Tumor Production in Mice
by Sodium Saccharin

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Randy R. Boespflug
and
John C. King
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This thesis for honors recognition has been approved for the Department of Biology by:

Dr. James J. Manion
Dr. James J. Manion, Director

John A. Christenson
Dr. John A. Christenson, Reader

Guido Bugni
Mr. Guido Bugni, Reader

Date

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ABSTRACT

Four groups of about 25 mice were fed 0%, 0.02%, 0.2% or 2% sodium saccharin (w/v) in their drinking water for up to 100 days. Two groups of 10 mice were injected with 0.2 ml or 4% or 8% sodium saccharin (w/v) interperitoneally. One group of 10 mice was given 0.2 ml of 8% sodium saccharin (w/v) subcutaneously. One group of 5 mice was given 0.2 ml of saturated saccharin subcutaneously. These groups were controlled with the administration of sterile normal saline in the corresponding amounts and manner. Significant enlargement of kidneys, hardened and reddened bladders and enlarged, distended, reddened uteri were observed.

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INTRODUCTION

Ever since Fahlberg and Remsen discovered saccharin in 1879 (Bryan, et al 1970, National Research Council, 1974) there have been periodic controversies over its use by humans. European workers in 1886 noted no harm to human subjects given single doses of up to 5 grams of sodium saccharin, administered orally (National Research Council 1974).

In 1911 the Secretary of Agriculture appointed a board of scientific advisors to investigate saccharin (Herter and Folin 1912). They found 0.3 grams/day of saccharin was safe. A higher dosage of 1 gram per day produced disturbances in digestion, slight increases in appetite, increased indican production, some leucocytes in the urine and an increase in acidity of the feces. They saw no reason to question the use of saccharin by the general public.

There were numerous toxicological studies done between 1920 and 1950, none of which produced results conclusive enough to question its use.

In recent years the controversy over saccharin has increased as the use of synthetic sweetners has increased. Synthetic sweeteners are used by diabetics, used as a flavoring agent for pharmaceuticals, used as a dieting aid and as a means of making new palatable high protein, easy to produce foods such as fish flour and edible algae.

With the increased use of saccharin, for the above reasons and with, what seemed at the time, scarce and contradictory literature, we decided the topic needed more investigation.

It has been estimated (Bryan and Yoshida 1971) that 75% of the people in the U.S. have ingested sodium saccharin of some form, and saccharin is becoming even more widely used since the ban of cyclamates in 1969. Saccharin is about 300 times as sweet as sugar whereas cyclamates are 30 times as sweet.

When used together, as in the typical 10:1 ratio of cyclamate to saccharin, the combination has a greater sweetness than the summation of the sweetness of the individual components (Taylor, Richards and Wiegand 1967). Saccharin has the drawback of a slightly bitter taste but is presently being used in many diet drinks as well as numerous non-carbonated beverages at a concentration of roughly 0.01 to 0.02%.

Both saccharin and cyclamates drew fire from numerous researchers in 1969 but research by Price, et al (1970) and Lessel (1971) indicated cyclamates to be the major causal agent, although some methods of determination of carcenogenicity show saccharin second only to cyclamates (Bryan and Yoshida 1971).

Two methods for determining bladder carcenogenicity are currently accepted. The first of these is an implantation procedure in which an amount of the suspected chemical is placed into a vehicle pellet and surgically implanted into the urinary bladder. This allows a specific amount of the chemical to act directly on the bladder mucosa for a variable length of time depending upon the vehicle used. This procedure allows a direct application of the chemical which has not been degraded by the biological functions of digestion. This method has been questioned due to the massive quantities of the chemical that can be administered and the irrelevance of applying the chemicals directly, whereas they would normally be subjected to the digestion processes. Also the surgery itself has been blamed for either starting tumors or activating incomplete ones already present.

The alternative method is administration of the chemical orally allowing natural digestion and excretion. Kennedy, and coworkers (1972) have shown that over 90% of the orally ingested saccharin is excreted within 48 hours, with the exception that fetal tissue retains considerable amounts. Mininegishi, Asahima and Yamaha (1972), and Byard and coworkers (1974) showed that very little of the saccharin administered in this manner was chemically changed in any way during digestion and excretion, and up to 98% was recovered from the feces and urine, the urine containing approximately 90%.

Positive carcinogenic effects of saccharin have been shown by the use of implantation technique (Bryan and Yoshida 1971). Somewhat variable results have been reported using the feeding techniques. An explanation might be available in the method of reporting dosages. Some researchers have reported a concentration in drinking water, others in food, others in mg. of chemical administered per kg. of body weight of the animal per day; and still others will give concentrations in weight of chemical per unit weight or unit volume of food or water with the latter being especially popular for water administered chemicals. Bryan and Yoshida (1971) stated in all chemicals tested, a positive carcenogenic effect on mouse bladders has been correlated with a positive result in the dog. This suggests rodent bladder carcinogenicity may accurately reveal a potential human hazard.

