[CANCER RESEARCH 40, 734–736, March 1980[
0008-5472/80/0040-0000$02.00

Saccharin-induced Hyperplasia of the Rat Urinary Bladder[1]

Shoji Fukushima[2] and Samuel M. Cohen[3]

Department of Pathology, St. Vincent Hospital, Worcester, Massachusetts 01604

ABSTRACT

Dietary administration of sodium saccharin to male Fischer rats increased the rate of DNA synthesis of the urinary bladder epithelium as measured by tritiated thymidine uptake. A multifocal mild epithelial hyperplasia was induced, which was most readily detected by scanning electron microscopy, with pleomorphic microvilli present on many of the hyperplastic cells.

INTRODUCTION

The 2-stage process of carcinogenesis involving initiation and promotion was initially demonstrated in the murine skin cancer model (1, 2, 19). A similar process has subsequently been demonstrated in other tissues including the liver (16, 17, 21) and the urinary bladder. Evidence supporting the 2-stage process in bladder carcinogenesis was provided by Hicks *et al.* (8–10) using *N*-methyl-*N*-nitrosourea instilled intravesically in rats as the initiating agent followed by either sodium cyclamate p.o. or sodium saccharin p.o. as the promoting agent. Recently, we demonstrated that sodium saccharin and DL-tryptophan acted as promoting agents following initiation by *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide p.o. in male Fischer rats (6). Tryptophan was also shown to have promoting activity for the bladder in mice (13) and dogs (18) following initiation by *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide or aromatic amines, respectively, and sodium saccharin has shown promoting activity in an *in vitro* assay system using fibroblasts (15). A property of promoting chemicals in various model systems has been their ability to induce hyperplasia in the target organ even without prior initiation (2, 19). Although sodium saccharin induces bladder tumors when administered p.o. to rats (5), especially if a 2-generation experiment is performed, the incidence is low, and few animals show a hyperplastic effect in the bladder. However, these studies have relied on light microscopic examination of the bladder. Utilizing the more sensitive techniques of autoradiography and scanning electron microscopy, we have examined the bladders of rats fed sodium saccharin for up to 18 weeks for evidence of hyperplasia.

MATERIALS AND METHODS

Sodium saccharin (Sigma Chemical Co., St. Louis, Mo.) synthesized by the Maumee procedure was fed to 6-week-old male F344 rats (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) as 5% of the powdered diet (Charles River rat chow). Food and water were available *ad libitum* during the experiment. Control rats received the same basal diet but without sodium saccharin. Three sodium saccharin-fed rats were killed after each of Weeks 1, 3, 5, 7, 9, 12, 15, and 18 from the beginning of the experiment, and 3 control rats were killed after each of Weeks 0 and 18. The bladders were inflated with 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) and divided in half longitudinally. One half was processed for light microscopy, and the other half was processed for scanning electron microscopy (JEOL JSM 35U scanning electron microscope) as previously described (11, 12). For autoradiography, 3 additional rats from each group were used after each of Weeks 1, 5, 9, and 18 of the experiment. Each rat received a single i.p. injection of [*methyl*-^{3}H]thymidine (New England Nuclear; 20 Ci/mmol) at a dose of 1 μCi/g body weight 1 hr before sacrifice. All rats were killed at the same time of day to avoid variations in the mitotic rate due to the circadian rhythm of the rat bladder (22). Five-μm sections of the bladder were mounted on glass slides, covered with Kodak NT-B2 emulsion, and exposed for 1 week. Sections for light microscopy and autoradiography were stained with hematoxylin and eosin. Several serial sections were examined.

RESULTS

The rats receiving sodium saccharin showed no evidence of toxicity although they grew at a slightly slower rate than did the controls.

Macroscopically, the urinary bladder showed no abnormalities on the luminal surface through the 18 weeks of the experiment, and no calculi were observed in the urine. No evidence of bladder parasites was observed. By light microscopy, vacuolar degeneration of the epithelial cells was observed after 3 weeks. At 5 weeks, mild simple hyperplasia appeared focally, the mucosa being 4 to 5 cells thick. By 9 weeks, it increased to 5 to 7 cells thick (Figs. 1 and 2) with mitotic figures present. These changes were present in the 3 rats examined at each of these times and were present to the same extent through Week 18. Papillary or nodular hyperplasia was not observed.

