Effect of Sodium Saccharin and L-Tryptophan on Rat Urine during Bladder Carcinogenesis

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ABSTRACT
We examined several parameters of urine excretion during a two-year initiation-promotion experiment in male Fischer rats using four weeks of N-(4-(5-nitro-2-furyl)-2-thiazolyl)formamide at 0.2% of the diet as the initiating agent and either 5% sodium saccharin or 2% L-tryptophan in the diet as promoting agents. Rats fed sodium saccharin increased their intake of water; this was accompanied by diarrhea and an increased urinary volume. Osmolality was decreased slightly. The total amount of sodium excreted was increased, although the concentration in the urine was similar to that of the controls or slightly increased. No abnormalities were observed in urinary potassium, calcium, urea, or other parameters measured except for the pH, which was slightly increased during the first three months of the experiment. There was no increase in the size or concentration of crystals in the urine of rats fed sodium saccharin, and no calculi were observed. Hypoglycemia and hypoglycosuria were present in sodium saccharin-fed rats and to a lesser extent in L-tryptophan-fed rats. No other abnormalities were seen in the urine of rats fed L-tryptophan. These data suggest that none of the urinary factors measured in our experiment, including crystal and calculus formation, correlated with the induction of urinary bladder lesions by sodium saccharin.

INTRODUCTION
Sodium saccharin has demonstrated weak carcinogenic (2, 7) or promoting (11, 14, 16) activity toward the urinary bladder of rats when fed for long periods of time at high doses. Various mechanisms have been postulated to explain the promotional and/or carcinogenic effects of saccharin, and they can be divided into 2 basic categories: (a) saccharin has a direct effect on the bladder epithelium; or (b) the ingestion of saccharin alters the physiology of the animal, such as changes in the constituents of the urine, in a way that leads to tumor production. It has been reported that rats fed saccharin have increased magnesium and phosphorus in the urine that could combine to form MgNH₂PO₄ crystals and possibly calculi (1, 2). Several investigators have indicated that vesical calculi, whether natural or implanted pellets, may act as a cocarcinogen (9, 10), a promoter (5, 10), or a mitotic stimulator of epithelial cells (10).

The present experiments were performed to evaluate various urinary parameters in rats fed sodium saccharin or other chemicals to determine what effect they have on the urine and whether the changes correlated with tumor formation.

MATERIALS AND METHODS
Five-week-old inbred male Fischer 344 rats (Charles River Breeding Laboratories, Wilmington, Mass.) comprised 8 groups (26/group) following the diet plans outlined in Chart 1. The animals were maintained at 24° and 50% humidity on a 12-hr light-dark cycle; food and H₂O were available ad libitum. FANFT² (Saber Laboratories, Morton Grove, Ill.), sodium saccharin (Lot 1174-0154, Sigma Chemical Co., St. Louis, Mo.), and L-tryptophan (Sigma) were administered to the rats in the diet (powdered Charles River rat chow) at levels of 0.2, 5.0, and 2.0% by weight, respectively. Sodium saccharin or L-tryptophan were fed continuously after feeding either FANFT for 4 weeks (Groups 1 and 7) or control diet (Groups 2 and 8). In Group 4, a 4-week administration of FANFT was followed by the control diet (Charles River rat chow). FANFT in Group 6 and sodium saccharin in Group 3 were fed continuously beginning at 0 time of the experiment. Group 5 was fed pelleted Charles River rat chow without added chemicals throughout the experiment. The rats were weighed weekly for 8 weeks and then monthly to the end of the experiment. Food and water consumption was determined periodically during this experiment.

Urinalysis was performed prior to the start of the experiment; then during Weeks 1, 2, 4, 5, 6, 7, 8, and 12; and monthly from Week 64 to the end of the experiment. The analysis began each time with the random selection of 4 rats/group. The animals were placed individually in metal metabolic cages with glass collection flasks surrounded by ice. Food and H₂O were provided for the animals, and they were left overnight (3 p.m. to 7 a.m.). Occasional 3-hr collections were done throughout the experiment to compare excretion parameters at different times of day. The urine was evaluated for blood, bilirubin, ketone, glucose, and protein with Ames Bili-Labstix. The urinary pH was determined with a Fischer Accumet Model 320 pH meter. After the color was noted, the volume was measured, and 3 ml of the urine were transferred to a plastic 15-ml centrifuge tube and spun for 5 min at 1000 rpm (Damon/IEC Division IEC PR-J centrifuge, swinging bucket rotor), at a temperature of 10–15°. The supernatant was poured off and retained in covered plastic tubes while the pellet was placed on glass slides, coverslipped, and immediately viewed with a light microscope at ×100 and ×400. The number of crystals,

