the Frederick Cancer Research Facility were bred and maintained within a barrier, housed four to a polycarbonate cage. They were fed Purina Autoclavable Rodent chow (Ralston Purina Co., St. Louis, Missouri) and were given acidified tap water (adjusted to pH 2.5 with HCl) *ad libitum*. At age 10 weeks the rats were treated twice a week under anesthesia [metofane (methoxyflurane), Pittman-Moore] with 0.2 ml of one of the nitrosamine solutions delivered by syringe with a flexible Teflon catheter (Abbocath-T 20G×1-1$\frac{1}{4}$″ iv) inserted directly into the bladder through the urethra. To help prevent infection, each catheter was used on only one animal and the catheters were stored in absolute ethanol. The urethra and surrounding skin was swabbed with an isopropyl alcohol preparation and prior to catheterization the bladder was compressed to expel most of the urine. This assured retention of most of the solution for some time.

Three groups of rats were each given one of the nitrosamine solutions and a fourth, control, group was treated only with sterile water. All treatments lasted 30 weeks. If clinical signs of infection or

Table I. Induction of Tumors of the Bladder in Female F344 Rats by Intraurethral Injection of Nitrosamines

| Compound and concentration | Weekly dose (2×mg) | Total dose (mmol) | Number of survivors at week | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0 | 10 | 20 | 30 | 40 | 50 | 60 | 70 |
| Nitroso- | | | | | | | | | | |
| methyl-3-carboxypropylamine 75 mg/ml | 2×15 | 6.2 | 12 | 12 | 12 | 11 | 11 | 6 | 4 | 0 |
| methyl-2-hydroxypropylamine 10 mg/ml | 2×2 | 1.0 | 12 | 11 | 11 | 11 | 11 | 11 | 11 | 10* |
| methyl-2-oxopropylamine 10 mg/ml | 2×2 | 1.0 | 12 | 12 | 12 | 9 | 7 | 0 | | |

*Sacrificed at 87 weeks.

inflammation appeared, the rat was treated with 5 mg amoxicillin once a day by gavage until signs disappeared, usually in 3–5 days. Animals were treated on a case-by-case basis and there was occasional minor inflammation in each of the groups, including controls.

At the end of treatment the animals were maintained until natural death, or killed when moribund, except four survivors in the NMHP group, which were sacrificed at week 87. Almost all of the controls were still alive at that time. All animals were necropsied and all lesions and major organs and tissues were fixed in formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin for histologic examination.

## Results and Discussion

As shown in Table I, most of the nitrosamine-treated rats died before week 70 following the beginning of treatment, except the group given NMHP and the controls. Many of the latter were still alive at week 110. Tumors induced by the treatments seemed to be the principal cause of death of the nitrosamine-treated animals, except three in the NMOP group which had acute hemorrhagic inflammation in the bladder due to infection and died before week 30. Other inflammatory or proliferative lesions of the urinary bladder were minor and included a few examples of focal or diffuse epithelial hyperplasia, squamous metaplasia or calculi. The NMOP-treated rats died earlier than the other groups which received equal or higher nitrosamine doses. The dose of NMCOP was more than six times as high as that of NMOP, yet most rats given the former were alive when all of those given NMOP were dead with tumors. By the criterion of reduced time-to-death with tumors, often the best measure of potency in dose-response studies with nitrosamines,[11] NMOP is clearly more potent in rats than NMHP or NMCOP when administered by bladder instillation. When given orally, NMOP was also a more potent carcinogen in rats than NMHP or NMCOP, inducing a different pattern of tumors[8]; the difference between NMOP and NMHP was not as great as in the present experiment.

The most common tumors induced in this study were transitional cell carcinomas and papillomas of the urinary bladder, which were present in two-thirds or more of the rats in all three chemically treated groups. Not all of the urinary bladder neoplasms were considered the cause of death. In those cases where neoplasms were large, death was considered to be due to urinary tract obstruction. The bladder tumors in the NMOP-treated group included 5 carcinomas, whereas only papillomas were seen in the other two groups. These tumors are extremely rare in untreated F344 rats, and none was seen in the controls of this experiment that have died to date. There were several transitional cell neoplasms of the kidney pelvis in rats treated with NMCOP.

Nasal neoplasms were a major contribution to the cause of death of NMHP- and NMOP-treated rats. Often the nasal neoplasms were large and involved the olfactory lobes of the cerebral cortex. Papillomas of the esophagus contributed to the death of two rats given NMHP.

| Median survival (weeks) | Effective no. of rats | Number of rats with tumors of | | | | |
|---|---|---|---|---|---|---|
| | | Urinary bladder | Kidney pelvis | Nasal mucosa | Esophagus | Kidney mesenchymal |
| 55 | 11 | 9 | 4 | — | — | — |
| 83 | 11 | 7 | — | 8 | 3 | 3 |
| 43 | 9 | 9 | 1 | 7 | — | 2 |

Both NMOP and NMHP appear to be absorbed from the bladder and to be distributed systemically, since tumors of the nasal mucosa appeared in a majority of the rats dosed intravesically; there were some mesenchymal tumors of the kidney and in the case of NMHP some tumors of the esophagus and two follicular cell tumors of the thyroid. On the other hand, there were no systemically induced tumors in the rats treated with NMCOP, which was of relatively low potency, suggesting that conversion of NMCOP to NMOP in the kidney or bladder occurred to only a small extent, so that there was little systemic distribution of NMOP from this source.

These results show that, as suggested previously,[7] nitrosomethylalkylamines with even-number carbon chains are oxidized by successive $\beta$-oxidations (probably in the liver) to NMCOP which is excreted by the kidneys. It is partially converted in the bladder or kidney, by $\beta$-oxidation and decarboxylation, to NMOP, which is a proximate carcinogen for the mucosa of the urinary tract. This process probably takes place in hamsters as well as rats, since the even-carbon chain alkylmethylnitrosamines also induce bladder tumors in hamsters when given by mouth,[12, 13] including nitrosomethyl-*n*-octylamine.[14]

The mechanism by which NMOP induces tumors in the urinary mucosa (or in any other organ) is not known. However, when given orally to rats, NMOP is a methylating agent for DNA in the liver and kidney, comparable with nitrosodimethylamine in nature and extent (Lijinsky, unpublished information). The alkylation by NMOP in the bladder mucosa of rats has not yet been examined, nor has the mechanism of formation of the alkylating agent, but the latter is being investigated using deuterium-labeled NMOP.

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