By scanning electron microscopy, occasional superficial cells were in the process of exfoliation after 1 and 3 weeks as evidenced by protrusion from the luminal surface. After 5 weeks, the bladders had focal small irregularly shaped flat mucosal lesions as seen by scanning electron microscopy. These appeared to represent areas in which several superficial cells had exfoliated, and the underlying layers of cells were exposed. The cells in these areas had their luminal surfaces covered with ropy rounded microridges rather than the peaked microridges seen on the luminal surface of normal bladders. Some cells had short uniform microvilli which are also seen on normal intermediate cells. After 9 weeks, cells on the mucosal surface displayed greater variability in size and shape. Small irregularly shaped foci with a slight degree of elevation of cells, giving the mucosa a cobblestone appearance, were observed

[1] This work was supported in part by USPHS Grant CA15945 from the National Cancer Institute through the National Bladder Cancer Project.

[2] Present address: First Department of Pathology, Nagoya City University Medical School, Nagoya 467, Japan.

[3] To whom requests for reprints should be addressed.

Received November 1, 1979; accepted December 10, 1979.

Table 1

Labeling index of urinary bladder epithelium in rats fed 5% sodium saccharin

Duration (wk)	Saccharin	Control
1	0.36 ± 0.18[a, b]	0.05 ± 0.02
5	0.28 ± 0.12[b]	0.06 ± 0.04
9	0.42 ± 0.20[b]	0.05 ± 0.03
18	0.41 ± 0.24[c]	0.04 ± 0.03

[a] Mean ± S.D. of labeled cells/100 cells.
[b] $p < 0.05$, saccharin *versus* control.
[c] $p < 0.06$, saccharin *versus* control.

(Fig. 3). Superficial cells in these foci were somewhat round, rather than polygonal like normal superficial cells, and smaller in size. Most of the cells in these foci were covered with short uniform microvilli and with ropy rounded microridges (Fig. 4). Some degenerated cells were in the process of exfoliation. In addition, several small round cells having pleomorphic microvilli on their luminal surface were observed in these foci (Fig. 5). Hyperplastic foci were observed in all rats fed sodium saccharin after each of Weeks 9, 12, 15, and 18, and they did not appear to increase in number or extent during those 9 weeks. Pleomorphic microvilli were observed in 2 of the 3 rats after each of these 3 periods. We interpret these lesions as areas of hyperplasia following damage to the mucosa with exfoliation of superficial cells. The control rats showed no abnormalities by light or scanning electron microscopy.

Autoradiographs were evaluated 1, 5, 9, and 18 weeks after feeding of saccharin began, and at all times, increased thymidine uptake in the mucosa was present compared to the control group (Table 1). The multifocal nature of the lesions demonstrated by light and scanning electron microscopy was also evident by autoradiography. The labeled cells were clustered in multiple small foci, occasionally 2 or 3 labeled nuclei in a high-power microscopic field (Fig. 2), a rare event in the mitotically quiescent normal bladder (22). Most of the labeled nuclei were observed in basal cells. Superficial cells were rarely labeled.

DISCUSSION

These data demonstrate that sodium saccharin induces a hyperplastic response in the rat urinary bladder, the target organ for its promoting activity. Although the ability to induce hyperplasia appears to be a necessary property of tumor promoters, it is not sufficient to explain the entire promotion process since various agents are known which induce hyperplasia but do not act as promoting agents (2, 19, 23). For sodium saccharin, the hyperplasia induced in the bladder is multifocal but slight, requiring sensitive techniques to detect. The demonstration of pleomorphic microvilli following sodium saccharin feeding suggests that more than simple regenerative hyperplasia has occurred since pleomorphic microvilli are seen in hyperplastic lesions induced by carcinogens (7, 11, 12, 20). The mechanism through which sodium saccharin causes bladder epithelial hyperplasia is unkown. Sodium saccharin does not appear to be metabolized (4) with 70% excreted unchanged in the urine and the remainder in the feces (14), nor does it appear to be mutagenic (5).

