ARTICLES

Long-term Feeding of Sodium Saccharin to Nonhuman Primates: Implications for Urinary Tract Cancer

*S. Takayama, S. M. Sieber, R. H. Adamson, U. P. Thorgeirsson, D. W. Dalgard, L. L. Arnold, M. Cano, S. Eklund, S. M. Cohen**

Background: **It was observed in the early 1970s that saccharin produced bladder cancer in rats. However, it has been unclear whether sodium saccharin when consumed by humans poses a substantial carcinogenic hazard. Numerous epidemiologic studies have not shown any evidence of increased urothelial proliferation associated with ingestion of sodium saccharin.** ***Purpose:*** **Our purpose was to determine the effects of long-term feeding of sodium saccharin to three species of nonhuman primates.** ***Methods:*** **Twenty monkeys of three species (six African green, seven rhesus, six cynomolgus, and one hybrid [of rhesus male and cynomolgus female parentage]) were treated with sodium saccharin (25 mg in the diet/kg body weight daily for 5 days a week) beginning within 24 hours after birth and continuing for up to 24 years. Sixteen monkeys (seven rhesus and nine cynomolgus) served as controls. During their last 2 years of life, urine was collected from selected treated and control animals and evaluated for various urinary chemistries and for the presence of calculi, microcrystalluria, and precipitate. Urinary bladders were examined by light microscopy and by scanning electron microscopy.** ***Results:*** **Sodium saccharin treatment had no effect on the urine or urothelium in any of these monkeys. There was no evidence of increased urothelial cell proliferation, and there was no evidence of formation of solid material in the urine.** ***Conclusion:*** **Although the dose of sodium saccharin administered to these monkeys was only five to 10 times the allowable daily intake for humans, the results provide additional evidence that sodium saccharin is without a carcinogenic effect on the primate urinary tract. [J Natl Cancer Inst 1998;90:19–25]**

Several studies on the carcinogenicity of sodium saccharin in rodents have been reported, beginning in 1970 with the report by Bryan et al. *(1)* that sodium saccharin increased the incidence of bladder tumors in mice treated by direct bladder implantation of sodium saccharin in a cholesterol pellet. However, this method has been questioned with respect to interpretation, and the result may be related directly to the effects of the pellet rather than to the chemical within the pellet *(2)*. The traditional single-generation 2-year bioassay in which high doses of sodium saccharin are used in the diet generally has been negative for increased incidence of bladder tumors in rats *(3–5)*, except for one report *(4)*. However, in two-generation experiments, sodium saccharin produced a statistically significant increase in bladder tumors in F_1-generation rats, and the effect was greater in males than in females *(5)*. Initiating administration at birth rather than before gestation also led to a comparable increased incidence of bladder tumors when sodium saccharin was administered in the diet for the remainder of the offsprings' lives *(6)*. On the basis of the observation that sodium saccharin increased the incidence of bladder tumors in rats, the U.S. Food and Drug Administration published a proposal to ban the food use as well as other use of this compound in 1977, but this proposal was overturned by a moratorium passed by the U.S. Congress; this moratorium has been renewed several times subsequently *(7–10)*. However, sodium saccharin use was banned in Canada.

In contrast to the weak tumorigenicity of sodium saccharin found in traditional bioassays evaluating a single agent, sodium saccharin demonstrated relatively strong cocarcinogenic action for the rat urinary bladder, either after concurrent administration with *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (FANFT) *(11)* or after initial administration with a strong bladder carcinogen, such as FANFT, *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN), *N*-methyl-*N*-nitrosourea (MNU), or cyclophosphamide, or after freeze ulceration *(5)*. Except for the experiments with MNU *(5)*, these studies were generally performed in male rats.

In contrast to the observations in rats, sodium saccharin administered orally in the diet, either alone or following preadministration with 2-acetylaminoflourene, had no effect in mice *(5,12)*. Also, administration of sodium saccharin to hamsters or guinea pigs had no effect *(5)*.

In addition to its tumorigenic effects in the rat, sodium saccharin was reported to produce a mild, simple hyperplastic re-

**Affiliations of authors:* S. Takayama, S. M. Sieber, R. H. Adamson, U. P. Thorgeirsson, Division of Basic Sciences, National Cancer Institute, Bethesda, MD; D. W. Dalgard, Covance Laboratories, Inc., Vienna, VA; L. L. Arnold, M. Cano, S. Eklund, S. M. Cohen, Department of Pathology and Microbiology and the Eppley Institute, University of Nebraska Medical Center, Omaha.

