Carcinogenicity of Uracil, a Nongenotoxic Chemical, in Rats and Mice and Its Rationale

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ABSTRACT

In Experiment 1, groups of thirty 6-wk-old male and female F344 rats were given diets containing 0 (control) or 3% uracil for 104 wk. In the uracil-treated groups, carcinomas, in particular transitional cell carcinomas, developed in the urinary bladder of 90% of the males and 19% of the females. Squamous cell carcinomas also developed in 10% of the males, but not in females. Striking findings were that calculi were present in the urinary bladder of almost all males, but in only 30% of the females, and that induction of urinary bladder carcinomas was related to the presence of calculi. In Experiment 2, groups of thirty 6-wk-old male and female C57BL/6 x C3H F, mice were given a diet containing 0 (control) or 3% from Wk 1 to Wk 6) and 2.5% (from Wk 7 to Wk 96) uracil. The total observation period was 96 wk. In the uracil-treated groups, transitional cell carcinomas developed in 76% of the females and 8% of the males. Squamous cell carcinomas developed in only 8% of the males. In Experiment 3, 6-wk-old male F344 rats were given diets containing 3% uracil, 3% uracil plus 5% or 10% NaCl, or 10% NaCl for 36 wk and then diet without chemicals for a recovery period of 4 wk. The incidences of carcinomas and calculi of the urinary bladder were 75% and 81% in the group given uracil alone, 6% and 6% in the group given uracil plus 5% NaCl, and both zero in the group given uracil plus 10% NaCl. Thus, the present study showed that the inductions of urinary bladder carcinomas by uracil, a nongenotoxic compound, in rats and mice showed sex differences and were related to the presence of calculi in the urinary bladder.

INTRODUCTION

In rodents, foreign bodies have long been known to be causes of urinary bladder carcinogenesis (1–10). For example, surgically implanted pellets of paraffin wax (1, 3–5), cholesterol (4), glass beads (3), or wood chips in the urinary bladder and endogenous urinary stones (8, 9) have been shown to be involved in the induction of urinary bladder tumors of rats and mice and have been proposed to act as cocarcinogens (11). Moreover, Melnick et al. (9) demonstrated that inclusion of the resin melanin in the diet induced not only stones but also tumors in the urinary bladder of rats. Their study showed a direct association between endogenous stone formation and tumorigenicity.

Lalich (12) first reported induction of stone formation in the urinary bladder of rats by p.o. administration of uracil, a component of RNA. Subsequently, Shirai et al. (13, 14) demonstrated that p.o. administration of uracil to rats induced mucosal papillomatosis of the urinary bladder associated with urolithiasis and urinary bladder carcinoma at low incidence after a relatively short time of treatment. They also found that uracil-induced calculi strongly promoted BBN-induced urinary bladder carcinogenesis in rats (15). Similar promoting activity of uracil has been observed in urinary bladder carcinogenesis initiated with N-methyl-N-nitrosourea in rats (16). Very recently, Okumura et al. (17) found that calculi induced in rats by uracil ingestion for 36 wk themselves induced a high incidence of carcinoma of the urinary bladder and also acted as a strong cocarcinogen of BBN in induction of urinary bladder carcinogenesis. Sakata et al. (18) observed urinary calculi and severe epithelial hyperplasia of the urinary bladder in Swiss and C3H mice after p.o. treatment with uracil for 15 wk.

In the present study, we evaluated the carcinogenicity of uracil by its administration to rats and mice for 2 yr. We also examined the effect of p.o. administration of uracil with sodium chloride (NaCl) to determine the reason for the carcinogenicity of uracil in the rat urinary bladder.

MATERIALS AND METHODS

Test Chemicals. Uracil, 2,4-dioxypyrimidine, was obtained from Yamasa Shoyu Co., Chiba, Japan. The purity of the preparation was over 95.0%. NaCl was from Wako Pure Chemical Ind., Osaka, Japan. Animals and Their Maintenance. Male and female F344 rats (Experiment 1), C57BL/6 x C3H F, (hereafter called B6C3F,) mice (Experiment 2), and 5-wk-old male F344 rats were purchased from Charles River Japan, Inc. (Atsugi, Japan), and quarantined for 7 days before experiments. Animals of the same sex were housed five to a polycarbonate cage with hardwood Beta Chips (Northeastern Product Co., Warrensburg, NY) for bedding. The animals had free access to food (Oriental MF powdered diet; Oriental Yeast Co., Tokyo, Japan) and tap water. Bedding and cages were changed 3 times/wk. The room temperature was controlled at 22 ± 2°C, and the relative humidity, at 55 ± 10%. The room air was changed more than 15 times/h. Fluorescent lighting provided a 12-h light/dark cycle. Animals were weighed and randomly assigned to dose groups the day before the start of experiments.

