Comparative Carcinogenicity and Metabolism of 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butane and N'-Nitrosonornicotine in Syrian Golden Hamsters

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ABSTRACT

The tobacco-specific nitrosamines 4-(methylnitrosamino)-1-(3-pyridyl)-1-butane (NNK) and N'-nitrosonornicotine (NNN) were tested for carcinogenic activity in Syrian golden hamsters. In Assay A, 30 hamsters were each given 19 s.c. injections of 0.048 mmol of NNK or NNN. In Assay B, 20 hamsters each received 75 s.c. injections of 0.012 mmol of NNK or NNN. Among the NNK-treated hamsters in Assay A, three developed carcinomas of the nasal cavity, and 19 had adenomas and/or adenocarcinomas of the lung. In the NNN group, one hamster developed a lung adenoma, and five had tracheal papillomas. In Assay B, 11 of the NNK-treated hamsters developed carcinomas of the nasal cavity, 16 had lung adenomas and/or adenocarcinomas, and seven had tracheal papillomas; in the NNN group, we recorded only one hamster with a lung adenoma and one with a tracheal papilloma. These findings in the Syrian golden hamster confirm that NNK is a more powerful carcinogen than NNN, as was shown previously in assays with rats and mice.

In metabolism studies, 96 to 98% of the radioactivity of the injected [1-14C]NNK was recovered in the urine, 4% was recovered in the feces, and less than 0.5% was recovered as exhaled 14CO2. The corresponding distribution for [2'-14C]NNN was 62 to 78% in urine, 10% in feces, and less than 0.5% in respiratory 14CO2. The levels of binding of [1-14C]NNK and [2'-14C]NNN to the trichloroacetic acid-insoluble fractions were highest in liver, lung, kidney, and adrenals. The urinary metabolites of NNK and NNN resulted from α-hydroxylation, from N-oxidation of NNN to N'-nitrosonornicotine-1-N-oxide, and from reduction of NNK to 4-(methylnitrosamino)-1-(3-pyridyl)butan-1-ol.

INTRODUCTION

Tobacco and tobacco smoke contain relatively high levels of nitrosamines derived from the tobacco alkaloids (7, 11). These tobacco-specific nitrosamines include NNN3 and NNK (see Chart 1), which are formed principally from nicotine (6), as well as N'-nitrosoanatabine, which originates from the minor alkaloid, anatabine. While the role of these nitrosamines in tobacco-related cancers is not known, their significant levels in tobacco and tobacco smoke as well as their carcinogenic activities suggest that they are important (13). Previous studies of the comparative carcinogenicity of NNK and NNN have demonstrated higher activity for NNK than for NNN. In strain A mice, both compounds induced lung adenomas, but NNK induced more tumors per animal than did NNN (6). In F344 rats, s.c. injection of NNK resulted in tumors of the nasal cavity, lung, and liver, whereas NNN induced nasal cavity tumors only (8). In the present study, we have compared the carcinogenicity and metabolism of NNK and NNN in Syrian golden hamsters and have found evidence that NNK is a potent carcinogen in the hamster, with much greater activity than NNN. Both compounds are extensively transformed in vivo, leading to mixtures of urinary metabolites which result partially from α-hydroxylation.

MATERIALS AND METHODS

Chemicals

NNN and NNK were synthesized and were more than 99% pure, according to analysis by gas chromatography, HPLC, thin-layer chromatography, and mass spectrometry (5, 12). [2'-14C]NNK (18.4 mCi/mmol) was obtained from New England Nuclear (Boston, Mass.) and purified by thin-layer chromatography on silica gel (E. Merck, Darmstadt, Germany) by elution with CHCl3:methanol (15:1). [1-14C]NNK (4.2 mCi/mmol) was synthesized and was refined to >99% purity by thin-layer chromatography on silica gel. Triocotain was obtained from Eastman Kodak Co. (Rochester, N. Y.) and was redistilled before use. NCS solubilizer (1) was obtained from Amersham/Searle Corp. (Arlington Heights, Ill.).

Bioassays for Carcinogenicity

Outbred Syrian golden hamsters (8 to 10 weeks old) from Sprague-Dawley Co. (Madison, Wis.) were housed in groups of 5 in solid-bottom polycarbonate cages with hardwood bedding and were kept at 20 ± 2°(S.D.) and 50 ± 10% relative humidity with a 12-hr light-dark cycle. The hamsters were given Purina laboratory chow and tap water ad libitum.