Correspondence to: U. P. Thorgeirsson, M.D., National Institutes of Health, Bldg. 37, Rm. 2D-02, Bethesda, MD 20892.

See "Notes" following "References."

sponse within a relatively short period when it was administered at high doses (≥2.5%) in the diet *(5)*. Hyperplasia was found to occur regardless of whether the chemical was administered in a two-generation protocol *(13)* or beginning after weaning in a traditional one-generation protocol *(14)*. The hyperplastic response was considered necessary for eventual development of bladder epithelial tumors *(15)*. Evidence *(16)* suggests that the hyperplasia occurs secondary to mild cytotoxicity of the superficial layers of the bladder epithelium from formation of a cytotoxic, calcium phosphate-containing precipitate in the urine after administration of high doses of sodium saccharin or other similar sodium salts, such as sodium ascorbate, sodium citrate, and sodium chloride.

Because of saccharin consumption by humans, an experiment was begun in nonhuman primates in 1970 to evaluate possible urinary bladder or other effects; preliminary findings have been published *(17,18)*. The nonhuman primates used in that experiment received the compound for 79 months; however, no abnormal pathologic findings were observed in the urinary bladder, kidneys, or testis *(19,20)*. Some of these animals were continued on the sodium saccharin until 1995.

The purpose of this study was to determine the effects of long-term feeding of sodium saccharin to nonhuman primates. Preliminary data have previously been presented by Sieber and Adamson in 1978 *(20)*, by Thorgeirsson et al. in 1994 *(21)*, and by Cohen et al. in 1996 *(22)*.

Materials and Methods

Monkeys

Monkeys were selected at the time of inception of this study in 1970 from those available in a monkey colony consisting of three species: *Macaca fascicularis* (cynomolgus), *Macaca mulatta* (rhesus), and *Cercopithecus aethiops* (African green). One hybrid monkey bred by the mating of a rhesus male with a cynomolgus female was also used in the study. Details of the maintenance and management procedures and the method used to rear neonates have been described elsewhere *(17,20)*.

The monkeys were cared for according to the standards established by the Association for Assessment and Accreditation for Laboratory Animal Care (AAALAC). The experimental protocols used were approved by the Animal Sciences Branch of the National Cancer Institute and reviewed on an annual basis. The animals were given a diet consisting of high-protein Purina monkey chow (5045 Standard), with a vitamin spread on sandwiches and apples. Euthanasia was performed by immobilization with ketamine hydrochloride (15 mg/kg, intramuscular), followed by sodium thiamylal (40 mg/kg, intravenous). The autopsies were performed immediately following the euthanasia.

Chemical and Treatment

Sodium saccharin (>99% purity) was purchased from Fisher Scientific Company (Fairlawn, NJ) and was used as obtained without further purification. Sodium saccharin was administered orally at a level of 25 mg/kg for 5 days a week. This dose represents 10 times the allowable daily intake for humans as determined by various agencies, e.g., JECFA (Joint Food and Agricultural Organization/World Health Organization Expert Committee on Food Additives). Use of 10 times the allowable daily intake was the standard choice of dose for the National Cancer Institute's nonhuman primate carcinogenicity testing program at the time of inception of this study *(21)*. For newborn monkeys, sodium saccharin was added to the Similac formula at the time of feeding. When the monkeys were 6 months old, the compound was incorporated into a vitamin mixture that was given to the monkeys as a vitamin sandwich on a slice of bread. The mixture consisted of powdered dry milk (5 pounds), Parvo (a folic acid supplement, 4 ounces, 20% with starch; Roche Agricultural Products), Cecon (a vitamin C supplement, 300 mL; Abbott Laboratories, Chicago, IL), molasses (2 L), and water (500 mL). The vitamin mixture was spread onto bread (1 teaspoon per slice of bread), after which the dose of sodium saccharin was added on top of the spread and the bread was folded in half to form a sandwich and then fed to the animal. Sodium saccharin administration was initiated within 24 hours after birth and was continued until the animals died or were euthanized at the end of the study. The sexes of the animals are listed in Table 1.