In our previous study with sodium saccharin (6), the bladder appeared normal as observed by scanning electron microscopy after 6 weeks of feeding, and although occasional blebs on cells were observed at the end of 2 years, the hyperplastic changes observed in the present experiment were not seen. This difference might be due to the younger age at which feeding of sodium saccharin was begun in the present study (6 *versus* 11 weeks of age), but it is more probably due to the discontinuance of sodium saccharin administration 21 weeks before the end of the previous study, sufficient time for the slight hyperplasia induced by sodium saccharin to regress. Reversibility is typical of the hyperplasia induced by promoting agents when administered without prior initiation (1, 2, 19).

The results of the present experiment provide additional evidence that sodium saccharin has properties consistent with a promoter for rat urinary bladder carcinogenesis (3). However, the possibility that it also has weak initiating activity and therefore weak complete carcinogenic activity still cannot be excluded.

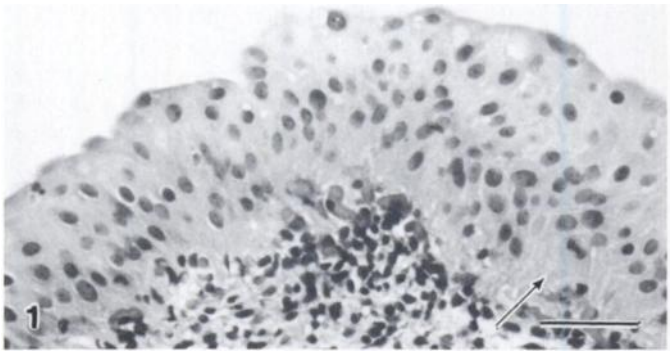
ACKNOWLEDGMENTS

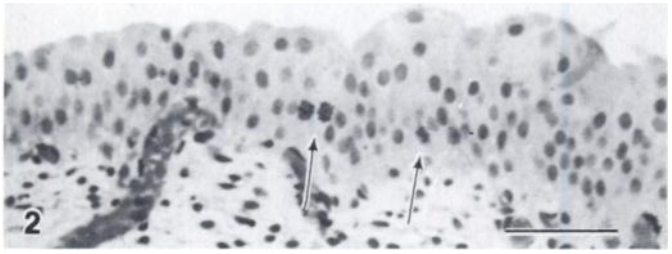
We thank Dr. Gilbert H. Friedell and Dr. Jerome B. Jacobs for their advice and comments and Denise Demers and Margaret St. John for their technical assistance.