Two experiments were performed. In Experiment A, 15 male and 15 female hamsters each received NNK and NNN (Groups 1 to 4). Injections (s.c.) of NNK [10.0 mg (0.048 mmol) in 0.3 ml triocotain] or NNN [8.6 mg (0.048 mmol) in 0.3 ml triocotain] were administered 3 times weekly, beginning at the age of 8 to 10 weeks. The total dose of NNK or NNN was 0.91 mmol/hamster. In Experiment B, 4 groups, consisting of 10 hamsters each, received 75 s.c. injections of NNK [2.5 mg (0.012 mmol) in 0.3 ml triocotain] or NNN [2.15 mg (0.012 mmol) in 0.3 ml triocotain] at a rate of 3 injections per week.

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3 The abbreviations used are: NNN, N'-nitrosonornicotine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butane; HPLC, high-pressure liquid chromatography.

4 A. Castonguay, S. S. Hecht, and D. Hoffmann, manuscript in preparation.
Thus, the total dose applied was also 0.91 mmol/hamster. The average weights of the male and female hamsters were 106 and 113 g, respectively, at the onset of the experiment. They rose to 140 and 158 g, respectively, in the course of 25 weeks of nitrosamine applications. The hamsters were weighed weekly, and moribund animals were sacrificed. Experiment A was terminated after 16 months and Experiment B was terminated after 17 months. Vehicle controls were terminated after 16 months. Upon necropsy, gross lesions and representative samples of all major organs were fixed in 10% buffered formalin and processed for microscopic examination. The nasal cavity was sectioned frontally after decalcification; portions of the anterior, middle, and posterior parts of the cavity were examined microscopically. Trachea and esophagus were embedded flat and cut longitudinally in step sections.

**Metabolism**

**Routes of Excretion.** To determine routes of excretion and distribution in tissues, groups of 2 male Syrian golden hamsters (160 to 180 g) were injected s.c. with either 10 mg (8.7 µCi) of \([1-\text{14C}]\text{NNK}\) or 10 mg (8.60 µCi) of \([2'-\text{14C}]\text{NNN}\) in trioctanoin. Label distribution was subsequently measured in urine at 6-hr intervals, in feces at 24-hr intervals, and in exhaled air and blood 48 hr after injection. Exhaled air was collected in traps containing 1 N NaOH, and \(^{14}\text{C}\) activity was determined by direct counting of 1-ml aliquots. Blood was collected by orbital bleeding. Tissues and organs were removed and weighed, solubilized in NCS, and decolorized with benzoyl peroxide. The radioactivity was determined by liquid scintillation counting in Scintolute (Isolab, Akron, Ohio). Feces were ground in ethanol and air dried, and \(^{14}\text{C}\) activity was determined by combustion analysis (New England Nuclear).

**Urinary Metabolites.** For determination of urinary metabolites, hamsters were given s.c. injections of \([1-\text{14C}]\text{NNK}\) (5.14 µCi, 2.5 mg) or \([2'-\text{14C}]\text{NNN}\) (5.32 µCi, 2.15 mg) in trioctanoin. The 48-hr urine, collected at −76, was lyophilized. The residue was sonically dispersed with methanol, and the supernatants were filtered and transferred to a 5-ml volumetric flask. An aliquot was counted to determine recovery, and a 0.03-ml aliquot was analyzed by HPLC using two 3.9-mm x 30-cm C\(_{18}\)-Bondapak columns in series and the following gradient: Solvent A for 10 min, and then linear to 60% Solvent B in 60 min at 1 ml/min. Solvent A was 30 ml of 1 M acetic acid, 12 ml of 1 M NaOH, and 18 ml of 1 M NaCl in a total volume of 0.5 liter of H\(_2\)O at pH 4.5 (NNN metabolites) or pH 6.0 (NNK metabolites). Solvent B was CH\(_3\)OH:H\(_2\)O (1:1). Standard metabolites (4, 9) were added as UV markers prior to HPLC analysis. Products were identified by coelution of UV markers and radioactivity.