Experimental Design

A total of 20 monkeys were treated with sodium saccharin. They included six African green, seven rhesus, six cynomolgus, and one hybrid rhesus male × cynomolgus female monkeys (Table 1).

Control, untreated breeder and vehicle-treated monkeys, ranging in age from 206 to 301 months, that were contemporary with the sodium saccharin-treated monkeys were used for comparison. Monkeys that died at earlier ages were not included as controls for this comparison. The controls consisted of seven rhesus (five males and two females) and nine cynomolgus (five males and four females) monkeys. The specific strains and sexes of the animals used are listed in Table 2. These control, aged monkeys were included to determine whether the observed changes were in fact age dependent.

The individual monkeys underwent complete physical examinations by a veterinarian every 6 months. In addition, the following routine blood examinations were performed at intervals of 3–6 months: hematocrit, hemoglobin, white blood cell count, platelet count, and other clinical parameters (i.e., alkaline phosphatase, total bilirubin, serum glutamic pyruvic transaminase, and serum glutamic oxalacetic transaminase).

Tissue Collection, Processing, and Evaluation

Monkeys that died or were euthanized were carefully necropsied. The following tissues and organs were fixed in buffered formalin: brain, pituitary, salivary gland, thyroid, tongue, cheek pouches, trachea, esophagus, lungs, heart, aorta, liver, gallbladder, spleen, kidneys, adrenals, stomach, pancreas, duodenum, jejunum, ileum, large intestine, lymph nodes, urinary bladder, testis, prostate, seminal vesicles (or ovaries and uterus), breast, skin, and bone marrow, as well as any grossly apparent tumor tissue. Tissue sections were routinely processed for paraffin embedding and stained with hematoxylin–eosin. Animals remaining for the terminal euthanasia were put under deep anesthesia, and their urinary bladders were inflated *in situ* by injection of 25 mL phosphate-buffered glutaraldehyde into the bladder to distend it for examination by both light microscopy and scanning electron microscopy *(23–25)*. The animals on which these procedures were performed are indicated in Tables 1 and 2.

Urine Collection and Analysis

One to 2 years before the animals were euthanized, urine samples were collected from two male and two female monkeys that had been treated with sodium saccharin and from two male and two female controls of the cynomolgus and the rhesus species. Collection was from 8 AM to 10 AM. Urinary pH was determined by use of the Beckman combination electrode (Beckman Instruments, Inc., Fullerton, CA), and urine chemistries were determined on an Ektachem 700 Chemistry Analyzer (Eastman Kodak Co., Rochester, NY), except for chloride determinations, which were measured on an Astra 4 Automated Analyzer (Beckman Instruments, Inc.), and protein determinations, which were measured by use of the Bradford protein assay (BioRad Laboratories, Richmond, CA). The urine samples were examined for solid material by means of scanning electron microscopy, using previously described methods *(16)*.

A sample of fresh-voided control urine was also collected and examined by use of chromatographic procedures detailed elsewhere *(24)* for the potential of sodium saccharin to associate (coelute) with urinary macromolecules. Urinary filters were examined by means of scanning electron microscopy (Phillips 515 Scanning Electron Microscope; Phillips, Inc., Eindhoven, The Netherlands) with attached energy dispersive spectroscopy (Kevex Micro-X 7000 Analytical Spectrometer with Quant-X Program; Kevex, Inc., Hayward, CA).

Results

Twenty monkeys underwent long-term treatment with sodium saccharin. These monkeys were arbitrarily divided into two groups: Group 1 consisted of eight monkeys that died during the course of the experiment between 103 and 282 months after the initiation of sodium saccharin administration. Group 2 consisted of 12 monkeys that did not show toxic effects and were