1 Supported in part by USPHS Grant CA15495 from the National Cancer Institute through the National Bladder Cancer Project and by USPHS Biomedical Research Support Grant RR05660.
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Received January 7, 1980; accepted September 26, 1980.

Abbreviations used: FANFT, N-(4-(5-nitro-2-furyl)-2-thiazolyl)formamide.
RESULTS

The rats in all groups gained weight at a rate similar to that of the control group except for the rats receiving sodium saccharin (Group 1 to 3), which gained weight at a rate approximately 10% less than did the controls (Table 1). The decreased growth with sodium saccharin is in contrast to our previous experiment (11) in which rats fed sodium saccharin gained weight similar to controls until near the end of the experiment and in which DL-tryptophan resulted in a decreased growth rate. Although diarrhea was not severe at most times, diarrhea was greater in the present experiment in rats fed sodium saccharin than in our previous experiment. No diarrhea was observed in the present experiment with L-tryptophan or with DL-tryptophan in the previous experiment (11).

The diet consumed by the various groups was similar but varied from day to day (15 to 24 g/day). Daily water consumption was occasionally determined and was similar for control and L-tryptophan-fed rats but was slightly increased in rats fed FANFT and increased 1.5- to 2-fold in rats fed sodium saccharin (Table 1).

The incidences of lesions in the urinary bladder (12) and other tissues in each group will be reported in detail separately. Briefly, the use of FANFT for 4 weeks instead of 6 weeks eliminated the incidence of bladder cancer at the end of the experiment (2 years) (11). The incidence in rats fed FANFT followed by either sodium saccharin or L-tryptophan were significantly increased, but the incidences were less than in the previous experiment that used FANFT for 6 weeks (11). No bladder tumors were seen in the rats fed either sodium saccharin or L-tryptophan without FANFT prefeeding. All rats fed long-term FANFT (Group 6) developed bladder carcinomas by Week 52.

Variations in urinary volume and the other parameters measured occurred in individual rats from day to day and also within the same 24-hr period in urines collected at different 3-hr intervals. Similar to the results reported by Cambar et al. (6), rat urinary excretion was greatest during the night; consequently, most of our data come from overnight urine collections. Although the parameters measured varied during the day, differences between groups remained consistent. Variations in excretion parameters were also observed if the urines were collected with food available as opposed to without food; the data presented are from urine collections with food and water available.

Feeding of sodium saccharin to rats in Groups 1 to 3 resulted in a rapid increase in urine volume, generally 1.5 to 2 times the amount observed in the control group, which persisted during most of the experiment (Chart 2). A lesser and inconsistent increase in urinary volume was seen in rats fed FANFT.
The volume of urine excreted by rats fed FANFT followed by sodium saccharin (Group 1) (O) or by control diet (Group 4) (•). The volume excreted by Groups 2 and 3 was similar to that of Group 1, whereas that excreted by Groups 5, 7, and 8 were similar to that of Group 4.

Color of the urine was a dark, clear, golden yellow in rats fed FANFT and was a lighter yellow color than control urine in rats fed sodium saccharin. The urine of rats fed L-tryptophan was similar to that of controls in appearance and in all of the parameters measured except glucose.

Although the concentration of urinary sodium was usually slightly greater in rats fed sodium saccharin than the control diet, the absolute amount excreted in the urine, as estimated by multiplying concentration times volume, was always considerably greater in rats fed sodium saccharin (Chart 3). In contrast, the urinary urea nitrogen and potassium concentrations in sodium saccharin-fed rats were less than in the control rats, but the absolute amounts excreted were similar to controls at all time periods (range: urea nitrogen, 100 to 180 mg/100 ml; K⁺, 150 to 250 mg/kg).