**Binding to Trichloroacetic Acid-Insoluble Fraction of Various Tissues.** Groups of 2 male Syrian golden hamsters were injected in the dorsal or cephalic vein with solutions of \([2'-\text{14C}]\text{NNN}\) (96 µg, 10 µCi) or \([1-\text{14C}]\text{NNK}\) (490 µg, 10 µCi) in dimethyl sulfoxide (0.3 ml). The animals were sacrificed 1 hr later, and approximately 32 to 500 mg of tissue were minced, suspended in 10 ml of H\(_2\)O, and homogenized with a teflon pestle. Ten ml of 10% trichloroacetic acid were added, and the mixture was sonically dispersed for 15 min. After centrifugation at 5000 × g for 15 min, the supernatant was decanted. This procedure was repeated twice with 20 ml of H\(_2\)O. The pellet was washed twice with a mixture of ethanol:ether (3:1), and the final pellet was dried over phosphorous pentoxide in a vacuum. Samples of 10 mg each were dissolved in a mixture of H\(_2\)O (0.1 ml) and NCS (1.0 ml). Each tissue was analyzed in duplicate.

**RESULTS**

**Carcinogenesis.** In Experiment A, treatment resulted in a high mortality rate for the NNK groups. After 10 weeks, 50% of the NNK-treated animals had died, and, by 14 weeks, only 4 of 15 males and 4 of 15 females were alive. In contrast, NNN treatment did not have apparent toxicity. In Experiment B, the survival rates in both treatment groups were 100% after 4 months, 80% (NNK) and 90% (NNN) after 7 months, and 75% (NNK) and 80% (NNN) after 10 months. After 13 months, 30% of the NNK-treated and 60% of the NNN-treated hamsters survived.

The bioassay results are summarized in Table 1. In Experiment A, NNK induced most frequently adenomas but also some adenocarcinomas of the lung and tumors of the nasal cavity. All 14 animals with lung adenomas and one with lung adenocarcinoma died within the first 11 weeks of the experiment. The remaining adenocarcinomas of the lung and carcinomas of the nasal cavity were induced in 4 of the 8 animals who survived the initial toxic effects. In the NNN-treated groups, tracheal tumors were observed in 5 animals.

In Experiment B, NNK induced tumors of the nasal cavity, trachea, or lungs in 90% of the treated animals; 70% of the males and 50% of the females had tumors in more than one of these organs. In contrast, NNN induced only one tracheal tumor and one adenocarcinoma of the lung.

The nasal cavity tumors (Fig. 1) were aggressive, destroying the bone and sometimes infiltrating the brain. Pure olfactory tumors were rarely found, since squamous cell carcinomas or adenocarcinomas originating in the respiratory-type epithelium interpenetrate them. However, in many cases, true rosettes, solid lobular patterns, and nuclear structures, suggesting esthesioneuroepithelioma, were clearly distinguished (Fig. 1, b to c), especially in areas adjacent to the roof of the nasal cavity. The tracheal tumors (Fig. 2a) were richly ramified papillomas, usually multiple, seeded along the length of the laryngotracheal tube. In some instances, they completely obliterated the tracheal lumen. The lung tumors were generated from the alveolar or possibly the bronchoalveolar epithelium. The difficulty in establishing the histogenesis was due, in part, to the fact that the hamster respiratory bronchiole is very short, covered primarily by Clara cells and, thus, histologically resemble the alveolar region. It appeared that the lung lesions usually started as nonspecific inflammatory changes (sometimes including squamous metaplasia), followed by bronchiolar and alveolar epithelial hyperplasia, leading to an adenomatoid aspect. Up to this stage, the bronchial or alveolar lumen could still be recognized. The image changed when the proliferative process went beyond this stage, producing a solid nodule. The structure of the "nodule" was most commonly adenomatous (solid adenoma). Early epidermoid changes could, however, turn the initial adenoma-like nodule into a combined adenoepidermoid (Fig. 2, b to c) or purely epidermoid tumor. Further development depended largely on the survival time of the animal; most of
Table 1

Number of Syrian golden hamsters with tumors after treatment with NNN, NNK, or vehicle control