Table 1. Tumors and other lesions in monkeys treated with sodium saccharin

Animal No.	Species*	Sex†	Age at first dose	Month dosed	Total dose, g	Major autopsy findings
Group 1: monkeys that died during the course of the experiment from non-neoplastic diseases						
827K	Rh	M	2 days	103	267.8	Bronchial aspiration
1206S	Cy	F	2 days	128	229.9	Bronchopneumonia; ulcer of the esophagus and stomach
1204S	Rh	M	1 week	157	457.5	Congestion of the lung, spleen, and kidney; edema of the lung
826K	Gr	F	2 days	168	299.7	Septic hepatitis
821J	Gr	F	Birth	170	307.7	Septic hepatitis
1214S‡	Gr	M	Birth	192	467.1	Myocardial degeneration; atelectasis of the lung; congestion of the liver, lung, spleen, and kidney; hypoplastic bone marrow; atrophy of the thyroid; cholelithiasis
1207S	Rh	M	Birth	214	721.0	Myocardial fibrosis; congestion of the liver and lung; renal tubular necrosis; kyphosis
828K‡	Gr	F	1 day	282	473.6	Chronic ileitis
Group 2: monkeys euthanized after >207 months in the experiment						
1213S	Cy	F	Birth	207	318.7	Fatty infiltration of the liver; colloid cyst of the thyroid; endometriosis; chronic ulcer of the rectum; hydronephrosis of the kidney
1215S‡	Cy	M	1 day	213	803.0	Myocardial fatty infiltration; fatty infiltration of the liver; atrophy of the testis
1205S‡	Hybrid	F	3 days	214	598.9	Fatty degeneration of the liver; myocardial fibrosis and fatty degeneration
1209S‡	Rh	F	1 day	214	633.1	Ductal hyperplasia of the pancreas
1211S‡	Cy	M	1 day	214	658.3	Myocardial fibrosis and fatty degeneration; fatty infiltration of the liver; hyalinization of the pancreatic islet cells; atrophy of the testis
1212S‡	Cy	F	1 day	214	461.2	Myocardial fatty degeneration; fatty infiltration of the liver; hyalinization of the pancreatic islet cells
829K‡	Gr	F	Birth	281	515.6	Leiomyoma of the uterus; diverticulosis of the colon
820J‡	Rh	F	Birth	282	1021.5	Congestion and fatty degeneration of the liver; ovarian cyst
823J‡	Rh	M	Birth	282	1136.2	Fatty degeneration of the liver; colloid cyst of the thyroid
824K‡	Rh	M	Birth	282	1054.3	Lymphoma of thyroid; cyst of the pituitary gland
825K‡	Gr	F	10 days	282	455.4	Papillary cystadenoma of the ovary; leiomyoma of the stomach
818J	Cy	M	1 day	283	970.1	Myocardial fibrosis; liver cyst

*Rh = rhesus (*Macaca mulatta*); Cy = cynomolgus (*Macaca fascicularis*); Gr = African green (*Cercopithecus aethiops*).
†M = male; F = female.
‡Bladder observed by scanning electron microscopy.

Table 2. Major autopsy findings in control monkeys

Animal No.	Species*	Sex†	Euthanized or died‡	Observation, mo	Major histologic findings
1234T§	Cy	F	E	206	Myocardial degeneration; hyalinization of the pancreatic islet cells
1188S§	Cy	M	E	217	Atrophy, testis
1156R§	Rh	F	E	224	
947M	Rh	M	E	234	
944M	Rh	M	E	237	Fatty infiltration of the liver; hemorrhage and edema of the lung; inguinal hernia
987M	Cy	F	E	240	Hypertrophy of the left kidney; absence of the right kidney; endometriosis; oil injury of the right eye
1017N	Cy	M	E	244	Fibrosis, papillary muscle, left ventricle
1041N§	Cy	M	E	248	Atrophy of the testis; fatty infiltration of the liver; hyalinization of the pancreatic islet cells; cystic dilation of the prostate glands
989M§	Rh	M	E	257	Nodular cortical hyperplasia of the left adrenal
6971	Rh	M	E	261	Diverticulitis; emphysema and interstitial fibrosis of the lung
899L	Cy	M	E	268	Chronic bronchitis
901L§	Cy	F	E	277	Proliferation, follicular epithelial cell of the thyroid
778J	Rh	M	D	284	
780J	Cy	M	D	285	Myocardial fibrosis; hyalinization of the pancreatic islet cells
779J	Rh	F	E	299	Fatty infiltration, clear cell foci of the liver; chronic gastritis; cholecystitis; adenomyosis of the uterus
678H	Cy	F	E	301	Fatty infiltration of the liver; hyalinization of the pancreatic islet cells; endometriosis; splenomegaly; hydronephrosis of the right kidney

*Rh = rhesus (*Macaca mulatta*); Cy = cynomolgus (*Macaca fascicularis*); Gr = African green (*Cercopithecus aethiops*).
†M = male; F = female.
‡E = euthanized; D = died.
§Urinary bladder examined by scanning electron microscopy.

euthanized between 207 and 283 months after administration of the compound had begun (terminal sacrifice).