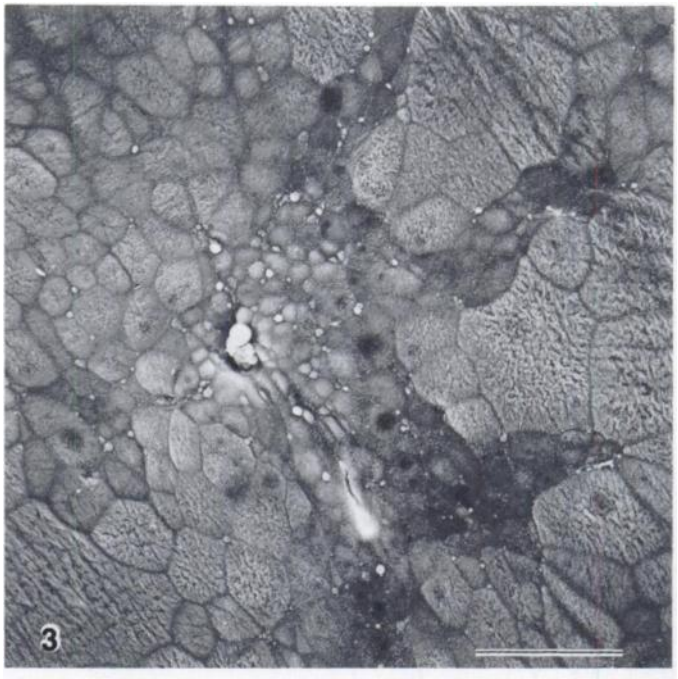
REFERENCES

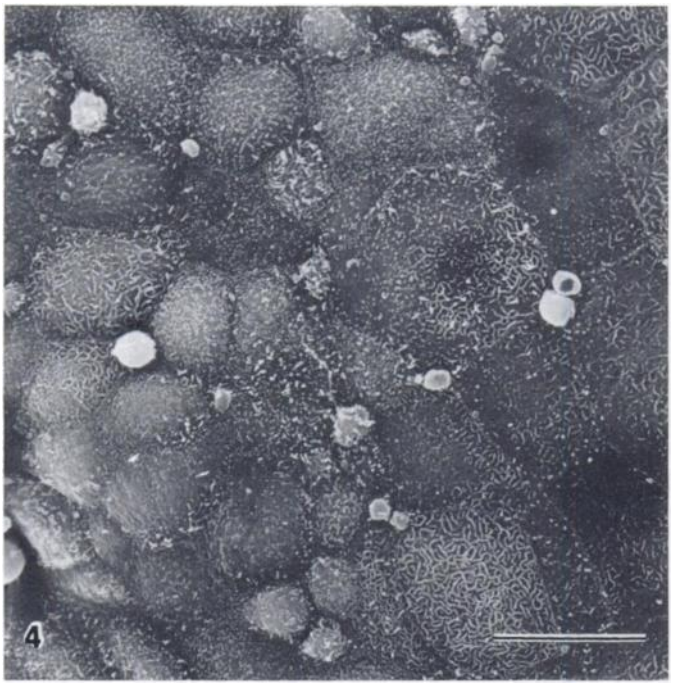
1. Berenblum, I., and Shubik, P. A new quantitative approach to the study of stages of chemical carcinogenesis in the mouse's skin. Br. J. Cancer, *1*: 383–391, 1947.
2. Boutwell, R. K. Some biological aspects of skin carcinogenesis. Prog. Exp. Tumor Res., *4*: 207–250, 1964.
3. Boyland, E. Saccharin from carcinogen to promoter. Nature (Lond.), *278*: 123–124, 1979.
4. Byard, J. L., and Goldberg, L. The metabolism of saccharin in laboratory animals. Food Cosmet. Toxicol., *11*: 391–402, 1973.
5. Cancer Testing Technology and Saccharin, Office of Technology Assessment. Washington, D. C.: United States Government Printing Office, 1977.
6. Cohen, S. M., Arai, M., Jacobs, J. B., and Friedell, G. H. Promoting effect of saccharin and DL-tryptophan in urinary bladder carcinogenesis. Cancer Res., *39*: 1207–1217, 1979.
7. Friedell, G. H., Jacobs, J. B., Nagy, G. K., and Cohen, S. M. The pathogenesis of bladder cancer. Am. J. Pathol., *89*: 431–442, 1977.
8. Hicks, R. M., and Chowaniec, J. The importance of synergy between weak carcinogens in the induction of bladder cancer in experimental animals and humans. Cancer Res., *37*: 2943–2949, 1977.
9. Hicks, R. M., Wakefield, J. S. J., and Chowaniec, J. Co-carcinogenic action of saccharin in the chemical induction of bladder cancer. Nature (Lond.), *243*: 347–349, 1973.
10. Hicks, R. M., Wakefield, J. S. J., and Chowaniec, J. Evaluation of a new model to detect bladder carcinogens or co-carcinogens: results obtained with saccharin, cyclamate and cyclophosphamide. Chem.-Biol. Interact., *11*: 225–233, 1975.
11. Jacobs, J. B., Arai, M., Cohen, S. M., and Friedell, G. H. Early lesions in experimental bladder cancer: scanning electron microscopy of cell surface markers. Cancer Res., *36*: 2512–2517, 1976.
12. Jacobs, J. B., Arai, M., Cohen, S. M., and Friedell, G. H. A long-term study of reversible and progressive urinary bladder cancer lesions in rats fed *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide. Cancer Res., *37*: 2817–2821, 1977.
13. Matsushima, M. The role of the promoter L-tryptophan on tumorigenesis in the urinary bladder. 2. Urinary bladder carcinogenicity of FANFT (initiating factor) and L-tryptophan (promoting factor) in mice. Jpn. J. Urol., *68*: 731–736, 1977.
14. Minegishi, K-I., Asahina, M., and Yamaha, T. The metabolism of saccharin and related compounds in rats and guinea pigs. Chem. Pharm. Bull. (Tokyo), *20*: 1351–1356, 1972.
15. Mondal, S., Brankow, D. W., and Heidelberger, C. Enhancement of oncogenesis in C3H/10T ½ mouse embryo cell cultures by saccharin. Science (Wash. D. C.), *201*: 1141–1142, 1978.
16. Peraino, C., Fry, R. J. M., and Staffeldt, E. Reduction and enhancement by phenobarbital of hepatocarcinogenesis induced in the rats by 2-acetylaminofluorene. Cancer Res., *31*: 1506–1512, 1971.
17. Peraino, C., Fry, R. J. M., Staffeldt, E., and Kisieleski, W. E. Effects of varying the exposure to phenobarbital on its enhancement of 2-acetylaminofluorene-induced hepatic tumorigenesis in the rat. Cancer Res., *33*: 2701–2705, 1973.