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Sex</th>
<th>Dose schedule</th>
<th>Total respiratory</th>
<th>Lung</th>
<th>Nasal cavity</th>
<th>Trachea</th>
<th>Other</th>
<th>Multiple sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>NNN</td>
<td>15 (15)</td>
<td>M</td>
<td>A</td>
<td>10</td>
<td>8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td>1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>NNN</td>
<td>15 (15)</td>
<td>F</td>
<td>A</td>
<td>12</td>
<td>11&lt;sup&gt;g&lt;/sup&gt;</td>
<td>1&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0</td>
<td>2&lt;sup&gt;h&lt;/sup&gt;</td>
<td>1&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>NNN</td>
<td>15 (14)</td>
<td>M</td>
<td>A</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NNK</td>
<td>10 (10)</td>
<td>M</td>
<td>B</td>
<td>10</td>
<td>10&lt;sup&gt;1&lt;/sup&gt;</td>
<td>6&lt;sup&gt;m&lt;/sup&gt;</td>
<td>3</td>
<td>2&lt;sup&gt;j&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td>NNK</td>
<td>10 (10)</td>
<td>F</td>
<td>B</td>
<td>8</td>
<td>6&lt;sup&gt;g&lt;/sup&gt;</td>
<td>5&lt;sup&gt;g&lt;/sup&gt;</td>
<td>4</td>
<td>0</td>
<td>5&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>NNK</td>
<td>10 (9)</td>
<td>M</td>
<td>B</td>
<td>2</td>
<td>2</td>
<td>1&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>NNK</td>
<td>10 (9)</td>
<td>F</td>
<td>B</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Triocanoin control</td>
<td>15 (14)</td>
<td>M</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2&lt;sup&gt;k&lt;/sup&gt;</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

-<sup>a</sup> Number in parentheses, number of animals autopsied.
-<sup>b</sup> A, single dose of 0.048 mmol 3 times weekly for 6.3 weeks, total dose = 0.91 mmol; B, single dose of 0.012 mmol 3 times weekly for 25 weeks, total dose = 0.91 mmol.
-<sup>c</sup> One with adenocarcinoma, one with atypical adenoma, 6 with adenoma.
-<sup>d</sup> Pleomorphic carcinoma.
-<sup>e</sup> Spindle-celled sarcoma in the right inguinal area.
-<sup>f</sup> One with nasal cavity tumor and spindle-celled sarcoma in the right inguinal area.
-<sup>g</sup> Three with adenocarcinoma, 8 with adenoma or atypical adenoma.
-<sup>h</sup> One with uterine horn adenocarcinoma and one with metastases in lung presumed to originate in nasal cavity.
-<sup>i</sup> Lung adenocarcinoma and nasal cavity adenocarcinoma.
-<sup>j</sup> Lung adenoma.
-<sup>k</sup> Undifferentiated carcinoma, right posterior leg.
-<sup>l</sup> Six with adenocarcinoma, 4 with adenoma.
-<sup>m</sup> Five with pleomorphic carcinoma, one with papilloma.
-<sup>n</sup> One with a large carcinoma of unknown origin in abdominal cavity with metastases to lung; one with adenocarcinoma, possibly in pancreatic area.
-<sup>o</sup> One with lung adenoma and nasal cavity tumor; 3 with lung adenocarcinoma and nasal cavity tumors; 1 with lung adenocarcinoma and tracheal papilloma; 1 with lung adenoma, nasal cavity tumor, and tracheal papilloma; 1 with lung adenocarcinoma, nasal cavity tumor, and tracheal papilloma; 1 with lung adenoma and abdominal carcinoma; 1 with lung adenocarcinoma and possibly pancreatic adenocarcinoma.
-<sup>p</sup> Four with adenocarcinoma, 2 with adenoma.
-<sup>q</sup> Two with lung adenocarcinoma and nasal cavity tumor; one with lung adenoma and nasal cavity tumor; 2 with lung adenocarcinoma, nasal cavity tumor, and tracheal papilloma.
-<sup>r</sup> Adenocarcinoma.
-<sup>s</sup> One with soft-tissue sarcoma of the right inguinal area; one with cortical adenoma of the adrenal gland.

The levels of radioactivity incorporated in the trichloroacetic acid-insoluble fraction of various tissues are summarized in Table 4. The highest levels of binding for both [1-<sup>14</sup>C]NNN and [2'-<sup>14</sup>C]NNN were detected in liver, lung, kidney, and adrenals.