The major necropsy findings for the eight monkeys in group 1 are summarized in the top one third of Table 1. Total doses of sodium saccharin consumed by these monkeys averaged 403.0 g and ranged from 229.9 to 721.0 g. Two African green monkeys had septic hepatitis. The most frequent histopathologic findings at necropsy of these monkeys involved the respiratory system. Five of these monkeys were found at necropsy to have lung infection, edema, congestion, or atelectasis. In one monkey, acute bronchial aspiration and pneumonia were present. In two monkeys, there was myocardial fibrosis associated with myocardial fatty degeneration. One monkey had chronic ulcers of the stomach and esophagus, and another monkey had histologically observed chronic ileitis. None of these monkeys had abnormalities of the urothelium, including the renal pelvis, ureters, urinary bladder, or urethra.

In group 2, the total dose of sodium saccharin consumed averaged 718.9 g and ranged from 455.4 to 1136.2 g. When the 12 remaining saccharin-treated monkeys were euthanized in 1995, three of them had gross evidence of tumors. Histologic examination revealed a thyroid lymphoma in the first monkey, leiomyoma of the uterus in the second monkey, and a papillary cystadenoma of the ovary and leiomyoma of the stomach in the third monkey. Other histopathologic findings in the monkeys in group 2 are summarized in the bottom two thirds of Table 2. In addition to the tumors, three of the 12 monkeys had myocardial fibrosis and three had myocardial fatty degeneration. In seven of these 12 monkeys, fatty degeneration of the liver was noted, and one animal had a liver cyst. Light microscopy showed no evidence in any of these monkeys of urothelial changes, i.e., in the renal pelvis, the ureters, the urinary bladder (Fig. 1), or the urethra.

The lesions observed in age-matched control monkeys are listed in Table 2. In general, these monkeys showed myocardial and hepatocellular changes similar to those observed in the sodium saccharin-treated animals, but no definite tumors were seen.

It should be noted that the three types of tumors found in the saccharin-treated monkeys have also been observed in breeders and normal controls in this monkey colony *(21)*. Among 373 autopsy records reviewed, one case of leiomyoma of the uterus, one case of papillary cystadenoma of the ovary, and three cases of lymphomas were found in breeders and normal controls *(21)*.

No abnormalities were seen in the urinary bladders of the 12 sodium saccharin-treated monkeys (e.g., Fig. 2) and the six control monkeys examined by scanning electron microscopy following glutaraldehyde fixation. The animals whose bladders were examined by scanning electron microscopy are indicated in Tables 1 and 2. The bladder mucosa was lined with the typical superficial cells of mammalian urothelium, consisting of large polygonal cells with a microridge system on their surface *(26)*.

For a few of the monkeys that were euthanized, the bladders were removed and remained on the autopsy table for 15–20 minutes before they were placed in formalin for fixation. They were not inflated *in situ*, and they were not opened for fixation in the formalin until after they had been fixed in formalin for greater than 24 hours. By light microscopy, these bladders all appeared normal. However, by the more sensitive technique of scanning electron microscopy, the surface of these bladders (whether from control or treated animals) showed changes (e.g., Fig. 3) that were identical to those seen in normal rat bladder following autolysis secondary to lack of immediate fixation *in situ*. These autolytic changes occur literally within minutes (5–15 minutes) of the death of the animal; thus, inflation of the bladder with glutaraldehyde (a proper electron microscopic fixative) is necessary for the proper evaluation of the bladder urothelial surface. Besides the autolytic changes, no alteration of the bladder surface was evident in any of the animals. In summary, by light microscopy and by scanning electron microscopy, the urothelium of the bladders of these sodium saccharin-treated monkeys was normal.

