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Induction of Urinary Bladder Tumors in Rats by Administration of Nitrosomethyldodecylamine[1]

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SUMMARY

Nitrosomethyl-*n*-dodecylamine, a product of the reaction of dimethyl-*n*-dodecylamine with nitrous acid, was given to Sprague-Dawley rats by gavage in olive oil solution. Twice-weekly treatments with 12 mg of the nitrosamine for 50 weeks gave rise to 100% incidence of transitional cell carcinomas of the urinary bladder. In contrast with the tests of other *N*-nitroso compounds, this compound seemed to be more effective in males than in females; females died later with the tumor although the dose per unit body weight was higher in females than in males.

INTRODUCTION

In their extensive study of carcinogenesis in rats by *N*-nitroso compounds, Druckrey *et al.* (1) included a variety of *N*-methyl-*N*-alkylnitrosamines of varying chain length extending to C-7 (*n*-heptyl-). In almost every case the most common site of tumor induction was the esophagus, leading to the generalization that the esophagus was the principal target organ of this group of carcinogens. Because of our interest in the formation of nitrosamines from tertiary amines and nitrous acid *in vivo*, we examined the reaction of a tertiary amine, dimethyldodecylamine, that has been used as an antisuckering agent on tobacco plants and might, therefore, become a component of tobacco and tobacco smoke. This amine reacts quite readily with nitrous acid in mildly acid solution to give 2 possible nitroso derivatives, dimethylnitrosamine and nitrosomethyldodecylamine (4). The former is a well-known and extensively studied carcinogen in rats, whereas the latter does not appear to have been tested. Since we could not buy sufficient *N*-methyldodecylamine for our study, we prepared nitrosomethyldodecylamine by nitrosation of commercial dimethyldodecylamine and tested it by P.O. administration to rats by gavage, since its solubility in water was too low to allow feeding in drinking water.

MATERIALS AND METHODS

Preparation of *N*-Nitroso-*N*-methyl-*n*-dodecylamine. Technical dimethyldodecylamine (Eastman Kodak Co., Rochester, N. Y.) was distilled and the major portion was collected as a center cut boiling at 131-133° at 15 mm. Twenty-five g of this were dissolved in 50 ml glacial acetic acid with cooling, followed by addition of 150 ml water. Then 50 g of sodium nitrite were added and the solution was heated overnight on a steam bath under reflux. A light-brown oil separated, which, after cooling, was separated off, diluted with 50 ml ether, and shaken twice with approximately 50 ml of 5 N HCl. (From the combined aqueous solutions approximately 1 g of dimethylnitrosamine could be recovered by extraction with methylene chloride.) The ether solution was dried with anhydrous sodium sulfate, and the ether was removed at room temperature in a stream of nitrogen. The residual oil was distilled under reduced pressure. The bulk of the material distilled at 138-140° at 1 mm as a light-yellow oil, which crystallized in the refrigerator but melted at room temperature. The yield was 12 g. The product, as seen by gas-liquid chromatography, contained approximately 10% of an impurity, which was not removed by subsequent distillation and further acid extraction. Mass spectral examination of the material revealed a small molecular ion at *m/e* 228 corresponding to nitrosomethyldodecylamine, with a much more intense (more than 100 times) fragment ion at M - 17 (corresponding to loss of OH). The same pattern was shown by a small sample of nitrosomethyldodecylamine prepared from the secondary amine. In addition there was a very small ion at *m/e* 241, a much larger one at *m/e* 226, and one at *m/e* 210. These were absent from the mass spectrum of pure nitrosomethyldodecylamine but were present in the mass spectrum of the initial dimethyldodecylamine that was nitrosated. Accurate mass measurement of the *m/e* 226 ion gave a composition $C_{14}H_{28}NO$, which we interpret as a fragment of the *N*-oxide of an aliphatic cyclic or unsaturated tertiary amine; the M - 16 ion at *m/e* 210 is consistent with an amine oxide structure.

Our interpretation of these chemical findings is that the starting material for the preparation contains an aliphatic amine *N*-oxide which, fortuitously, distills both with dimethyldodecylamine and with nitrosomethyldodecylamine and is too weakly basic to be removed by acid extraction.

The UV absorptivity of this preparation was correspondingly lower (80 *versus* 90 at 346 nm) than that of a small sample of nitrosomethyldodecylamine (b.p. 136-138° at 1 mm) prepared from the secondary amine. Since other nitroso compounds were absent, as shown by mass spectral examination of the preparation, we decided to use the

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material in our test, bearing in mind that ~10% inactive material was present.

