Tumor Induction in Rats by Feeding Heptamethyleneimine and Nitrite in Water

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SUMMARY

Groups of 15 male and 15 female Sprague-Dawley rats were given 20 ml of drinking water solution containing either 0.2% heptamethyleneimine hydrochloride or this salt together with 0.2% sodium nitrite, 5 days a week for 28 weeks. Another group of 27 male and 30 female rats was given 0.2% sodium nitrite solution for 104 weeks. Most of the animals given heptamethyleneimine hydrochloride or sodium nitrite alone survived 2 years or more after the beginning of the treatment, and no tumors attributable to the treatment were seen at death; tumors appearing were those of endocrine origin found commonly in untreated controls. In the group receiving the combined treatment, most females were dead at 50 weeks and most males were dead at 80 weeks, 27 of 30 having tumors not seen in either control group. A total of 16 had squamous carcinomas in the lung; 25 had tumors of the oropharynx, tongue, esophagus, and forestomach; and there were a few animals with tumors in the nasal cavity and trachea. The experiment showed that squamous tumors of the lung could be induced by ingestion of an amine and sodium nitrite.

INTRODUCTION

It is now reasonably certain that in some circumstances amines and nitrite can interact to form nitroso compounds, many of which are carcinogens. That this could be a source of carcinogens for man and other animals has been amply demonstrated by Sander (4, 5) and others (2) who have fed amines and nitrites to rodents and observed the development of the same tumors characteristically induced by the nitroso derivative of the amine. Tumors induced in this way have been described in the rat esophagus (5), rat liver (5), rat central nervous system (4), and as an increase in lung adenomas in mice (2).

Tumors corresponding in histological type to the most common malignant tumors of man have not been induced in this way, however. In our previous studies of tumorigenicity of a series of cyclic nitrosamines, we found that nitrosopentamethyleneimine induced a high incidence of squamous cell carcinomas in the lungs of rats given the compound in drinking water. It was logical to speculate that such tumors might also be induced by feeding the progenitors of the nitrosamine, heptamethyleneimine and sodium nitrite, to rats over a long period. Assuming that the 2 compounds reacted to a reasonable extent in the stomach, this system could serve as a model for the induction of lung cancer in man from amines present in tobacco smoke and nitrites in food.

Accordingly, we performed such an experiment feeding 3 groups of rats the amine and sodium nitrite, both separately and together, in drinking water. A preliminary report of the results has been made (3).

MATERIALS AND METHODS

Chemicals. Heptamethyleneimine hydrochloride was prepared by slowly adding the calculated volume of concentrated hydrochloric acid (10 N) to heptamethyleneimine (Aldrich Chemical Co., Milwaukee, Wis.) which was cooled in ice. The resulting solution was concentrated to a small volume in a rotary evaporator at 70°. When cooled the salt crystallized as a white solid which was filtered off, partially dried by vacuum, and allowed to dry thoroughly in a vacuum desiccator. The melting point was 177–180°, and aqueous solutions of the solid were neutral. Commercially available sodium nitrite was used (Fisher Scientific Co., Silver Spring, Md.).

Animal Treatments. Experimental animals were male and female Sprague-Dawley rats born in a closed colony in this laboratory and maintained under specific-pathogen-free conditions. All animals were 8 to 10 weeks old at the start of the experiment. They were segregated by sex, housed 3 to a cage, and fed Purina laboratory chow ad libitum. Two days per week the animals received regular tap water. Five days per week, each cage of animals was offered 60 ml of one of the following test solutions in neutral (pH 6.8 to 7.0) distilled water: (a) heptamethyleneimine hydrochloride, 2.0 g/liter, plus sodium nitrite, 2.0 g/liter; (b) heptamethyleneimine hydrochloride, 2.0 g/liter; (c) sodium nitrite, 2.0 g/liter. Each day the residual drinking solution was measured so that the consumption of each test solution could be calculated. Treatment was continued on the 1st 2 groups for 28 weeks and on the sodium nitrite group for 104 weeks.

All animals were allowed to survive until natural death or were killed when moribund, except that 9 males and 9 females of the heptamethyleneimine control group were killed at the 104th week of the experiment. Necropsy
examination was performed immediately after death, and tissues from all lesions and major organs were prepared for histological examination by routine formalin fixation, paraffin embedding, and hematoxylin and eosin staining.