Saccharin has been shown to have a synergistic effect on chromosomal breakage when it was used in conjunction with alcohol or caffein (Matthews, Fields and Fishbein 1973). There is a case where saccharin was proved to be responsible for an allergenic reaction (Miller, White and Schwartz 1974).

LITERATURE REVIEW

The first feeding study of note was done in 1923 by Fantus and Hektoen (1923). The study involved 75 rats in three groups, 0%, 1%, and 10% saccharin (w/w) in their food. The study lasted 36 weeks, after which time the surviving rats were sacrificed. The major finding from the first experiment was that the higher the saccharin content in the rat's diet, the more likely the rat was to get pneumonia. In two additional feeding experiments (Frantus and Hekton, 1923), saccharin levels of 0%, 0.1% and 1.0% (w/w) were given to rats. The results of these experiments were inconclusive and appeared to contradict the results of the first experiment.

In 1951, Fitzhugh et al., studied toxicity of four common artificial sweeteners including saccharin and sodium cyclamate. The study involved 20 animals for each feeding group. The saccharin groups were 0%, 1%, and 5% saccharin (w/w) in the food. The animals were fed for 104 weeks. The 5% saccharin-fed rats showed an unusually high incidence of abdominal lymphosarcoma. The lower dosage rats were unaffected.

Burbank and Fraument (1970) reported that the increase in artificial sweeteners did not reflect a significant increase in bladder cancers in the human population between 1950 and 1967. They noted that the human consumption of saccharin was less than the amount needed to produce bladder cancer in laboratory animals and the time period might have been too short to show significant fluctuation. The report was rather inconclusive.

Bryan, Ertürk and Yoshida (1970) implanted cholesterol and saccharin pellets into the urinary bladder of mice. They found that the saccharin in the pellet was only in the bladder a short time. Fifty percent of the saccharin was eluded from the pellet in about 5.5 hours. The pellets were surgically implanted into the bladders of 100 mice. The control animals received plain cholesterol pellets. Animals that survived more than 175 days had their bladders examined. There was a significantly greater incidence of carcinoma in the saccharin mice (47 and 52 percent) as opposed to the control mice (13 and 12 percent). These tumors were more severe than those produced by sodium cyclamate.

Roe, Levi and Carter (1970) did an 18-month feeding study. The mice were divided into two groups of 50 mice. One group was given a single dosage of 0.2 ml polyethylene glycol (PEG) and the other had 50mg of benzo(a)pyrene (a known carcinogen) added to the 0.2 PEG to test for cocarcinogenicity. The mice were fed a mixture of 5% saccharin (w/w) in their food. Any mice that died early or that were sacrificed at the end of the study were examined macroscopically, except in the cranial area and the spinal chord, for tumors. They found no significant increase in the production of tumors and concluded that the saccharin was noncarcinogenic.

Tisdel and coworkers (1974) did a 100-week feeding study commissioned by the International Sugar Research Foundation. Four groups of 40 weanling rats (20 male and 20 female) were fed 0%, 0.05%, 0.5% and 5% saccharin (w/w) in their food. The rats were sacrificed after 100 weeks. The rats had a complete examination macroscopically and microscopically. Heart, liver, spleen, kidneys and gonads were weighed and organ weight to body weight ratios were calculated. They found that the 5% rats had a significantly higher rate of tumors of the uterus and urinary bladder.

This material gave the impression to the subcommittee that the work was poorly prepared and that the experiment itself was poorly executed.

Two other reports were presented at the same time to the subcommittee of the National Research Council (1974). One of the studies was done in Canada and showed no significant increase in tumor production by saccharin. The study was considered a well-designed and carried out experiment.

The third study was done by the FDA and showed no carcinogenicity on the part of saccharin. It was considered to be a poorly-executed experiment.

Studies show that saccharin is excreted unchanged (Minigishi, Asahina and Yamaha 1972; McChesney and Goldberg et al., 1973; Byard and Goldberg 1973, and Byard, 1974) or with only a slight amount metabolized

(Kennedy 1972). The metabolites found were the hydrolytes of saccarin, o-sulfamoylbenzoic acid and ammonium o-sulfobenzoic acid. McChesney and Goldberg (1973) claim that the ammonium o-sulfobenzoic acid is actually ammonium o-carboxybenzensulfonate and the hydrolytic metabolites found were impurities in the original sample of saccharin before it was introduced into the animal.

Saccharin is absorbed into the blood through the stomach and intestine (Kennedy et al. 1972). The absorption rate is directly related to the pH of the stomach and intestine, i.e., the lower the pH the more rapidly the saccharin is absorbed (Minegishi et al. 1972).