The osmolality of the urine of rats fed sodium saccharin varied considerably between rats, varying by as much as 600 mOsmol/kg at a given collection period. With such large variations, statistically significant differences were not observed. Nevertheless, the trend of the findings was for an increased osmolality during the first 6 to 8 weeks for sodium saccharin-fed rats compared to controls followed by a generally lower osmolality after that time. At all times measured, the osmolality of a urine sample collected in the morning (9 to 12 noon) was lower by approximately one-third less than a sample collected in the evening (4 to 7), regardless of the diet that the rat received. The range of osmolality in all groups was between 500 to 2500 mOsmol/kg, but the usual range was 1400 to 2200 mOsmol/kg in overnight collections. The osmolarities that were lower than 1400 were observed in the younger rats at the start of the experiment.

With the onset of either sodium saccharin or FANFT feeding, there was an immediate increase in the pH of the collected urine as compared to the control group. The average pH for the control group during the first 4 weeks of the experiment was 6.5 to 7.1, for rats fed sodium saccharin it was 7.4 to 7.8, and for FANFT it was 7.8 to 8.0. After that time, the pH fluctuated between 6.5 to 7.5 in all groups with rats receiving sodium saccharin or FANFT having a somewhat higher pH. The evaluation of bilirubin, ketone, glucose, and protein with Bililabstix showed no variation between diet groups. Dipstick indication of blood content occurred in Groups 1, 6, and 7 in rats with bladder tumors.

Most of the crystals in the urine of rats from all groups were typical MgNH₄PO₄ crystals ("triple phosphate crystals") (4). They ranged between 10 and 300 μm in greatest dimension, and there were 5 to 50 crystals per high-power field (× 400) in the sediment after centrifugation of 3 ml of urine. There were no differences in the size or quantity of these crystals between the groups at any time. Occasionally, calcium carbonate crystals were seen in the urine of some rats from each group, but they were inconsistent and without differences between the start of the experiment.
Groups. P, and magnesium were not determined. No calculi were observed in the urines of any of the rats throughout the experiment. No nematodes were seen, but occasionally bacillary bacteria were observed in the urines of any of the rats throughout the groups. PI and magnesium were not determined. No calculi lary bacteria were seen, and in these specimens fecal contamination could not be excluded. There was no evidence of bacterial cystitis in any of the rats, and no nematodes or calcifications were observed in the bladder with or without lesions.

Hyline casts were infrequently present, and occasionally WBC and RBC casts were observed in older rats. BC, RBC, and epithelial cells were present in low numbers, as seen in the urinary sediment. There were no differences between groups until the appearance of hematuria in Group 6 at 26 weeks and in Groups 1 and 7 at 88 weeks in rats subsequently shown to have bladder tumors. Cytological examination of urines showed RBC, WBC, and infrequent transitional epithelial cells, but malignant tumor cells were not observed, a finding seen in mice (23) and rats (17) previously.

Urinary glucose in all diet groups was 15 to 50 mg/dl. The level of glycosuria was consistently less in rats fed sodium saccharin than in control rats, calculated on the basis of concentration or absolute amount excreted (Chart 4). L-Tryptophan-fed rats also had decreased urinary excretion of glucose but not as low as in sodium saccharin-fed rats. FANFT-fed rats had glucose urinary excretion similar to that of control rats. Although measured only at the end of the experiment, serum glucose was decreased in rats fed sodium saccharin or L-tryptophan, either after control diet or after FANFT, but the decrease was significant statistically only in Groups 2 and 7 (Table 2). Blood urea nitrogen and the other serum chemistries were similar for all groups.