Diet Preparation. The diets containing uracil in Experiments 1 and 2 were prepared by mixing weighed quantities of uracil and Oriental MF powdered diet with 2% corn oil in a stainless-steel mixer for 30 min. Control diet also contains 2% corn oil. The diets were prepared every 1 or 2 wk and stored at 4°C until use. Diets containing uracil with or without NaCl in Experiment 3 were also prepared in the same way.

Experimental Procedure. In Experiment 1, groups of thirty 6-wk-old male and female rats were given diets containing 0 (control) or 3% uracil for 104 wk. In Experiment 2, groups of thirty 6-wk-old male and female mice were given a diet containing 0 (control) or 3% (Wk 1 to Wk 6) and then 2.5% (Wk 7 to Wk 96) uracil. The total observation period was 96 wk. The animals were examined daily, and those showing abnormalities were isolated and returned to their groups if their condition improved but otherwise killed and autopsied. Individual body weights were recorded weekly for the first 14 wk and then every other wk. Food and water consumptions were measured for 2-day periods before each time of weighing. In Experiment 2, urine samples were obtained from 12 animals in each group in Wk 96, and their pH, urinary electrolytes, specific gravity, and contents of protein, glucose, bilirubin, ketone, occult blood, and urobilinogen were measured with
were embedded in paraffin wax, sectioned, and stained with hematox-
skin, mammary glands, skeletal muscle, spinal cord, sciatic nerve, and
ether anesthesia by exsanguination from the abdominal aorta. In Ex
were deprived of food, but not water, overnight and then killed under
either anesthesia or exsanguination from the abdominal aorta. In Ex
were deprived of food, but not water, overnight and then killed under

Gross examinations were performed at autopsy, and detailed exam-
inations of the urinary bladder were also carried out after fixation of
the tissue. The following organs of each animal were weighed, and their
organ/body weight ratios were calculated: the brain; heart; liver; spleen;
kidneys; adrenals; and testes or ovaries.

Samples of these organs and of the salivary glands, trachea, lungs,
thyroid, lymph nodes, stomach, small intestine, pancreas, urinary bladder, pituitary, thyroid, prostate, seminal vesicles,
skin, mammary glands, skeletal muscle, spinal cord, sciatic nerve, and
any other tissues with an abnormal appearance were fixed in 10%
phosphate-buffered formalin. For microscopical examination, tissues
were embedded in paraffin wax, sectioned, and stained with hematox-
ylin and eosin. Histopathological examinations were also performed
on animals that died or were killed when they became moribund during
the experiments.

In Experiment 3, 6-week-old male F344 rats were divided into 4 groups:
16 rats in Groups 1 and 2; 15 rats in Group 3; and 10 rats in Group 4.
The animals were given diets containing 3% uracil (Group 1), 3% uracil
plus 5% NaCl (Group 2), 3% uracil plus 10% NaCl (Group 3), or 10%
NaCl (Group 4) for 36 wk and then diet without chemicals for a
recovery period of 4 wk. The total observation period was 40 wk. The
diets and drinking water were freely available. The concentrations of
NaCl added to the diet were determined from a preliminary experiment
in which rats were given diets containing various concentrations of
NaCl and 3% uracil for 4 wk. The animals were observed daily for
abnormalities. In particular, palpation for detection of stone formation
in the urinary bladder was carried out biweekly. Body weights and food
and water consumptions were also measured biweekly.

In Wk 12 and Wk 24, the urinary pH was measured with a pH meter
(Model F-7DP pH meter; Hitachi-Horiba, Tokyo, Japan), and urinary
osmolality was measured with an Osmette A instrument (Percision
System, Inc., Natick, MA). Urinary uric acid contents in Wk 12 were
measured at the Japan Food Analysis Center, Tokyo, Japan. Before
the end of uracil treatment in Wk 36, the urinary bladder of all animals
in each group were examined grossly. For this, the rats were anesthe-
tized with ethyl ether, and the lower part of the abdomen was opened
by a midline incision to expose the urinary bladder. The urinary bladder
was then examined macroscopically for the presence of calculi. The
abdomen was then closed with metal clips.