The saccharin reaches a peak concentration in the blood after 7-15 min. (Matthews et al. 1973). The only normal tissues that build up any significant concentrations of saccharin were those of the excretory system. The kidney had a concentration five times greater than that of any other tissue, indicating that the kidneys play a role in filtering saccharin from the body system (Matthews et al. 1973). The exception to the rule is fetal tissue. Saccharin passes rapidly across the placenta (Pitkins, Reynolds, Filer and Kling 1971). The fetal body slowly clears the saccharin from it, with appreciable levels of saccharin in the body two hours after maternal levels had become negligible in Rhesus monkeys, according to Pitkin and co-workers (1971). The Saccharin is excreted 82.65-96.95% in the urine (Kennedy, Fancher, Calandra, and Keller 1972).

METHODS AND MATERIALS

Feeding Study

We used 99 Swiss-Webster mice from Rocky Mountain Laboratories. The Sodium Saccharin was saccharin sodium derivative dihydrate CH:CHCH:CHC:$CSO_2NNaCO \cdot 2H_2O$ (SX 10, 5303) from Matheson, Coleman and Bell, (Los Angeles, California).

The mice were fed 0%, 0.02%, and 2.0% saccharin in their drinking water (w/v). The control consisted of 20 mice (0% saccharin). Twenty-eight mice received 0.02% saccharin, 23 mice received 0.2% saccharin and 28 mice received 2.0% saccharin. The mice were fed Purina Rat Chow. Both food and saccharin solutions were given ad libitum.

The mice were kept on the feeding program for a maximum of 100 days. An intermediate check was made after 84 days. Mice that died during that period were autopsied. We were unable to examine some of the mice because they had been eaten by their cage-mates or had been dead too long to render meaningful results.

An autopsy started with an overall external examination, followed by a macroscopic examination of the inside of the skin, body wall and viscera. No microscopic examinations were attempted.

All mice surviving at the end of the test period were sacrificed by cervical fracture and autopsied. In addition to the regular examinations, a random sampling of the mice was weighed to get an average weight for calculating the mg of saccharin ingested per kg of body weight per day. The kidneys were weighed for calculating ratios of organ weight to body weight at the time of sacrifice. Daily water consumption was checked on a random basis throughout the experimental period for calculating the average dosage of saccharin per mouse per day.

Injection Study

All mice were injected with a Plastipak disposable syringe, 26 gauge, 3/8 inch, intradermal bevel needle.

The mice were generally injected three times per week on Monday, Wednesday and Friday. The injection schedule was suspended over Christmas break, Dec. 19, to Jan. 12.

When a mouse appeared sick or to have an obvious tumor, it was sacrificed and autopsied.

The 4% injection study was done with 10 mice divided equally between the sexes and Swiss-Webster and C-57 B-10 mice. The mice were injected with 0.2ml of an autoclaved 4%(w/v) solution of saccharin. The injections were made interperitoneally in the posterior-left ventral side of the mouse.

The 8% injection study was done with 10 mice of both sexes and assorted strains. The mice were injected with 0.2 ml of an autoclaved 8%(w/v) solution of saccharin. The injections were made interperitoneally in the posterior-left ventral side of the mouse.

Two groups of 10, 4-week old Swiss-Webster male mice were injected subcutaneously mid-dorsally with 0.2 ml of an autoclaved 8% (w/v) solution of saccharin.

The saturated injection study was done on five, 10-week old Swiss-Webster male mice. The mice were injected subcutaneously and mid-dorsally with 0.2 ml of a saturated saccharin solution (at 27° C).

For control of the injection study 10 mice of assorted sexes and strains were utilized. The mice were injected with 0.2 ml of an autoclaved solution of 0.9% salt (w/v). The injections were made in the same place and manner as the other mice.

TABLE 1

Abnormalities in Feeding Study Mice at 84 days

Abnormality	0%[1]	.02%[2]	0.2%[3]	2.0%[4]
Tumors - Foreleg			3	1
Tail			1	
Back			1	1
Skin				
Glandular at extremeties	1	1	2	4
Skin ulceration			1	
Jelly-like growth			2	
Bladder - Thick, hard & enlarged	3	2	15	2
Uterus - Red, & enlarged		4		6
Fundus hardened		1		
Swollen lymph nodes	1		2	
Poor-looking skin			16	1
Poor-looking body wall			6	1
Internal hemorrhage				1
Cecum shriveled				
Enlarged stomach	1			1
Enlarged pancreas	1			1
Enlarged spleen		1	1	
Soft &/or grey fat		6		2
Enlarged kidneys	5		13	4
Enlarged penis			3	
Enlarged prostate glands			6	
Enlarged thyroid				1
Intestinal blisters	1			1
Bladder - Reddened				1