**DISCUSSION**

Experiments evaluating the carcinogenic (2, 7) or promoting (11, 16) activity of sodium saccharin in rats have utilized high doses of the chemical administered p.o. for long periods of time. The appearance of calculi in the urine and/or foci of calcification in bladder lesions in some of the experiments (1, 2, 8, 11, 14, 16) has led to the hypothesis that the high doses of sodium saccharin result in changes in the characteristics of the urine, such as increased crystalluria or calculus formation, which cause the urothelial abnormalities. However, in the present study, neither increased crystalluria nor increased calculi were observed, but several changes were observed in the urine of rats fed sodium saccharin, most of which were expected on the basis of the administration of a high dose of the sodium salt of a weak acid accompanied by an increased p.o. intake of water. Our results differ from those reported by others in which calculi were found in the urine of rats given p.o. sodium saccharin (8). This may be due to a number of factors including the strain of rat or the source and method of synthesis of chemical. Sodium saccharin synthesized by the Remsen-Fahlberg method results in relatively large amounts (>600 ppm) of o-toluene sulfonamide as an impurity (2, 7, 8, 16). If synthesized by the Maumee procedure, as was the sodium saccharin used by us, o-toluensulfonamide is not present as an impurity (2, 7, 11). Also, the marked increase in water intake by the rats fed sodium saccharin in the present experiment resulted in an increased volume of slightly more dilute urine than in the controls, which may have acted to prevent the formation of calculi (15, 25).

The results of the present experiment indicate that calculi, crystalluria, and other parameters of the urine that were measured do not explain the hyperplastic (13) or promoting effect of sodium saccharin toward the urinary bladder mucosa. These results do not exclude the possibility that, when such factors such as calculi are present, they may enhance the effect induced by saccharin. Nevertheless, our results are consistent with the hypothesis that saccharin induces the bladder lesions by directly acting on the bladder epithelium rather than indirectly by altering the urine. Although other urinary parameters or other physiological changes in the rats may yet be found that cause bladder lesions following sodium saccharin administration, other experimental systems provide additional evidence for a direct effect. Sodium saccharin at comparatively high concentrations *in vitro* has promoting activity toward 10T½ cells (18), affects differentiation of 10T½ cells (19), induces genetic alterations in *Saccharomyces cerevisiae* in recombination assays (20), and inhibits metabolic cooperation between Chinese hamster V79 cells in culture (24), properties displayed by other chemicals with promoting activity in the mouse skin system.

Both tryptophan and saccharin have been shown previously to have hypoglycemic (21, 22) and hypoglycosuric effects, and decreases in serum and urinary glucose were found in the present experiment in rats receiving either chemicals. No mechanism has yet been demonstrated, although increased insulin levels or potentiation of the effects of insulin have been suggested. The relevance of these changes in the carcinogenic process, if any, is also unknown.

**Effects on Urine during Bladder Carcinogenesis**

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum glucose (mg/100 ml)</th>
<th>Serum blood urea nitrogen (mg/100 ml)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>69.2 ± 15.4</td>
<td>8.0 ± 2.5</td>
<td>0.001</td>
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<tr>
<td>2</td>
<td>57.0 ± 7.9</td>
<td>23.6 ± 4.1</td>
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</tr>
<tr>
<td>4</td>
<td>76.4 ± 8.4</td>
<td>21.2 ± 4.5</td>
<td>0.001</td>
</tr>
<tr>
<td>5</td>
<td>84.2 ± 7.5</td>
<td>18.0 ± 2.1</td>
<td>0.001</td>
</tr>
<tr>
<td>6</td>
<td>54.5 ± 7.2</td>
<td>19.3 ± 3.1</td>
<td>0.001</td>
</tr>
<tr>
<td>7</td>
<td>86.3 ± 17.4</td>
<td>22.8 ± 1.3</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*Mean ± S.D. of 5 rats. Calculated on the basis of Student’s t test compared to the serum glucose of Group 5. There was no significant difference between groups.*

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**Table 2**

Effects on Urine during Bladder Carcinogenesis

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*Mean ± S.D. of 5 rats. There was no significant difference between groups.*

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**Chart 4**

Total amount of glucose in the urine of rats of Group 1 and Group 4 was estimated by multiplying the concentration (mg/100 ml) by the volume (ml). The glucose of other rats fed sodium saccharin was similar to that of Group 1, glucose of rats fed FANFT or control diet (Groups 5 and 6) was similar to that of Group 4, and glucose of rats fed L-tryptophan (Groups 7 and 8) was between Groups 1 and 4.
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ACKNOWLEDGMENTS

Expert technical assistance was provided by Robert Spiewak and Cindy Maher, Dr. Thomas Barry and his associates in the Chemistry Laboratory, and Dr. Robert Bain and his associates in the Cytology Laboratory of St. Vincent Hospital. We thank Dr. Gilbert H. Friedell for his continuing advice and comments and James P. Theberge for translating articles in foreign languages.

REFERENCES

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