The urinary bladder was then examined macroscopically at autopsy
at the beginning of Wk 37. Then it was examined microscopically in
the same way as in Experiment 1.

Statistical Analysis. Data on cumulative mortality were analyzed by
the generalized Wilcoxon test and Cox-Mantel test (19, 20). Tumor
incidences were analyzed by a one-sided Fisher's exact probability test.
Other data were analyzed by Student's t test.

RESULTS

Experiment 1. No clinical signs related to uracil treatment
were apparent in any of the rats during the 104-wk experiment.
The survival rates of rats fed 0 (control) and 3% uracil were
97% and 60% in males and 100% and 90% in females in Wk
78 and 80% and 0% in males and 73% and 60% in females in
Wk 104. Thus, there were significant differences between the
mortalities of controls and uracil-treated males during the 104-
wk experiment. The main causes of death of control rats were
spontaneous tumors, particularly malignant lymphomas in females.

The mean body weights of rats of both sexes given uracil
were consistently less than those of controls (Fig. 1). The mean
absolute and relative urinary bladder weights (excluding bladder
stones) of rats of both sexes fed a diet containing uracil were
significantly more than those of the controls (Table 1).

Macroscopically, polyoid or papillomatous tumor masses
of various sizes with thickening of the wall were seen in the
urinary bladder of rats of both sexes treated with uracil. A
remarkable finding was that almost all the males, but only 30% of
the females, in uracil-treated groups had calculi in the urinary
bladder (Table 1). These urinary calculi, which were yellowish-
white and granular, filled the lumen of the urinary bladder.
Moreover, unilateral or bilateral hydronephrosis was observed
in rats with calculi. Marked changes were apparent in all but
one male rat.

The histological types of lesions of the urinary bladder in the
rats are summarized in Table 2. The epithelial lesions found in
the urinary bladder were classified into PN hyperplasia, papil-
loma, carcinoma, and papillomatosis as described previously
(13, 21). Carcinomas, in particular transitional cell carcinomas,
were observed at very high incidence (90%) in males, but at low
incidence (19%) in females in the groups treated with uracil.
All the carcinomas were papillary and noninvasive. Squamous
cell carcinomas developed in 10% of the uracil-treated males,
but in no females. No metastases to other organs were observed.
The incidence of transitional cell papilloma was 80% in males
and 30% in females in uracil-treated groups. PN hyperplasias,
which are putative neoplastic lesions, were also seen in uracil-
treated males and females. The incidence of papillomatosis was
almost the same as that of PN hyperplasia and involved the
entire mucosa. These lesions were associated with the presence
of calculi. In one of 30 males and one of 27 females in uracil-
treated groups, the urinary bladder appeared normal with no
urinary calculi. Hydronephroses were observed in males and
females and were associated with hydrourter in both sexes.

In the renal pelvis, carcinomas or papillomas were observed
in uracil-treated males and females, the incidence of carcinomas
in uracil-treated males being significantly different from that in
controls (Table 3).

Benign and malignant tumors were found in various other
organs of rats of both sexes in all groups. The incidences of

![Graph](https://example.com/graph.png)

**Fig. 1.** Growth curves of rats treated with uracil. Ç, control males; Ç, uracil-
treated males; Ç, control females; Ç, uracil-treated females.

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*Unpublished data.*
URACIL-INDUCED BLADDER CANCERS

Table 1 Mean urinary bladder weights and urinary calculi in rats and mice fed a diet with or without uracil