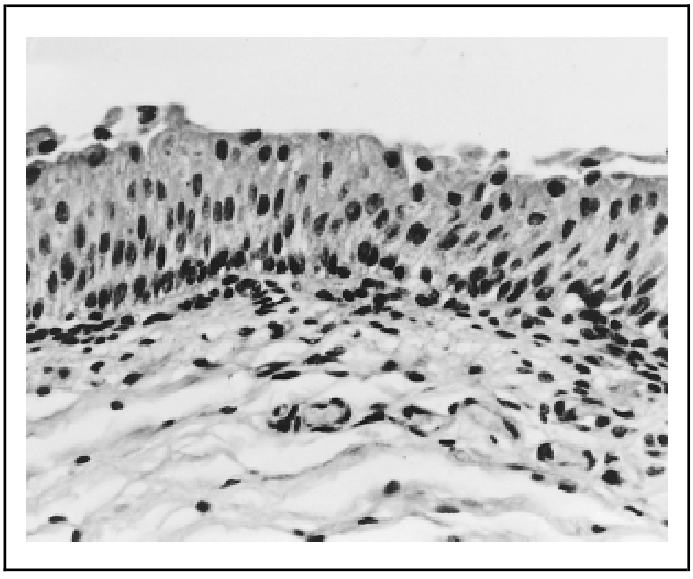

Fig. 1. Light microscopy of the urinary bladder of a monkey (monkey 1212S) fed sodium saccharin. A similar appearance was noted for all monkeys (treated and controls) in the study.

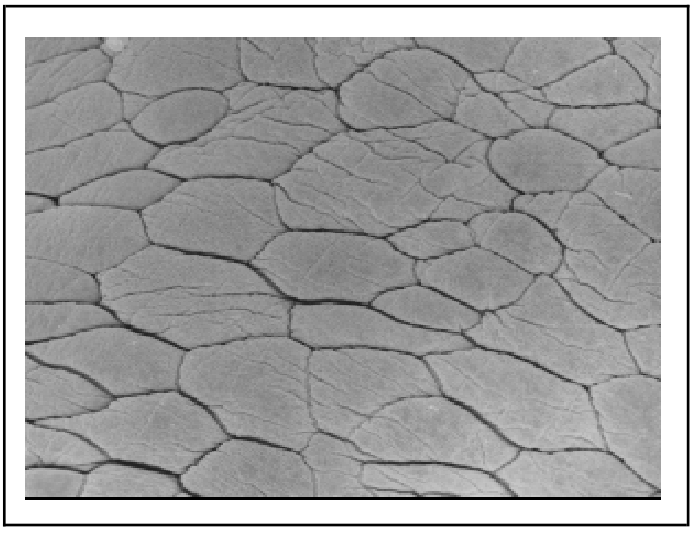

Fig. 2. Scanning electron micrograph of the surface of the urinary bladder of a monkey (monkey 1211S) administered sodium saccharin. The bladder was inflated *in situ* with phosphate-buffered glutaraldehyde while the animal was under deep anesthesia but not yet dead. A similar appearance was observed in the urinary bladders of control monkeys processed the same way (original magnification ×810).

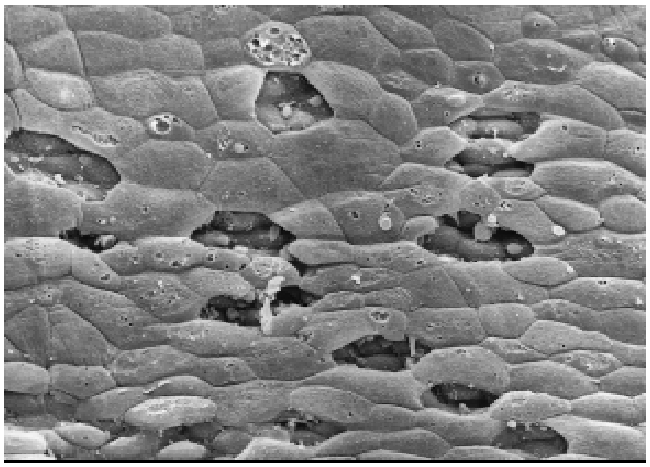

Fig. 3. Scanning electron micrograph of the surface of the urinary bladder of a monkey (monkey 823J) administered sodium saccharin. Changes observed are due to autolytic changes occurring within minutes after euthanasia. The bladder was removed and remained on the autopsy table for 15–20 minutes before being placed in buffered formalin. Similar changes occurred in the urinary bladders of other saccharin-treated and control monkeys processed in the same way. Also, similar changes were seen in rat urinary bladders if they were not placed in fixative until 5–15 minutes after death (original magnification ×400).

