SUMMARY

Pregnant mice were given s.c. injections of 4-nitroquinoline 1-oxide once only on Day 9, 13, or 17 or of N-methyl-N-nitrosourethan on Day 9, 11, 13, 15, or 17. Offspring of mice that received 4-nitroquinoline 1-oxide on Day 13 or 17 developed lung tumors in significantly high incidence, and offspring of those treated on Day 9 bore some malformations. However, tumors were not observed in the offspring when N-methyl-N-nitrosourethan was given on Day 11 or later, although lung tumors and malformations were induced when it was given on Day 9. When 4-nitroquinoline 1-oxide was given to lactating mice, their sucklings developed lung tumors and hepatomas.

INTRODUCTION

It has been shown that exposure of experimental animals to certain chemicals during pregnancy (1, 4, 6, 7, 11, 13, 14, 17–22, 25–27, 29–34) or during lactation (5, 15, 18, 21) induces tumors in their offspring. Nomura et al. (23) administered various doses of urethan to pregnant mice on various days of gestation, utilizing its fast action and complete placental penetration. Tumors were induced in the lung and liver of the offspring when even a very small amount of urethan was given during late organogenesis and fetal growth of each organ (18, 19, 21). This forms a striking contrast to the findings that malformations were observed in each organ when a large amount of urethan was given during early organogenesis (19). When urethan was given to lactating mice, very small amounts were detected in the milk and also in the organs of their sucklings (23), and tumors were induced (18, 21). This paper deals with the carcinogenic effects of 4NQO and MNUT on progeny of mice given these drugs during pregnancy or lactation. Mutagenic action of these chemicals has been well established (8, 9, 12, 24).

MATERIALS AND METHODS

Animals. Eight- to 10-week-old ICR/ICR female mice, weighing 26 to 30 g, were obtained from Japan Central Laboratory for Experimental Animals, Tokyo, Japan. They were placed in a cage with 1 breeder male, and vaginal plugs were checked daily to determine Day 1 of gestation. Details have been previously reported (21).

Carcinogens. A 0.5% suspension of 4NQO (m.p. 154°, provided by the late Dr. K. Mori, Department of Medical Biology, Showa University Medical School, Tokyo, Japan) was prepared by the previously reported method (20). A 0.05 or 0.01% solution of MNUT (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan) in distilled water was also prepared just before use.

Treatment during Pregnancy. Pregnant mice received a s.c. injection of 4NQO (25 μg/g body weight) on Day 9, 13, or 17 of gestation. MNUT (5 μg/g body weight) was given to pregnant mice on Day 9, 11, 13, 15, or 17 of gestation. MNUT (1 μg/g body weight) was also given on Day 9. Live offspring were separated from mothers 4 weeks after birth.

Treatment during Lactation. Lactating mothers received a s.c. injection of 4NQO (25 μg/g body weight) within 12 hr after delivery. Five mothers received 2 additional treatments with 4NQO at 5 day intervals. Sucklings were nursed by these lactating mice and were separated from them 4 weeks after birth.

These offspring were weighed at 2-week intervals and maintained on Mouse Diet CA-1 (CLEA Japan Inc., Tokyo, Japan) and water. 4NQO-treated and MNUT-treated groups were sacrificed at 32 or 38 weeks after birth, respectively, and gross pathological lesions were examined. Specimens were fixed in a 20% neutral formaldehyde solution and subjected to microscopic examinations.

RESULTS

Teratogenesis and Carcinogenesis. When 4NQO was given to pregnant mice once only on Day 13 or 17, lung tumors were
induced in significantly higher incidence than with the controls (Table 1). There was a significant difference in tumor incidence between the offspring of mice receiving 4NQO once only on Day 17 and those receiving it within 12 hr after delivery ($\chi^2$-test with Yates's correction, $p < 0.02$; $t$-test with approximation of Cochran-Cox, $p < 0.05$ in Tables 1 and 3). Histologically, most of the lung tumors were papillary adenomas. There was no significant tumor incidence when 4NQO was given on Day 9, although the average number of live births decreased and some malformations were observed (Table 1). In contrast to 4NQO, tumors were not observed in the offspring of mice receiving MNUT on Day 11 or later (Table 2). When a MNUT dose of 5 µg/g body weight was given to pregnant mice on Day 9, the average number of live births decreased and lung tumors, hepatomas, and some malformations were observed. However, these findings were not observed when a smaller amount of MNUT (1 µg/g) was given on Day 9 (Table 2). There was no difference in the average body weight between the offspring of mice receiving 4NQO or MNUT during pregnancy and the controls.

**Carcinogenesis via Mother's Milk.** When lactating mice received a single injection of 4NQO within 12 hr after delivery, the incidence of lung tumors in their sucklings was higher than for controls although not statistically significant (Table 3). When 2 additional treatments with 4NQO were given to lactating mothers, deaths of sucklings increased. Live offspring developed lung tumors in significantly high incidence and hepatomas were also observed (Table 3).