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Animal</th>
<th>Sex</th>
<th>Treatment with uracil</th>
<th>No. of animals</th>
<th>Absolute (g)</th>
<th>Relative of body wt (%)</th>
<th>No. with urinary calculi</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rat</td>
<td>Male</td>
<td>+</td>
<td>30</td>
<td>0.17 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.04 ± 0.01</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td></td>
<td>20</td>
<td>2.00 ± 1.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.66 ± 0.31&lt;sup&gt;c&lt;/sup&gt;</td>
<td>29 (97)&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>+</td>
<td>30</td>
<td>0.13 ± 0.07&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.05 ± 0.03</td>
<td>1 (3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td></td>
<td>27</td>
<td>0.56 ± 0.79&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.26 ± 0.43&lt;sup&gt;f&lt;/sup&gt;</td>
<td>8 (30)&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>Mouse</td>
<td>Male</td>
<td>−</td>
<td>22</td>
<td>0.06 ± 0.02&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.15 ± 0.05</td>
<td>ND&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>+</td>
<td>16</td>
<td>0.16 ± 0.05&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.24 ± 0.23&lt;sup&gt;h&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>−</td>
<td>26</td>
<td>0.05 ± 0.02&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.10 ± 0.04</td>
<td>ND&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>+</td>
<td>23</td>
<td>0.13 ± 0.06&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.42 ± 0.16&lt;sup&gt;h&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± SD.
<sup>b</sup> P < 0.01 (significance of difference from the control by Student's t test or Fisher's exact probability test).
<sup>c</sup> Numbers in parentheses, percentage.
<sup>d</sup> P < 0.05 (significance of difference from the control by Student's t test or Fisher's exact probability test).
<sup>e</sup> ND, not determined.

Table 2 Histological types of lesions of the urinary bladder in rats given diets with and without uracil

<table>
<thead>
<tr>
<th>Male</th>
<th>Female</th>
<th>Control (n = 30)</th>
<th>Uracil (n = 30)</th>
<th>Control (n = 27)</th>
<th>Uracil (n = 27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PN hyperplasia</td>
<td>0</td>
<td>29 (97)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3 (9)</td>
<td>27 (93)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27 (97)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Transitional cell papilloma</td>
<td>0</td>
<td>24 (80)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7 (27)</td>
<td>8 (30)</td>
<td></td>
</tr>
<tr>
<td>Squamous cell papilloma</td>
<td>0</td>
<td>1 (3)</td>
<td>1 (3)</td>
<td>2 (7)</td>
<td></td>
</tr>
<tr>
<td>Transitional cell carcinoma</td>
<td>0</td>
<td>27 (90)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5 (19)&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>0</td>
<td>3 (10)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leiomyosarcoma</td>
<td>0</td>
<td>1 (3)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Numbers in parentheses, percentage.
<sup>b</sup> P < 0.01 (significance of difference from the control by Fisher's exact probability test).
<sup>c</sup> P < 0.05 (significance of difference from the control by Fisher's exact probability test).

Table 3 Incidences of pelvic tumors in rats and mice treated with uracil

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Animal</th>
<th>Sex</th>
<th>Treatment with uracil</th>
<th>Effective no. of animals</th>
<th>No. of tumors of the pelvis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rat</td>
<td>Male</td>
<td>−</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>+</td>
<td>30</td>
<td>4 (13)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>−</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>+</td>
<td>27</td>
<td>3 (11)</td>
</tr>
<tr>
<td>2</td>
<td>Mouse</td>
<td>Male</td>
<td>−</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>+</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>−</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>+</td>
<td>29</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Numbers in parentheses, percentage.
<sup>b</sup> P < 0.05 (significance of difference from the control by Fisher's exact probability test).

Table 4 Histological lesions of the urinary bladder in mice treated with uracil

<table>
<thead>
<tr>
<th>Male</th>
<th>Female</th>
<th>Control (n = 30)</th>
<th>Uracil (n = 26)</th>
<th>Control (n = 29)</th>
<th>Uracil (n = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PN hyperplasia</td>
<td>0</td>
<td>8 (31)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>12 (41)&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Carcinoma in situ</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (3)</td>
<td></td>
</tr>
<tr>
<td>Transitional cell carcinoma</td>
<td>0</td>
<td>2 (8)</td>
<td>0</td>
<td>22 (76)&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>0</td>
<td>2 (8)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Leiomyosarcoma</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (3)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Numbers in parentheses, percentage.
<sup>b</sup> P < 0.01 (significance of difference from the control by Fisher's exact probability test).

Interstitial cell tumors of the testes were significantly higher (97%) in the controls than in the uracil-treated animals (60%). This difference was due to the difference in survival of the two groups. There were no significant differences in the incidences of other tumors in control and uracil-treated rats of either sex.