No serum, hematologic, or chemical abnormalities were detected in these animals during their lifetime. Measurement of urine chemistries showed no differences between treated monkeys and controls (Table 3). Examination of the urinary sediment by filtration and scanning electron microscopy showed no increase in microcrystalluria or no evidence of abnormal microcrystals, precipitate formation, or calculi. The few microcrystals that were present were typical of mammalian urine and were morphologically identical to magnesium ammonium phosphate crystals seen in other mammalian species *(26)*. Some of these crystals may also be crystals of magnesium potassium phosphate. In contrast to the urinary proteins of rats and mice *(24,27)*, the urinary proteins of the monkeys examined showed no evidence of an association with saccharin. Similarly, we did not find evidence of an association at these saccharin concentrations with human urinary proteins *(28)*. This finding may be related to the much lower concentrations of protein of any kind in the urine of primates, whether monkey or human, compared with those in rodents *(26–30)* and may also be related to the qualitative differences in the proteins of different species.

Discussion

Sodium saccharin administered to rats beginning at weaning, birth, or before conception (i.e., to their mothers) and continuing throughout their lives produces a low incidence of bladder tumors, and the effect is greater in males than in females *(5)*. This finding is associated with a mild superficial cytotoxicity of the urothelium with a mild regenerative hyperplasia *(14)*. This outcome appears not to be due to the concentration of saccharin in the urine, but rather to the marked physiologic alterations produced by high doses of sodium saccharin administered in the diet *(5,16)*. Critical factors in the urine appear to be pH, volume, and protein, calcium, and phosphate concentrations as well as other contributory factors *(5,16)*. Ultimately, sodium saccharin, along with numerous other sodium salts *(16)*, administered at high doses in the diet of male rats produces a urinary precipitate that contains predominately calcium phosphate but also contains large amounts of mucopolysaccharides and small amounts of silicate, saccharin, and proteins. Calcium phosphate precipitate is cytotoxic to bladder epithelial cells in tissue culture *(31)*.

Since the observations in the early 1970s that sodium saccharin produced bladder cancer in rats, the question has persisted as to whether sodium saccharin poses a significant carcinogenic hazard when it is consumed by humans *(3–8)*. Numerous epidemiologic studies in humans have consistently failed to show any effect on urinary tract tumor incidences [reviewed in *(32)*], and they also have not shown any evidence of increased urothelial proliferation in response to sodium saccharin ingestion *(33)*.

To ascertain whether a carcinogenic hazard is present for sodium saccharin when consumed by humans, we need to understand the mechanism involved. On the basis of more than two decades of extensive research, it appears that the phenomenon in rats is related to the development of a calcium phosphate-containing precipitate in the urine, which occurs following ingestion of high doses of any sodium salt, i.e., saccharin, ascorbate, aspartate, or a wide variety of other salts *(5,16)*. A high

Table 3. Urinary chemistries from monkeys ≤2 years before being euthanized*

	Cynomolgus				Rhesus			
	Male		Female		Male		Female	
	Control	Saccharin treated	Control	Saccharin treated	Control	Saccharin treated	Control	Saccharin treated
pH	7.9; 6.6	7.6; 6.5	6.5; 6.1	7.1; 6.4	6.9; 7.1	7.2; 6.7	7.5; 6.1	7.9; 5.1
Protein	0.03; 0.0	0.07; 0.10	0.17; 0.11	0.24; 0.10	0.18; 0.08	0.07; 0.13	0.06; 0.08	0.16; 0.01
Sodium, mEq/L	20; <10	<10; <10	10; 83	23; <10	17; <10	20; <10	<10; <10	<10; <10
Potassium, mEq/L	24; 2	91; 4	29; 88	12; 26	34; 8	56; 6	16; 6	17; 2
Calcium, mg/dL	5; 3	110; 6	1; 109	9; 17	14; 10	16; 4	5; 6	4; 2
Phosphorus, mg/dL	2; 1	1; 1	—; 1	2; 2	1; <1	1; 1	1; 1	2; 1
Magnesium, mg/dL	2; 1	26; 2	1; 22	4; 11	5; 5	9; 2	2; 3	2; 1
Chloride, mEq/L	<15; 15	17; <15	<15; 63	13; <15	<15; <15	35; <15	<15; <15	<15; <15
Urea, mg/dL	217; 21	873; 62	204; 1380	263; 490	334; 104	575; 77	146; 129	155; 33
Creatinine, mg/dL	21; 9	130; 13	29; 233	23; 37	37; 14	65; 16	17; 19	22; 10
Osmolality, mOsm/kg	194; 25	527; 84	—; 959	237; 305	255; 90	384; 81	156; 184	159; 45