[1]This group contained 15 mice

[2]This group contained 11 mice

[3]This group contained 18 mice

[4]This group contained 17 mice

TABLE 2

Abnormalities in Feeding Study Mice at 100 days

Abnormality	0%[1]	.02%[2]	0.2%[3]	2.0%[5]
Tumors - Foreleg				
Tail				
Back				
Skin			1	1
Glandular at extremeties		2		3
Jelly-like growth				
Bladder - Thick, hard and enlarged			3	5
Reddened		6		1
Uterus - Red and enlarged		6		4
Fundus hardened				
Swollen lymph nodes			2	
Poor-looking skin			4	
Poor-looking body wall			1	
Internal hemorrhage				
Cecum shriveled				1
Enlarged stomach				
Enlarged pancreas		1		
Enlarged spleen				
Soft and/or grey fat		5	1	4
Enlarged kidneys		4	5	4
Enlarged prostate glands				
Enlarged thyroid				
Intestinal blisters	4	10	3	10

[1]This group contained 5 mice

[2]This group contained 12 mice

[3]This group contained 5 mice

[4]This group contained 11 mice

TABLE 3

Summary of Abnormalities as a Percentage of the Total Number of Mice

Abnormality	0%	.02%	0.2%	2.0%
Tumors - Foreleg			13	4
Tail			4	
Back			4	4
Skin			4	
Glandular at extremeties	5	11	9	25
Skin Ulceration			4	
Jelly-like growth			9	
Bladder - Thick, hard and enlarged	15	7	78	25
Reddened		21	4	7
Uterus - Red, and enlarged		36		36
Fundus hardened		4		
Swollen lymph nodes	5		11	
Poor-looking skin			87	4
Poor-looking body wall			30	4
Internal hemorrhage				4
Cecum shriveled				4
Enlarged stomach	5			
Enlarged pancreas	5	4		4
Enlarged spleen		4	4	
Soft and/or grey fat		42	4	43
Enlarged kidneys	25	17	78	29
Enlarged penis			9	
Enlarged prostate glands			26	
Enlarged thyroid				4
Intestinal blisters	20	36	17	36

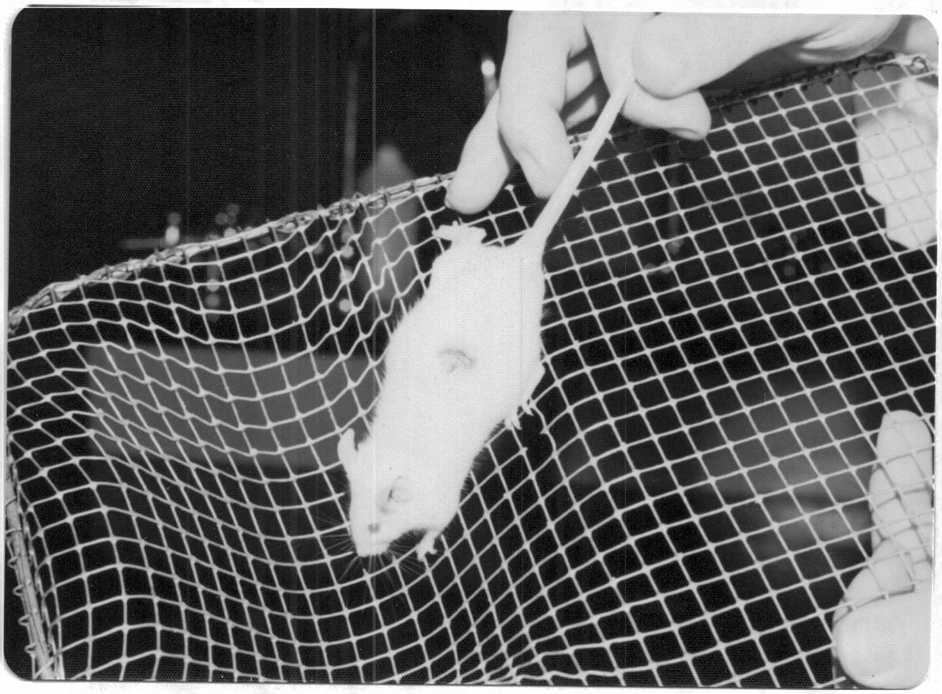
TABLE 4

Summary of Mice in Injection Study

	Control	4%	8%	8% SC	Sat. Sco.
Total Number of Mice	10	10	10	10	5
Abnormality					
Bladder - Thick, hard and enlarged		2	2		
Tumors - Foreleg		1			
White tissue on body wall		1			
Urethra			2		
Skin growth			1	1	
Skin ulcerations			1	10	5
Swollen lymph nodes			2		
Mute		4			
Poor-looking skin		1			
Internal hemorrhaging			1		
Large cecum		1			
Soft and/or grey fat		7	6	10	
Enlarged kidneys	1		4		
Swollen area around genitalia			1		
Enlarged penis			2		
Intestinal blisters	2			2	