Experiment 2. No clinical signs related to uracil treatment were observed in any mice during the 96-wk experiment. The survival rates of mice in the control and uracil-treated groups were 90% and 80% for males and 97% and 97% for females in Wk 52 and 73% and 53% for males and 87% and 77% for females in Wk 96. Thus, there was no significant difference between the mortalities of control and uracil-treated mice of either sex during the 96-wk experiment.

Hematological examination showed no remarkable changes in mice of either sex treated with uracil. Blood chemical analyses showed that the values of albumin and GPT in uracil-treated males were slightly higher than those of control males (data not shown). However, judging from background data, these differences did not seem significant. Urine analyses showed slightly lower pH values and decreases of Na+, K+, Ca<sup>2+</sup>, Mg<sup>2+</sup>, and CI<sup>-</sup> concentrations and lower specific gravity due to the increase of urine volume in uracil-treated mice of both sexes (data not shown). There were no differences between values for other urinary parameters in control and uracil-treated mice of either sex.

Reduced body weight gains were apparent in the groups treated with 3% uracil (1 to 6 wk). The mean body weights of both sexes were 20% less than those of controls in Wk 6 and were consistently less than those of controls throughout the experiment. The mean urinary bladder weights of mice of both sexes treated with uracil were significantly higher than those of the controls (Table 1).

On gross observation of the urinary bladder after its fixation for about 3 wk, thickening of the wall was apparent in uracil-treated groups, but no polypoid lesions were seen on the luminal surface of the urinary bladder. The incidences of urinary bladder calculi in the urinary bladder of uracil-treated groups could not be determined exactly through the serosal wall at the time of autopsy, and no calculi were found in the urinary bladder in any fixed preparations, because they disappeared during fixation with formalin for about 3 wk. No remarkable changes were observed in the urinary bladder of controls.

The histological findings in the urinary bladder of mice are summarized in Table 4. In the uracil-treated groups, transitional cell carcinomas were observed at high incidence in females, but at low incidence in males. No papillomas were found in either sex. PN hyperplasia developed in both sexes. Unlike in rats, papillomatosis did not develop in mice. No epithelial lesions were found in control mice of either sex. No tumors were observed in the renal pelvis in uracil-treated mice (Table 3). Benign and malignant tumors were found in various other organs of mice with and without uracil treatment (Table 5). The incidence of hepatocellular carcinoma in males was signif-
Macroscopically, tumors were seen in the urinary bladders of many rats given uracil alone, but not in those of rats given uracil plus 10% NaCl. The histological types of lesions of the urinary bladder are summarized in Table 8. Carcinomas were observed at high incidence (75%) in the group given uracil alone and at very low incidence (6%) in the group given uracil plus 5% NaCl, while no carcinomas were found in the group given uracil plus 10% NaCl. The incidence of papillomas was 100% in the group given uracil alone, 6% in that given uracil plus 5% NaCl, and zero in that given uracil plus 10% NaCl. Slight papillomatosis was observed only in rats treated with uracil alone. The urinary bladder in the group given NaCl alone showed no proliferative lesions.

**DISCUSSION**

In this study, long-term, p.o. administration of uracil induced urinary calculi and urinary bladder carcinomas in both rats and mice. The frequencies of urinary bladder carcinomas were higher in male than female rats and in female than male mice, and the carcinomas were associated with urinary calculi.

Previously, Shirai et al. (13) and Masui et al. (16) reported low yields (10 to 20%) of rat urinary bladder carcinomas after administration of diet containing 3% uracil for 20 to 30 wk. Wang et al. (22) also reported induction of urinary bladder cancers in rats at relatively low incidence (27%) after administration of diet containing 3% uracil for 36 wk and then normal diet for 19 wk. Recently, Okumura et al. (17) found that administration of diet containing 3% uracil for 36 wk and then normal diet for 4 wk resulted in a high incidence (73%) of urinary bladder cancers in rats associated with urinary calculi, whereas administration of diet containing 1% uracil and then normal diet by the same protocol did not. Urolithiasis induced by uracil administration has a strong promoting effect on BBN urinary bladder carcinogenesis in rats (15, 23). Sakata et al. (18) also reported that administration of diet containing 3% uracil, but not 1% uracil, to mice for 15 wk resulted in formation of urinary calculi and hyperplastic lesions of the urinary bladder. In the present 2-yr experiment, we confirmed that the presence of calculi in the urinary bladder of rats and mice was associated with the development of urinary bladder cancers. This is the first report of high induction of urinary bladder cancer by uracil in mice.