**DISCUSSION**

The results of both tumorigenicity assays clearly demonstrate that NNK is a more powerful carcinogen than NNN in the Syrian golden hamster. In Experiment A, the dose of NNK proved toxic, but despite the fact that only 27% of the original 30 animals survived the first 10 weeks of the experiment, 75% of the males and 80% of the females had respiratory tract tumors. The higher incidence of nasal cavity and tracheal tumors in the NNK groups in Experiment B was most likely related to increased survival. In Experiment B, the 90% incidence of respiratory tract tumors in the NNK groups contrasts sharply with the 10% incidence observed in the NNN groups. In a previous bioassay of NNN in Syrian golden hamsters, a dose of 2.1 mmol gave a 63% incidence of tracheal tumors (10). In the present experiment, the dose was 0.91 mmol. The more powerful tumorigenicity of NNK compared to that of NNN has also been observed in rats and in strain A mice (6, 8).

The target organs of NNN in the F344 rat were liver, nasal cavity, and lung. The induction of tracheal tumors and the absence of liver tumors in the present experiment with the Syrian golden hamster are typical of bioassays with several nitrosamines (16). The nasal cavity tumors in the NNK-treated...
hamsters were more commonly of mixed structure (olfactory and respiratory), whereas in NNK-treated rats the olfactory element was predominant. The lung tumors in the hamsters consisted of peripheral bronchoalveolar carcinomas with combined adenoepidermoid components, similar to the tumors induced by other respiratory carcinogens, such as polonium-210 and benzo(a)pyrene (14, 15). Some of the lung adenomas were generated relatively early (up to 11 weeks), and, as mentioned, the incipient lesions had a rather nonspecific chronic inflammatory character reminiscent of viral infections or dust aspiration.

α-Hydroxylation is presumed to be the mechanism of acti-

Table 2

Levels of 14C in tissues of male Syrian golden hamsters 48 hr after administration of [1-14C]NNK or [2'-14C]NNN

Groups of 2 male Syrian golden hamsters were given s.c. injections of [1-14C]NNK (10 mg, 8.79 μCi) or [2'-14C]NNN (10 mg, 8.6 μCi) in trioctanoin. The dose was the same as in the bioassay (Experiment A) for carcinogenicity. Tissues were isolated, and radioactivity was determined as described in "Materials and Methods."

<table>
<thead>
<tr>
<th>Tissue</th>
<th>NNK (dpm/mg tissue)</th>
<th>NNN (dpm/mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>1.41 ± 0.54</td>
<td>0.54 ± 0.25</td>
</tr>
<tr>
<td>Liver</td>
<td>16.80 ± 0.71</td>
<td>16.71 ± 0.26</td>
</tr>
<tr>
<td>Adrenal glands</td>
<td>7.15 ± 0.37</td>
<td>3.73 ± 0.18</td>
</tr>
<tr>
<td>Lacrimal glands</td>
<td>3.28 ± 0.15</td>
<td>1.53 ± 0.08</td>
</tr>
<tr>
<td>Lungs</td>
<td>2.14 ± 0.11</td>
<td>1.76 ± 0.08</td>
</tr>
<tr>
<td>Kidneys</td>
<td>4.03 ± 0.21</td>
<td>2.93 ± 0.14</td>
</tr>
<tr>
<td>Nasal cavity</td>
<td>2.23 ± 0.11</td>
<td>2.62 ± 0.14</td>
</tr>
<tr>
<td>Eyes</td>
<td>1.36 ± 0.08</td>
<td>2.26 ± 0.12</td>
</tr>
<tr>
<td>Heart</td>
<td>1.72 ± 0.09</td>
<td>0.64 ± 0.04</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.99 ± 0.14</td>
<td>1.08 ± 0.05</td>
</tr>
<tr>
<td>Trachea</td>
<td>1.77 ± 0.11</td>
<td>2.21 ± 0.12</td>
</tr>
<tr>
<td>Colon</td>
<td>2.49 ± 0.12</td>
<td>3.73 ± 0.18</td>
</tr>
</tbody>
</table>

* Mean of 2 values. S.D. was less than 25%.