RESULTS

Each day the rats consumed all of the solution that contained sodium nitrite alone, while they drank only about one-half to two-thirds of both heptamethyleneimine hydrochloride solutions. Males drank more of the latter solution than did the smaller females.

The animals receiving the combination of heptamethyleneimine hydrochloride and sodium nitrite were all dead at 100 weeks after the start of the experiment (Table 1). Survival time was shorter and tumor incidence was higher in females than in males of this group. Squamous cell carcinomas and squamous papillomas appeared in high incidence in the lungs, upper respiratory tracts, and upper digestive tracts of these animals (Table 1). In the lungs, squamous cell carcinomas (Fig. 1) appeared to arise from metaplastic plaques in small bronchioles and peripheral alveoli. They consisted of nests of squamous cells with clearly visible intercellular bridges and keratohyaline granules, and in many areas large masses of keratin were produced. The growth pattern and cell types of most of these tumors appeared benign, but in some places anaplasia, bizarre mitotic formations, and infiltrative growth were seen. Metastases of squamous cell carcinomas to the kidneys (Fig. 2) and mediastinal lymph glands in 2 animals gave evidence that they were malignant.

Tumors of the nasal cavity were locally invasive squamous cell carcinomas which arose from metaplastic foci in the epithelium of the turbinates. Mostly squamous papillomas, but also a few locally invasive squamous carcinomas, were seen in the tongue, oropharynx, esophagus, and nonglandular stomach. Of these locations the esophagus contained, by far, the majority of such tumors. A few papillomas were present in the larynx and trachea, but metaplastic plaques of keratinizing squamous epithelium were much more commonly seen (Fig. 3).

Various benign endocrine tumors were present in the 2 control groups, but no tumors of squamous origin or comparable in location to the 1st group were present. No tumors in these groups were attributed to the treatment.

DISCUSSION

As others have shown, sodium nitrite, which is a commonly used food additive and preservative, showed no evidence of carcinogenicity in this test, although each animal received near the maximum tolerated dose (higher doses cause fatal methemoglobinemia) and ingested more than 20 g of the compound during the 2-year treatment. Neither was there a noticeable reduction in life-span, since at the age of 30 months two-thirds of the animals were alive. This is an excellent survival rate for laboratory rats. Heptamethyleneimine hydrochloride was not detectably
carcinogenic in this test either, each animal having been
given more than 5 g of the compound.

The combination of heptamethyleneimine hydrochloride
and sodium nitrite, however, was potently carcinogenic at
the dose administered. This contrasts with an earlier study
(1) in which the same combination was given to rats at
considerably lower doses and in which no tumors were
induced. This illustrates that the factor of dose is critical in
this type of experiment, in which sufficient carcinogen has
to be formed to give rise to tumors within the short lifetime
of a rat. In man, on the other hand, much smaller doses of
nitrosamine could be formed, but their effects would
accumulate over a much longer life-span. The dependency
of the yield of nitrosamine on the nature of the amine
reacting with nitrous acid is shown by the failure of similar
doses of piperidine hydrochloride (a lower homolog of
heptamethyleneimine) together with sodium nitrite to give
rise to tumors when given to rats in drinking water (W.
Lijinsky, and H. W. Taylor, unpublished observations).

The fact that a combination of the innocuous amine and
sodium nitrite ingested by rats in drinking water can give
rise to squamous lung tumors, one of the most common
tumors in man, through formation of a nitrosamine in the
stomach would seemingly have implications as a model for
human lung cancer. Nitrites are commonly ingested by man
in food, although in smaller doses than we gave to rats;
tobacco smoke contains secondary and tertiary amines,
most of which are unidentified. Some of these could be
swallowed and interact with nitrous acid in the stomach to
form nitroso compounds that happen to be lung carcinogens
in man. We are trying to gain more insight into this matter
by analysis of tobacco smoke for nitrosatable amines.

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Fig. 1. Lung from rat receiving heptamethyleneimine and nitrite. Squamous cell carcinoma. H & E, × 200.

Fig. 2. Kidney from rat receiving heptamethyleneimine and nitrite. Metastatic squamous cell carcinoma in cortex. Arrow, triploid mitotic figure H & E, × 200.

Fig. 3. Trachea from rat receiving heptamethyleneimine and nitrite. Squamous metaplasia of mucociliary epithelium. H & E, × 200.
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