*Values are listed for individual monkeys (value from one monkey; value from second monkey).

dose appears to be required, and no effects are seen when the diet administered contains 1% sodium saccharin. The following factors appear to be necessary in rat urine for the development of this precipitate: a pH above 6.5, high urinary protein concentration (possibly acting as a nidus for generation of the precipitate), and high concentrations of calcium and phosphate (often increasing despite an overall dilutional effect of the urine because of the increased water ingestion associated with consumption of high sodium salts in the diet) *(5,16,27).*

The results from this long-term study clearly show that there is no adverse effect of sodium saccharin administered in the diet of three species of monkeys beginning at birth and continuing for nearly the entire lifetime of the animals. There was no evidence of urothelial tumor formation, and there was no evidence of increased urothelial proliferation as judged either by light microscopy or by the more sensitive scanning electron microscopy. In addition, there was no evidence of formation of a calcium phosphate-containing urinary precipitate. A major difference between primate urine and rat or mouse urine is the low concentration of protein in the former *(26,29,30).* This was again found in the three strains of monkeys in this study and has been reported previously in nonhuman and human primates *(26,30).* It has been suggested that there is a 100 to 1000 times difference between the concentration of protein in the urine of rodents and the concentration of protein in the urine of primates *(30).* In addition, rodent urine is highly concentrated overall, with osmolalities ranging from 1000 to 3000 mOsm/L *(26).* This concentration is considerably higher than that seen in primates—whether in the monkeys, as in the present study, or in humans. The highest theoretical concentration of urine that can be attained in humans has been calculated to be approximately 1200 mOsm/L, but it is usually in the range of 100–500 mOsm/L. Even upon severe dehydration, the urine generally does not attain osmolalities above 1000 mOsm/L.

The combination of findings in this long-term study strongly supports the conclusion that sodium saccharin administered in the diet does not pose a carcinogenic hazard to nonhuman primates. The conditions necessary for formation of the urinary calcium phosphate-containing precipitate that is an intermediary associated with the development of urothelial tumors in rats do not occur in these nonhuman primates; there is no evidence of formation of the precipitate, and there are no changes suggesting proliferative or tumorigenic effects in the urothelium. Since human urine is similar to nonhuman primate urine, we anticipate that the urinary precipitate would not form in humans and, consequently, there would not be an increased proliferation or an increased incidence of tumors of the urinary tract associated with sodium saccharin consumption. Although the dose of sodium saccharin administered to these monkeys was only five to 10 times the allowable daily intake for humans, the results provide additional evidence that sodium saccharin is without a carcinogenic effect on the nonhuman primate urinary tract.

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Notes

Present address: S. Takayama, Showa University School of Medicine, Tokyo, Japan.

Present address: R. H. Adamson, National Soft Drink Association, Washington, DC.

L. L. Arnold, M. Cano, S. Eklund, and S. M. Cohen have received partial research support from the International Life Sciences Institute, the National Cancer Institute, and the state of Nebraska. Also supported in part by Public Health Service grants CA32513 and CA36727 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services; and by contract No. 1-BC-4051 0 from the National Cancer Institute for the care of the monkeys at Covance Laboratories, Inc. in Vienna, VA.

We gratefully acknowledge the technical assistance of Barbara Mattson, Margaret St. John, and Cindy Lear and the assistance of Ms. Ginni Philbrick and Ms. Bettie Sugar in the preparation of this manuscript.

Preliminary results were presented at the 87th annual meeting of the American Association for Cancer Research in Washington, DC, April 20, 1996, and in Thorgeirsson et al. (1994).

Manuscript received November 25, 1996; revised April 22, 1997; accepted October 1, 1997.