Chart 2. High-pressure liquid chromatogram of urinary metabolites of NNN in the Syrian golden hamster. Unlabeled metabolites 3, 15, and 16 (see Chart 1) were added as UV markers prior to chromatography. Radioactivity (→) was detected after absorbance at 254 nm (→). See "Materials and Methods" for chromatographic conditions. (Radioactivity counting was time delayed compared to the UV absorbance).

Chart 1. Metabolic transformation of NNN and NNK. Structures in brackets are hypothetical intermediates.
NNK and NNN (4, 9). α-Hydroxylation of NNK gives the unstable α-hydroxynitrosamines 4 and 5 (Chart 1), which decompose to the electrophilic diazohydroxides 8 and 10 and possibly to the corresponding carbonium ions. Both of these pathways eventually produce keto acid 15. The origin of hydroxy acid 16 as a urinary metabolite of NNK is likely through α-hydroxylation of 2, a major in vivo reduction product of NNK, although reduction of 15 is also possible. In any case, keto acid 15 and hydroxy acid 16 are useful indicators of total α-hydroxylation of NNK and/or 2. Similar mechanisms hold for NNN, as summarized in Chart 1; 15 and 16 are indicators of total α-hydroxylation. In addition, NNK is metabolized to the N-oxide 3.

The data in Charts 2 and 3 and Table 3 indicate that, upon s.c. injection, α-hydroxylation is one major metabolic process for both NNN and NNK in the Syrian golden hamster. The levels of the known urinary metabolites resulting from α-hydroxylation of NNN exceed those from the more powerful carcinogen, NNK. Thus, these levels are not indicative of the carcinogenic potential of either NNN or NNK. The higher carcinogenicity of NNK compared to that of NNN may be due to the nature of the specific carcinogen-macromolecule adducts formed in target tissues. These adducts are likely to result from oxobutyl diazohydroxides, such as 10 and 11 (Chart 1) from both NNN and NNK, and from methyldiazonium hydroxide 8 from NNK. The possible role of methylation in determining the strong carcinogenicity of NNK requires further investigation, as does the potential involvement of the unknown NNK metabolites in its activation.

The levels of radiolabel bound to the trichloroacetic acid-insoluble material of selected tissues after i.v. administration of [1-14C]NNK or [2'-14C]NNN (Table 4) do not provide an explanation for the greater carcinogenicity of NNK. Since we used only [1-14C]NNK in the present experiments, we did not measure bound products resulting from formation of methyldiazonium hydroxide or formaldehyde. Thus, total binding of NNK to tissue macromolecules is undoubtedly higher than shown in Table 4. In contrast, radiolabel is retained in both α-hydroxylation steps for NNN.

Two recent autoradiographic studies on the distribution of [2'-14C]NNN in mice have shown that the metabolites of this labeled nitrosamine are bound to macromolecules of tracheobronchial and nasal mucosa, liver, sublingual and submaxillary glands, esophagus, and the melanin of the eye (3, 17). In this study with male hamsters, we found the highest β activity of bound metabolites of [2'-14C]NNN in liver, lung, kidney, and adrenal glands. These findings, however, should not be compared with the above cited data from studies with mice since different methods were used and since the organotropic activities in mice and hamsters are quite different (2, 6, 10).

The present studies of comparative carcinogenicity and metabolism of NNK and NNN are of mechanistic interest because of the higher carcinogenicity of NNK, which has now been demonstrated in hamsters, mice, and rats (6, 8). However, of greater importance is the relatively high exposure of smokers and tobacco chewers to NNK, a carcinogen more potent than the strongly carcinogenic N-nitroso-pyrrolidine (17). The level of NNK in the mainstream smoke of a typical U. S. 85-mm nonfilter cigarette is 0.11 µg; fine-cut chewing tobacco contains 2.4 µg/g (11). We do not know the minimum level of exposure to NNK necessary to produce tumors, although dose-response studies are currently in progress. Nevertheless, exposure to NNK in tobacco and tobacco smoke should certainly be minimized.
ACKNOWLEDGMENTS

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REFERENCES

Fig. 2. a, squamous papilloma of tracheal epithelium; x 20. b, lung tumor; adenomatous area; x 200. c, lung tumor; metaplastic squamous area; x 200.

NNK and NNN Carcinogenicity in Hamsters

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