**Tumors in Mothers.** Mice receiving 4NQO or MNUT during pregnancy or lactation developed lung tumors in significantly high incidence (Table 4). Ovarian cystadenomas were also observed in these mice.

**DISCUSSION**

Although 4NQO is only slightly soluble in water, when injected s.c. it induced lung tumors in mice, indicating that 4NQO or its active intermediate enters into circulating blood and reaches the lung (10, 16, 28). Small amounts of these substances penetrating the placental barrier can be very hazardous to the embryo, because of extremely embryonic sensitivity to various toxic agents. When 4NQO was given after the appearance of the fetal lung, tumors were induced in the lung. This finding is compatible with the previous results of Nomura (18, 19) and Nomura and Okamoto (21). Tumor incidence in the offspring of mice receiving 4NQO only once during pregnancy was significantly high than tumor incidence in those receiving it only once during lactation. It may be suggested from this finding that 4NQO penetrates the placental barrier and reaches the fetus. However, amounts of transplacentally transferred 4NQO or its active intermediate may be very small, because many more tumors were observed when 4NQO was injected directly into the mouse fetus (20) than when 4NQO was given to pregnant mice. In the case of MNUT, such an increase in tumor incidence was not observed. Alexandrov (2) also reported the same results in rats, although Tanaka (30) observed an increased incidence of tumors in the offspring of rats treated with MNUT during pregnancy. Tumor induction in the offspring by the intraplacental injection of MNUT (2) and also in the mother mice receiving it (Table 4)
Table 2

**Tumors in the offspring of mice receiving MNUT during pregnancy**

<table>
<thead>
<tr>
<th>Day of gestation</th>
<th>Dose (µg)</th>
<th>No. of pregnant mice</th>
<th>No. of offspring alive at 16 wk</th>
<th>Incidence</th>
<th>p (χ² test)</th>
<th>Tumor-bearing mice/litter</th>
<th>p (t test)</th>
<th>Tumors/mouse</th>
<th>p (t test)</th>
<th>Other tumors and malformations</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>5</td>
<td>5(5)</td>
<td>44</td>
<td>8.8</td>
<td>10/44 (22.7)</td>
<td>&lt;0.01</td>
<td>2.00 ± 0.32</td>
<td>NS</td>
<td>0.027 ± 0.082</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>3(3)</td>
<td>35</td>
<td>11.7</td>
<td>3/35 (8.6)</td>
<td>NS</td>
<td>1.00 ± 0.58</td>
<td>NS</td>
<td>0.086 ± 0.048</td>
<td>NS</td>
</tr>
<tr>
<td>11</td>
<td>5</td>
<td>5(5)</td>
<td>65</td>
<td>13.0</td>
<td>6/63 (9.5)</td>
<td>NS</td>
<td>1.00 ± 0.00</td>
<td>NS</td>
<td>0.111 ± 0.046</td>
<td>NS</td>
</tr>
<tr>
<td>13</td>
<td>5</td>
<td>4(4)</td>
<td>43</td>
<td>15.8</td>
<td>4/41 (9.8)</td>
<td>NS</td>
<td>1.00 ± 0.41</td>
<td>NS</td>
<td>0.098 ± 0.047</td>
<td>NS</td>
</tr>
<tr>
<td>15</td>
<td>5</td>
<td>2(2)</td>
<td>20</td>
<td>10.0</td>
<td>3/20 (15.0)</td>
<td>NS</td>
<td>1.50 ± 0.41</td>
<td>NS</td>
<td>0.150 ± 0.081</td>
<td>NS</td>
</tr>
<tr>
<td>17</td>
<td>5</td>
<td>2(2)</td>
<td>25</td>
<td>12.5</td>
<td>2/24 (8.3)</td>
<td>NS</td>
<td>1.00 ± 0.00</td>
<td>NS</td>
<td>0.083 ± 0.058</td>
<td>NS</td>
</tr>
<tr>
<td>Control*</td>
<td>Water</td>
<td>11(11)</td>
<td>127</td>
<td>11.5</td>
<td>10/125 (8.0)</td>
<td>0.91 ± 0.09</td>
<td>0.080 ± 0.024</td>
<td>2 hepatomas, 4 kinky tails, 1 ovarian cystadenoma</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

* Control mice received s.c. injection of distilled water during pregnancy, and their offspring were sacrificed at 40 weeks after birth.
### Table 3