Animal Treatments. A solution of nitrosomethyldodecylamine containing 60 mg/ml of olive oil was administered by gastric intubation at the rate of 0.2 ml twice weekly to groups of 15 male and 15 female Sprague-Dawley rats. The experimental rats were born in a closed colony at this laboratory and maintained under specific-pathogen-free conditions. The animals were housed 3 to a cage, segregated by sex, and fed Purina Laboratory chow and tap water *ad libitum*. Treatment was started when the animals were 12 weeks old and continued for 50 weeks. All animals were allowed to die naturally or were killed when moribund. Postmortem examinations were performed on all animals immediately after death, and all lesions and several organs were examined microscopically.

RESULTS

Survival times and tumor incidence in treated rats are shown in Table 1. All animals but 1 (which died at 48 weeks) were alive at the end of the treatment period, and all animals but 1 died with tumors during an 80-week period after initiation of treatment. The remaining animal, which was killed 96 weeks after the experiment started, also had a tumor typical of the group. Tumors that were observed in the animals, and which were the cause of death, were transitional cell carcinomas that arose from the epithelium of the urinary bladder.

In contrast with most of our tests of *N*-nitroso compounds, in which female rats given an equal dose with males (and, therefore, a higher dose per unit body weight) died with tumors earlier or at the same time as the males, in this experiment the females survived longer than the males.

Macroscopically, affected bladders were 3 to 5 cm in diameter and were filled with soft solid tumors which completely obliterated the lumen. Centers of these tumors were necrotic and hemorrhagic. The hindrance of urine passage resulted in hydroureter and hydronephrosis with bilateral loss of functional renal parenchyma. Metastatic tumor nodules were visible in the lungs of 4 animals as 1- to 3-mm nodules.

Microscopically, the transitional cell carcinomas consisted of solid nests and cords of epithelial cells as well as papillary stromal projections covered by thick layers of transitional cells (Fig. 1). Mitotic figures and bizarre hyperchromatic nuclei were commonly seen in invasive areas of the tumors, and in some areas differentiation into adenocarcinomas with acinar formation and into squamous cell carcinomas with keratin formation occurred. Tumors appeared to arise from multiple foci in the urinary mucosa. Metastatic foci in lungs consisted of solid nests of epithelial cells, which at their periphery grew along the alveolar septa (Fig. 2).

In 7 of the 30 tumor-bearing animals, alveologenic adenomas were also present in the lungs (Fig. 3), and in 1 animal there were papillomas in the esophagus. Endocrine tumors were present in small numbers in the older survivors, but these were no more numerous than in control rats in this laboratory.

DISCUSSION

It is unusual for a carcinogenic nitroso compound to induce such a uniform tumor response. Comparison can be made, for example, with another bladder-tumor-inducing nitrosamine, di-*n*-butylnitrosamine, which induces a high incidence of bladder tumors in rats (2), especially when administered by s.c. injection, but also induces an equally high incidence of tumors in the liver. This has led to extensive studies of the mechanism of bladder tumor induction by di-*n*-butylnitrosamine resulting in the hypothesis that di-*n*-butylnitrosamine is metabolized in the liver to the 4-hydroxy compound, part of which is supposedly oxidized to other products, including a carboxylic acid, that are excreted via the kidney and collect in urine in the bladder where they exert their carcinogenic action (3). No liver lesions were observed in rats treated with nitrosomethyldodecylamine, while the incidence of bladder tumors was 100%. We feel that the mechanism of action of nitrosomethyldodecylamine is worth investigation, since the compound is a good model for bladder tumor induction in rats.

Although it is desirable to repeat our test with a pure sample of nitrosomethyldodecylamine, since the results were unexpectedly interesting, we feel that the impurity can have made little or no contribution to the outcome. The same compound was present in the tertiary amine from which the nitrosomethyldodecylamine was prepared, and a test of this sample of tertiary amine in which 150 mg have

Table 1

Survival time and tumor incidence in Sprague-Dawley rats given nitrosomethyldodecylamine by gastric intubation

Dosage of carcinogen (total mg/animal)	No. of animals	Survivors at below weeks after initiation of treatment					No. of animals with tumors[a]			
		50	60	70	80	90	Transitional cell carcinoma of urinary bladder	Metastasis to lung	Esophageal papillomas	Adenomas of lung
1200	15 M	14	11	2	0		15	2	0	1
1200	15 F	15	13	9	2	1[b]	15	2	1	6

[a] Several animals had more than 1 type of tumor.
[b] Animal was killed 96 weeks after start of the experiment.

been given to a group of 30 rats each week for more than 1 year has not led to the death of any animal with tumors.