FIGURE 1

Photographs of 8% Subcutaneous Injected Mice Before Sacrifice



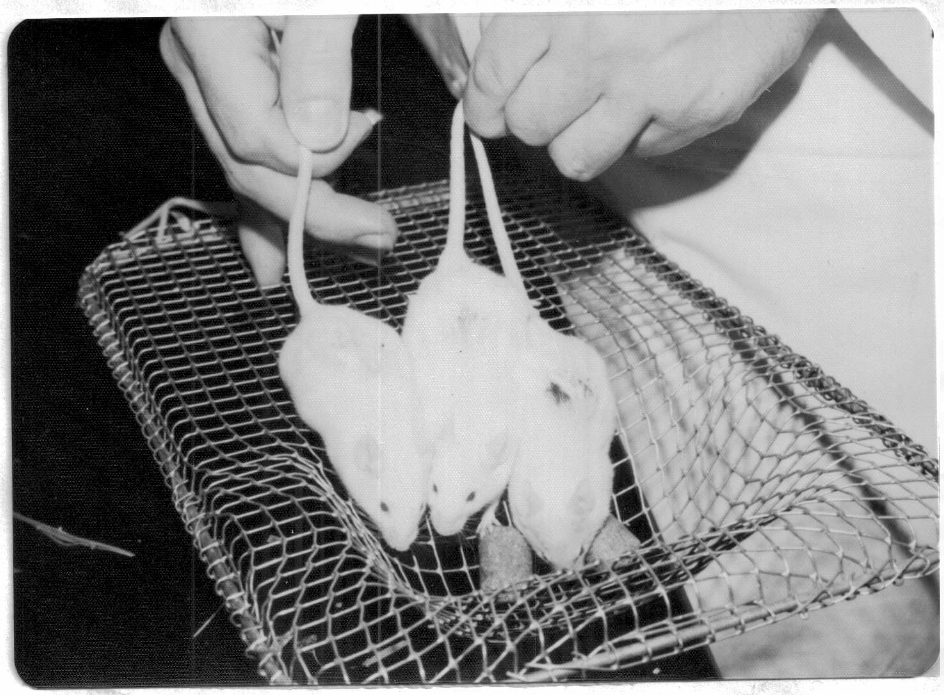


FIGURE 2

Photographs of Skins of 8% Subcutaneous Injected Mice.

FIGURE 3

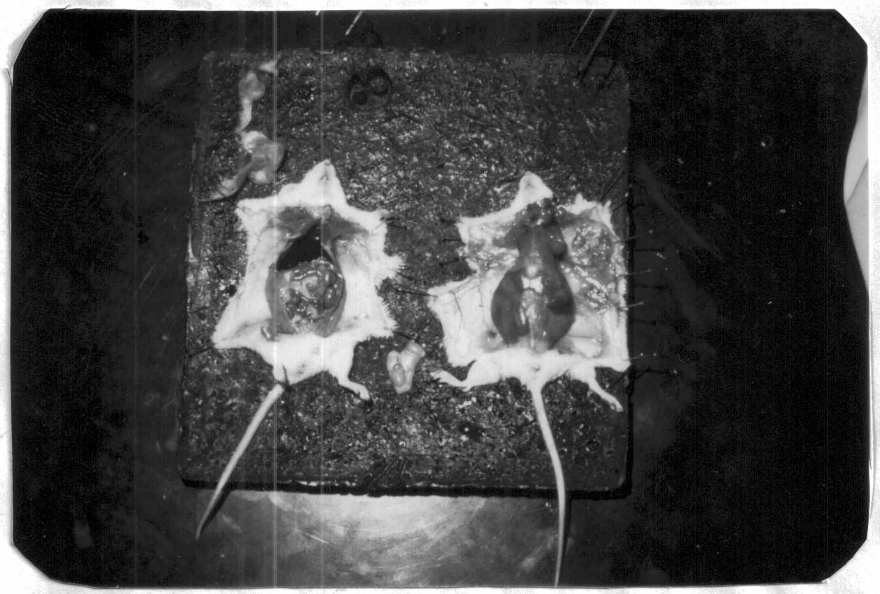
Photographs of Saturated Saccharin Subcutaneous Injected Mice Before Sacrifice.

FIGURE 4

Photographs of Saturated Saccharin Subcutaneous Injected Mice and Skins.

FIGURE 5

Photograph of a Mouse With Skin and Shoulder Tumors



RESULTS

Feeding Study

0.02% Saccharin Study

The mice in this study recieved an average dosage of 37 milligrams of saccharin per kg body weight. They had an average kidney weight to body weight ration of 0.01139.

After 84 days, the only significant deviation from the controls was the incidence of red and distended uteri. One had a hardened, calcified fundus of the uterus, which was probably a tumor of some kind. The fat deposits of these mice were of a softer texture, with a grey color rather than the more solid, white, flaky deposits of the controls.

After 100 days 50% of the mice had reddened bladders, indicating some irritation. There was also an increase in the incidence of reddened and distended uteri. The fat deposits showed a continued tendency toward the slushy grey deposits.

One mouse died after 25 days of the study, but was unavailable for autopsy.

0.2% Saccharin Study

The mice in this study received an average dosage of 686 mg/kg of body weight.

They had an average kidney weight to body weight ration of 0.0190.