**Tumors in the sucklings of mice receiving 4NQO during lactation**

<table>
<thead>
<tr>
<th>Treatment&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. of lactating mothers</th>
<th>No. Av. litter</th>
<th>No. of sucklings alive at 16 wk</th>
<th>Incidence</th>
<th>&lt;sup&gt;p&lt;/sup&gt; (χ² test)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Tumor-bearing mice/litter</th>
<th>&lt;sup&gt;p&lt;/sup&gt; (t test)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Tumors/mouse</th>
<th>&lt;sup&gt;p&lt;/sup&gt; (t test)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Other tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3</td>
<td>40 13.3</td>
<td>37</td>
<td>6/37 (16.2)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>&lt;0.02</td>
<td>2.00 ± 0.58&lt;sup&gt;e&lt;/sup&gt;</td>
<td>&gt;0.05</td>
<td>0.189 ± 0.076</td>
<td>&gt;0.05</td>
<td>1 hepatoma</td>
</tr>
<tr>
<td>B</td>
<td>5</td>
<td>67 13.4</td>
<td>45</td>
<td>11/45 (24.4)</td>
<td>&lt;0.01</td>
<td>2.20 ± 0.58</td>
<td>&lt;0.05</td>
<td>0.311 ± 0.089</td>
<td>&lt;0.01</td>
<td>1 hepatoma, 1 liver hemangioma, 6 lymphomas (splenic type), 2 ovarian cystadenomas</td>
</tr>
<tr>
<td>Control&lt;sup&gt;f&lt;/sup&gt;</td>
<td>25</td>
<td>265 10.6</td>
<td>253</td>
<td>11/253 (4.3)</td>
<td>0.44 ± 0.10</td>
<td></td>
<td>0.043 ± 0.013</td>
<td>6 lymphomas (node type)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Lactating mice received a s.c. injection of 4NQO within 12 hr after delivery (A), or received 2 additional treatments on the 6th and 11th day after delivery (B).

<sup>b</sup> χ² test was applied with Yates's correction.

<sup>c</sup> t test was applied after testing variance ratio. If variance ratio was over F value at 5%, t test was applied with approximation of Cochran-Cox.

<sup>d</sup> Numbers in parentheses, percentage.

<sup>e</sup> Mean ± S.E.

<sup>f</sup> Control mice received a s.c. injection of distilled water during pregnancy or lactation, and their offspring were sacrificed 32 weeks after birth.

### Table 4

**Tumors in mice receiving 4NQO or MNUT during pregnancy or lactation**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Carcinogen</th>
<th>Dose (μg)</th>
<th>No. of mice</th>
<th>Ovarian cystadenomas</th>
<th>Lung tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>During pregnancy</td>
<td>4NQO</td>
<td>25</td>
<td>12(3)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>MNUT</td>
<td>5</td>
<td>18(11)</td>
<td>1</td>
<td>5.9</td>
</tr>
<tr>
<td>During lactation</td>
<td>4NQO</td>
<td>25</td>
<td>8</td>
<td>3</td>
<td>37.5</td>
</tr>
<tr>
<td>Control&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Water</td>
<td>37</td>
<td>0</td>
<td>0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> χ² test was applied with Yates's correction.

<sup>b</sup> t test was applied after testing variance ratio. If variance ratio was over F value at 5%, t test was applied with approximation of Cochran-Cox.

<sup>c</sup> Numbers in parentheses, number of mice that died during experiment.

<sup>d</sup> Numbers in parentheses, percentage.

<sup>e</sup> Mean ± S.E.

<sup>f</sup> Control mice received a s.c. injection of distilled water during pregnancy or lactation.
may suggest that it is difficult for MNUT to reach fetuses via the placenta when MNUT is given to pregnant mice and rats, probably because of either the high hydrolytic decomposition on the way to fetuses (2) or the placental barrier to MNUT. Tomatis et al. (31) reported that only 0.9% of 3-methylcholanthrene in maternal concentration was detected in the fetus. Similar findings were also observed by Alexandrov and Shendrikova (3) with 7,12-dimethylbenz(a)anthracene. Slightly water-soluble carcinogens may have difficulty penetrating the placental barrier (3, 31), whereas highly water-soluble urethan can pass through the placental barrier freely at any stage of pregnancy (23).

Both 4NQO and MNUT showed teratogenic effects when given during early organogenesis. Decreased numbers of live births in the same groups may be due to embryo deaths. MNUT and 4NQO could reach the embryo directly, because Day 9 corresponded to the stage before the appearance of the placenta (19). Exposure of embryos to a large amount of MNUT on Day 9 might result in a change of detoxication and immunosurveillance after birth and might enhance the susceptibility to tumor induction, as was suggested by the results when urethan was given on Day 9 (18, 19).

Induction of tumors in sucklings suggests that 4NQO or its active intermediate is excreted in the milk and transferred into sucklings via nursing. There has been no report of the induction of hepatomas in mice by 4NQO. It may be due to the rapid enzymatic decomposition of an active intermediate in the adult liver (10, 28). Less catabolic activity in the liver of newborns may be a possible cause of tumor induction in the liver.

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Tumor Induction in the Progeny of Mice Receiving 4-Nitroquinoline 1-Oxide and \( N \)-Methyl-\( N \)-nitrosourethan during Pregnancy or Lactation

Taisei Nomura, Eizo Okamoto, Noriko Tateishi, et al.

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