Ashby and Tennant (24) reported that some nongenotoxic chemicals, which do not interact either directly or indirectly with cellular DNA in a biologically significant manner, are carcinogenic to both rats and mice. These nongenotoxic compounds are usually carcinogenic only at high doses (25–27). Nongenotoxic compounds, such as melamine (9), and 4-ethylsulfonylnaphthalene-1-sulfonamide (28) induced urinary bladder tumors in rats when given p.o. at high doses for long periods. But these compounds induced calculi and epithelial cell hyperplasia of the urinary bladder in rats in shorter periods. Urinary calculi are formed when the urine is oversaturated with a compound, and the importance of their formation in the induction of carcinogenesis by nongenotoxic agents has been pointed out (10). Uracil is nongenotoxic (29), and there are reports that administration of a diet containing 3% uracil induced significant epithelial cell proliferation of the urinary bladder in rats and mice (13, 15, 18). The finding that the dose-response curves for induction of calculi by uracil in rats were extremely steep (17, 18) is interesting, because calculi are formed when the urine is oversaturated with...
components of calculi, as mentioned above. In Experiment 3 of our study, administration of 10% NaCl with 3% uracil prevented induction of bladder calculi and cancers, although the urinary content of uracil was similar to that on administration of 3% uracil alone. This effect of NaCl was due to its induction of polyuria, resulting in excretion of microscopically sized uracil calculi and preventing formation of bigger uracil calculi. Large calculi damage the urinary bladder epithelium mechanically and increase DNA synthesis in the cells (14, 18). Thus, prolonged stimulation of excessive proliferation of the cells in rats and mice presumably results in carcinoma formation. This mechanism is consistent with a recent report of Cohen and Ellwein (30) that increased cell proliferation can account for the carcinogenicity of nongenotoxic chemicals. The present uracil model may be useful for examining how tumors develop from hyperplastic lesions.

Sakata et al. (18) reported sex and strain differences in the hyperplastic responses of uracil-treated mice. They found that, on administration of diet containing 3% uracil to Swiss and C3H mice, the [3H]thymidine labeling index was higher in males than in females in Wk 10, but not in Wk 15. Moreover, the males tended to show more severe histological changes than the females. However, in the present study, the incidence of carcinomas in mice was higher in females than in males. The urinary bladder calculi disappeared during processing of the tissues of mice for histological examination, so we could not compare the incidences of urinary calculi in male and female mice exactly, but judging from another experiment in which mice were treated with 2.5% uracil for 8 wk, females had more urinary microscopical calculi and greater urothelial hyperplasia than did males.4 These results were inconsistent with those of Sakata et al. (18). Male rats were more susceptible than females to urinary bladder carcinogenesis induced by uracil. These urinary bladder cancers were associated with the formation of urinary calculi. The reason for the sex differences in susceptibility to induction of calculi and cancer by uracil is unclear. However, it seems clear that there are anatomical reasons why male rats are more susceptible uracil calculi formation than are females.

The growth patterns of urinary bladder carcinomas induced by nongenotoxic compounds in rats and mice were different. In rats, the calculi-induced bladder cancers showed polypoid, papillary growth within the lumen of the urinary bladder, like those induced by genotoxic carcinogens such as BBN, N-[4-(5-nitro-2-furyl)-2-thiazoyl]formamide, and N-methyl-N-nitrosourea (31-33). On the other hand, the carcinomas induced by uracil in mice showed downward growth in the urinary bladder wall, namely, nonpapillary and invasive growth, like those induced by BBN (34).

As already stated, there must be a threshold level of a nongenotoxic carcinogen for induction of carcinogenesis in the urinary bladder, as a high dose but not a low dose induces cancers, and the mechanism of the induction is clear (35). In the present study, a very high dose of uracil was required for formation of calculi and carcinomas of the urinary bladder in both rats and mice. Therefore, a threshold level of uracil is required for calculi formation, as in the case of melamine (36).

REFERENCES


URACIL-INDUCED BLADDER CANCERS


Carcinogenicity of Uracil, a Nongenotoxic Chemical, in Rats and Mice and Its Rationale

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