One week into the study, one mouse appeared to dominate and killed seven of his cage mates. He was destroyed and the mice were replaced with eight, 4-week-old Swiss-Webster male mice.

Some mice had to be sacrified after 57 days. Tumors in the tail, back and forelegs were noted in autopsy of these mice. One mouse exhibited 15 small tumors, in varied locations.

At 84 days the mice showed a significant incidence of thick, red and hard bladders. A heavy growth of glandular tissue was noted around the forelegs in two mice and a jelly-like, orange substance was noted in two other mice. A skin ulceration was found in a third mouse. The kidneys were enlarged above the normal 1.5% of body weight. Three mice had enlarged penises, which we suspected contained tumors. Thirty-three percent of these mice had enlarged prostate glands. We observed one large tumor of the prostate measuring 9mm x 6mm x 5mm and which was encapsulated in the left gland. Two other smaller tumors were noted, one median to the lobes and exclusive of them, and a small one in a lobe of the prostate gland making the lobe 1.5 times its normal size.

After 100 days the bladders were mostly all thickened, red and enlarged. Two showed a whitish enlargement at the end with a white crystal of saccharin, partially imbedded in the mucosa. The crystals were hard, although not as hard as the pure crystals. Their size was 2mm x 3mm x 4mm, with a small point at one end projecting into the mucosa. One animal had a skin growth of fibrous tissue on its back, and a flat, hard 4mm diameter tumor between the shoulder blades. The fat deposits were quite different from the normal. The mice showed a tendency towards soft, grey fat in the pads of the testes and medial-dorsal deposits. They also showed a distrophy of the two seminal vesicles. All the mice had enlarged kidneys, about 1.8 to 2.0% of body weight. Toward the end of the study their coats became very motley, bitten and lost hair because of their constant fighting. This was reflected in enlarged lymphnodes and over-all poor coat condition.

2.0% Saccharin Study

The mice in this study recieved an average dosage of 4700 mg of sodium saccharin per kg of body weight.

They had an average kidney weight to body weight ratio of 0.0149.

After 84 days some mice had a reddening of the bladder and a darkening and enlargement of the uteri. Some showed a glandular growth in the forelegs, poor skin and coat condition, a body wall with a measeled appearance, and one had considerable internal hemorrhaging. They had a tendency toward the slushy, grey fat. Twenty five percent of these mice had enlarged kidneys.

After 100 days 50% of the mice had reddened, thickened, and enlarged bladders and nearly as many had reddened and distended uteri. Three mice had a growth of glandular nature around the forelegs. One mouse had a skin tumor. This was reflected as numerous white fiberous growths between the skin and body wall. Most of the mice had grey, slushy fat. Only one mouse in this group had normal or fairly normal fat deposits. Nearly 50% of the mice showed enlarged kidneys.

Injection Study

4% Saccharin Injection

The mice in this group were given 229 mg of sodium saccharin per kg of body weight. As mice died or were sacrificed in this group, we found that they could not be replaced due to the hostility of the cage mates, so only the original mice were studied.

Twenty-six days into the study, one mouse developed a tumor on her front leg and was sacrificed. The tumor was quite liquid, white and encapsulated.

Sixty days into the study, a female mouse developed a very large tumor in her lower abdomen. The tumor had grown rapidly and encompassed the bladder, the fundus of the uterus and much of the small intestine. It measured approximately 3cm x 1.5cm x 1.5cm and was encapsulated and fairly hard.

After 77 days, a female mouse developed an obvious abdominal tumor. The tissue was pinkish and tough, located in the peritoneum.

After 97 days, a male was found dead but had been badly eaten by his cage-mates and nothing could be determined from the autopsy. One mouse died about five minutes after an injection and, because he showed no abnormalities on autopsy, was disregarded from the experiment.

8% Saccharin Injection

The mice in the 8% study received 457 mg of saccharin per kg of body weight.

After 62 days, a male developed a tumor in the urethra. It was very hard, and cylindrical in shape, sharply defined, white in color, and fibrous in nature. Another male had a quite similar tumor, somewhat smaller, but surrounded by a white liquid. The tumor measured 12mm x 4mm x 4mm. The bladder of this mouse appeared to be about twice normal size and was thickened.

After 78 days one mouse died and two, with the same symptoms, were sacrified. All of these mice had a protrusion around the penis. Internal examination showed an enlarged penis, about twice normal size, with some white liquid but not a clearly defined tumor. All three had enlarged kidneys. One mouse was killed by its cage-mates early in the experimental period. Four mice survived the 100 day experimental period.

8% Subcutaneous Injection

During the Christmas break the mice in the 8% subcutaneous study were judged to be obviously diseased and sacrificed for humanitarian reasons. The data concerning the subcutaneous study are from a group of mice that was started on the injection program on January 17, 1976.