In view of the suggestion of Druckrey *et al.* (1) that nitrosomethyl-*n*-alkylamines tend to favor the esophagus as a target organ for tumor induction, it is strange that this particular nitrosamine of this class induced only 1 esophageal tumor.

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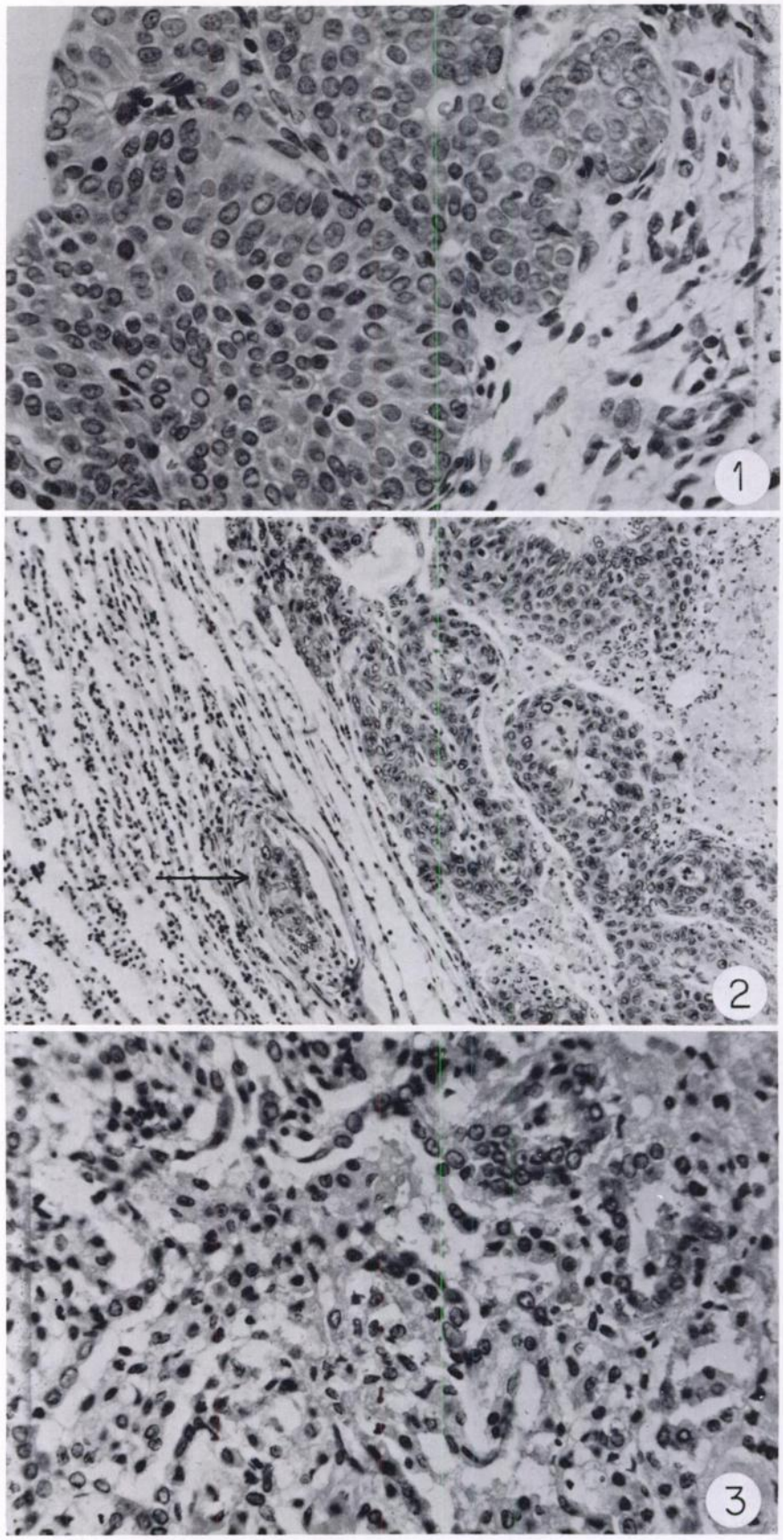

Fig. 1. Urinary bladder from rat that received nitrosomethyldodecylamine. Animal died at 70 weeks of experiment. Transitional cell carcinoma. H & E, × 500.

Fig. 2. Lung from rat that received nitrosomethyldodecylamine. Animal died at 72 weeks of experiment. Metastatic transitional cell carcinoma in lung. *Arrow*, tumor cells in pulmonary vessel. H & E, × 200.

Fig. 3. Lung from rat that received nitrosomethyldodecylamine. Animal died at 63 weeks of experiment. Alveologenic adenoma. Alveolar pattern is visible. H & E, × 500.