18. Radomski, J. L., Radomski, T., and MacDonald, W. E. Carcinogenic interaction between D, L-tryptophan and 4-aminobiphenyl or 2-naphthylamine in dogs. J. Natl. Cancer Inst., *58*: 1831–1834, 1977.
19. Scribner, J. D., and Suss, R. Tumor initiation and promotion. Int. Rev. Exp. Pathol., *18*: 137–198, 1978.
20. Shirai, T., Murasaki, G., Tatematsu, M., Tsuda, H., Fukushima, S., and Ito, N. Early surface changes of the urinary bladder epithelium of different animal species induced by *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine. Gann, *68*: 203–212, 1977.
21. Sirica, A. E., Barsness, L., Goldsworthy, T., and Pitot, H. C. Definition of stages during hepatocarcinogenesis in the rat: potential application to the evaluation of initiating and promoting agents in the environment. J. Environ. Pathol. Toxicol., *2*: 21–28, 1978.
22. Tiltman, A. J., and Friedell, G. H. Effect of feeding *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide on mitotic activity of rat urinary-bladder epithelium. J. Natl. Cancer Inst., *48*: 125–129, 1972.
23. Weinstein, I. B., and Troll, W. National Cancer Institute Workshop on Tumor Promotion and Cofactors in Carcinogenesis. Cancer Res., *37*: 3461–3462, 1977.









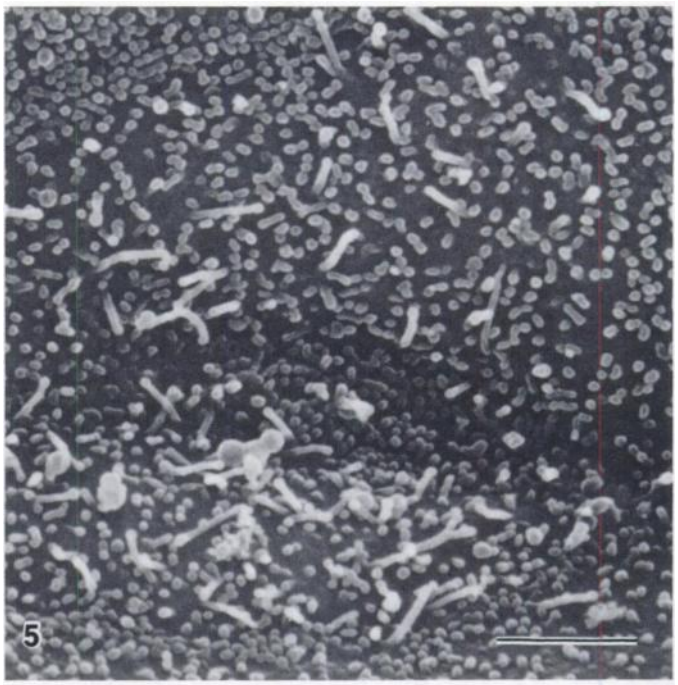


Fig. 1. Simple hyperplasia of the bladder epithelium after 18 weeks of saccharin feeding. *Arrow*, a mitotic figure in a basal cell; *bar*, 50 μm. H & E.

Fig. 2. Labeled cells (*arrow*) in the hyperplastic epithelium of the bladder after 18 weeks of saccharin feeding. *Bar*, 50 μm. H & E.

Fig. 3. Small irregularly shaped lesion with a cobblestone appearance on the luminal surface of the bladder after 9 weeks of saccharin feeding. *Bar*, 50 μm.

Fig. 4. Higher magnification of Fig. 3. Pleomorphic microvilli, short uniform microvilli, and ropy rounded microridges are observed on the luminal surface. *Bar*, 10 μm.

Fig. 5. Pleomorphic microvilli on the luminal surface of the bladder after 9 weeks of saccharin feeding. *Bar*, 2 μm.

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