In both groups, at 14 days into the study, or at about six injections, at least 60% of the mice developed a scabbed-over ulceration of the skin about 5mm in diameter. These scabs developed directly over the area of injection and could be produced with a single injection if the injection sites were moved. These ulcerations healed when the injection sites were moved but otherwise lasted for the remainder of the study. Due to the loss of the first group, we were unable to study these mice for more than seven weeks, and could not predict whether tumors would have been produced due to the constant irritation of the saccharin solution.

Saturated Solution Injection

Only five Swiss-Webster 10-week-old males were used in this study. These mice were injected as usual for the subcutaneous mice, using a saturated solution of saccharin (in water at 20°C). All of these mice developed massive ulcerations and open sores approximately 1.5cm in diameter following the first injection. Due to the size of the open sore and the pain involved, this study was discontinued and the animals sacrificed for study. Underlying muscle tissue was discolored to a greenish appearance where it was not open to the air.

We found 0.1 ml of the saturated solution to be lethal in about five min. when injected intraperitoneally; consequently no more mice were subjected to this treatment.

DISCUSSION AND CONCLUSION

Feeding Study

The 0.02% (w/v) saccharin fed mice had definite irritation in the urinary bladder. The irritation would be consistant with the fact that the saccharin is almost totally excreted in the urine. The bladder would build up a concentration of saccharin in it that would be much greater than found in other parts of the body. The saccharin in the concentrated solution would have a limited amount of time to work on the walls of the bladder.

The kidneys of this group tended to have a smaller weight to body weight ratio than the controls. We believe that this was due to the fact that the mice were all female, and that averaging of the total group tended to normalize the results.

The uteri were definitely showing signs of irritation. The only explanation of this seems to indicate a blood level of saccharin which is capable of irritation because Matthews et al. (1973) stated there was no significant build-up in the uterine tissue.

The fat formation was definitely affected by the presence of the saccharin in the diet. The fat deposits were softer and greyer in color than normal fat deposits. There was no definite shape to these formations in contrast to the normal tissues.

We conclude that the limited doses of saccharin did begin to act as an irritant to the mouse's viscera of which the urinary bladder and the uterus seemed to be the most affected. There were also signs that the body chemistry of the mouse is disrupted by the saccharin was witnessed by the change in the formations of fat deposits. These changes might become manifest in other ways given a more prolonged exposure to the saccharin.

The 0.2% (w/v) saccharin-fed mice also show the irritation of the urinary bladder and the urethra. The urinary bladder and the urethra showed signs of thickening with a very likely possibility that there was tumor production taking place in these areas.

There were crystals of saccharin that were found in the urinary bladder. The irritation caused by these crystals might play a very important role in the formation of bladder tumors. These crystals indicate an extremely high concentration of saccharin in the urine of the mouse.

The kidneys of these mice showed signs of being very enlarged. The enlargement might be caused by the concentrating of the saccharin in the kidneys as it is filtered out of the blood, thereby causing an irritation.

The prostate glands showed the effects of the saccharin also. This tissue shows fairly high susceptibility to the saccharin in the blood or possibly from the urine.

In general we found a significantly greater incidence of tumor production in the mice showing a higher blood level of saccharin. Blood levels are indicated because of their incidence even in the skin.

The coats of these animals were in poor condition. Saccharin may have played a role in the degeneration of the coats but since the 2.0% group did not show a higher incidence than did the 0.02% group the major cause of this seems to be that of fighting among the mice which was observed more regularly in the cage containing only males, fed 0.2% saccharin.

The fat deposits were degenerate, especially in the mice which received higher saccharin concentrations. When they were present they seemed to be of the soft grey formations. Here, the saccharin seems to be interfering with normal fat deposition and retention.

The mice in this group showed significant mortality during the experiment. Saccharin, at these levels in the mice's diet, had harmful effects on their normal physiological functionings.

The 2% (w/v) saccharin-fed mice showed a high number of individuals with the reddened, thickened and enlarged urinary bladders. There was also a significant number of mice with reddened and enlarged uteri. This would be expected with the higher concentration of saccharin in the blood of the animals if saccharin is an irritant to these tissues.

Many of the kidneys were enlarged in size, visually, but this enlargement did not reflect itself in the organ weight to body weight ration when averaged for all the mice of the group.

The appearance of a glandular growth in the region of the forelegs becomes significant with the higher concentrations. The growth was between skin and the body wall. It was usually in the same region as the forward mammary gland tissue of nursing mice, which these mice were not, but was of a different appearance and constitution.

The body wall of some of these mice had a measled appearance which did not always coincide with the poor coat condition. This gives some support for the speculation that the saccharin may in some way be affecting the coat also.

Injection Study

The 4% (w/v) saccharin injected mice showed tumorigenicity, in three individuals. These tumors showed in such areas as the forelegs, body wall and abdomen as early as 77 days after injection. One of the tumors started in the bladder or fundus of the uterus and rapidly grew to encompass most of the lower abdomen including the bladder, uterus and lower portions of the large and small intestine.

Four of the mice in the program developed interesting, but seemingly unrelated abnormalities. They had become mute after 19 days. We did not observe this reaction in any other group and have no explanation as to why it might have occurred here. The condition was not permanent and some eventually recovered the use of their voices.

These mice, too, showed a high incidence of degenerate fat, with a tendancy towards the soft, grey shapeless deposits noted earlier. This is an indication that the saccharin was having some of the same over-all effects as in the other mice.

The 40% mortality rate in this group is made even more significant by the fact that all survivors were females. This is almost repeated exactly in the 8% saccharin injection study with a single male and three females surviving the 100 day experimental period. The survivors were also the B-10 strain indicating that these mice might be more resistant to the irritation of the saccharin solution than were the Swiss-Webster mice. The 8% saccharin injection study showed a marked incidence of urethral tumors in the male mice which accounted for most of the mortality.

The study with 8% (w/v) saccharin subcutaneously injected mice showed significant results. After six injections 60% of the mice had developed skin lesions. After nine injections all the mice had developed the lesions. After the lesions began to appear in the area, each injection caused a new lesion, indicating a lowering of the resistance of the mouse to the effects of the sodium saccharin. When the injections were stopped the lesions began to heal over, the fur began to grow back. The examination of the hide showed signs of sores still present underneath the scabbed area. We feel that the saccharin produced the sores by killing or impairing significant tissue in the area of the injection.

We feel it would have been interesting to have then allowed the mice a longer period of time to heal in order to see whether or not any permanant damage was done to the mice.

We conclude that saccharin is an irritant when injected subcutaneously. It takes a smaller dose of saccharin injected to bring about a harmful effect than it does when the saccharin is ingested orally. This effect is probably due to the short period of time that saccharin is retained in the body, the total dose being in the body for as little as 15 min. in the injection study, whereas the dose is spread out over 24 hrs. in the feeding study.

The saturated saccharin study showed a rapid acceleration of the results obtained at lesser concentrations. We injected one mouse with 0.1 ml of a saturated saccharin solution intraperitoneally, and the mouse died within five min. of the injection. Intraperitoneally, this dose was definately a lethal one. We then injected five mice with the same dosage subcutaneously. The results were similar to those in which the mice recieved subcutaneous injections of the 8% saccharin, only much more drastic. The lesion was several times larger than those produced by the 8% (w/v) saccharin injections and were severe enough to expose the muscle on the mouse's back following a single injection.

We felt that the saturated saccharin solution was too concentrated for any long term study, because the mice would die of complications that would occur during such a program long before any tumorigenic effects could be produced in the mouse.

General Discussion and Conclusion

We noted in the 100 day study, "intestinal blisters" in most of the animals that we examined. They were in the 2% saccharin-fed mice and in the control animals, and all groups in between. In some mice they were more numerous and severe than in other mice. The severity depended on the individual mouse and not the group from which it came. We do not know the cause of these blisters. Perhaps they are a product of some infection, viral or otherwise. They might also be the result of a deficiency in the food they were given, as it was a rat diet.

There is an area of definite weakness in our study in that good slide preparations might have been able to confirm some tumors, along with the cause of the blisters, and might have allowed us to find many more tumors or early stages of tumors in the thickened bladders. Also we would have been able to determine the type of tumors produced.

This study did show areas that may tend to explain the deviation of results from those of previous studies. Different strains of mice showed different reactions to the same concentrations of saccharin. Males showed an increased susceptability even beyond that of the strain of the mouse.

Although it is not usually mentioned, there are different types of saccharin, all marketed under the name Saccharin. The sodium saccharin that we used in this study was much more soluble than the Soluble Saccharin marketed by Merck Chemical Co. or that offered commercially by S.C. Squibb, or Norwich Pharmacal Co. In preliminary research with these saccharin compounds, we found varying results. Many studies have simply used saccharin, which implies that it was not a sodium or potassium derivative and would not have been soluble, which could produce very different results.

We feel there was a weakness in our experiment in that we did not have males in two of our feeding studies. The weakness is more apparent when one notes that throughout this study males have reacted more often and more severely to the saccharin than have the females. The male mice had a higher incidence of uro-genital tumors than did the females. Many of the previous studies were done on a single sex, usually females because they are easier to house in large numbers. We see this as a serious weakness in our study and in previous work.

We realize that our study can not be directly equated to human danger. We also realize while we were using concentrations equal to, or just above, those encountered in human consumables, the mice in the feeding study were allowed no choice except the saccharin solutions. We forced them to consume levels that might be well over what an average human would ever encounter. Still our results would move us to caution much expanded use of saccharin in the human diet. There are daily maximums established (National Research Council 1974), and these seem to be within safe limits. However, many products contain saccharin in small amounts where we would not expect it - popcorn salt and toothpaste, for example - and its use is projected to expand considerably. Diabetics stand a considerable risk of exceeding these limits and may be ingesting questionable amounts of saccharin that could do serious harm over a lifetime.

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