Hypoglycemic Effects of Saccharin in Experimental Animals

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SACCHARIN (o-sulfobenzoic acid imide) is commonly used as a physiologically inert sweetening agent and, as such, is extensively used by diabetics as a substitute for sucrose in the diet. The British Artificial Sweeteners in Food Order (1953) states that saccharin is known to be harmless, while the use of other sweeteners may be detrimental to health. Studies of the toxic effect of saccharin in animals has been reported by Fitzhugh *et al.*,[1] Fantus and Hektoen,[2] and Carlson and associates,[3] and in many by Herter and Folin.[4] In these studies no attempt was made to determine the effect of saccharin on the blood sugar. Several other early reports describe a decrease in the blood sugar following administration of saccharin,[5–8] several report on significant change,[9–11] while Syllaba[12] observed an increase in rabbits and in man following administration by stomach tube. The majority of these experiments were performed on fasted normal human subjects. Unfortunately, the earlier reports show not only a paucity of experimental detail but also a lack of statistical inference.

It therefore appeared useful to determine the effect of both chronic and acute administration of saccharin on the blood sugar levels of both fed and fasted rats and mice. The mode of administration was varied to determine whether the effect was independent of the mode. A second report will describe the effect of saccharin in human subjects.

In view of the report by Macallum and Sivertz[13] that sulfonamides, including saccharin, potentiate and accelerate the hypoglycemic action of insulin when injected concomitantly, it seemed of especial interest to observe the effect on the obese-hyperglycemic mice (Bar Harbor *obob* strain) being studied in this laboratory. These animals present among other metabolic characteristics an insulin-resistant hyperglycemia, an increased pancreatic content of both insulin and glucagon,[16] and increased circulating insulin.[17]

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MATERIALS AND METHODS

Three types of animals were used in these experiments: obese-hyperglycemic mice of both sexes (40 to 70 g, averaging 57 g), their lean littermates (24 to 40 g, averaging 30 g), and female Wistar adult rats (250 to 300 g). All animals were kept in separate cages at constant environmental temperature (78°F) and under regular illumination. The effect of both chronic and acute administration was studied.

For the blood sugar determination, 0.1 ml of whole blood was obtained from the tail vein; assay was performed using the method of Somogyi,[14] with the colorimetric reagent of Nelson.[15] Blood was taken from animals under mild Nembutal anesthesia (10 mg/Nembutal/100 g mouse and 5 mg/rat).

I. Chronic Experiment

Two diets were used in the chronic experiment, a "synthetic" high-carbohydrate diet and ground Purina lab chow. The "synthetic" diet was of the following composition: casein 25%, corn oil 3%, cod liver oil 2%, sucrose 65.7%, Hegsted salt mix 4%, cystine 0.2%, and choline chloride 0.1%. The diet was supplemented with the following vitamins per kilogram diet: thiamine 10 mg, pyridoxine 10 mg, riboflavin 20 mg, niacin 50 mg, pantothenic acid 100 mg, biotin 0.2 mg, and folic

TABLE I

Food Consumption (g) of Obese-Hyperglycemic Mice and Their Lean Littermates on a Ground Purina Lab Chow plus 25 g Saccharin/kg Diet

Week	Obese			Lean		
	Control (N = 10)	Test (N = 10)	p	Control (N = 10)	Test (N = 10)	p
1	37 ± 4	36 ± 8	—	29 ± 9	26 ± 6	—
2	40 ± 7	40 ± 7	—	36 ± 6	34 ± 4	—
3	49 ± 17	62 ± 9	<0.05	35 ± 6	35 ± 6	—
4	38 ± 8	50 ± 7	<0.05	35 ± 5	39 ± 6	<0.05

TABLE II

Blood Glucose Levels (mg per 100 ml) of Obese Hyperglycemic Mice and Their Lean Littermates in Chronic Experiment: High-Carbohydrate Diet with Saccharin Added in Amounts Indicated

Day of experiment	Saccharin (g/kg diet)	Obese			Lean		
		Control (N = 10)	Test (N = 10)	p	Control (N = 10)	Test (N = 10)	p
t_0	—	149 ± 21	165 ± 5	—	81 ± 19	82 ± 19	—
t_7	5	153 ± 20	148 ± 22	—	76 ± 8	76 ± 11	—
t_{14}	5	178 ± 22	169 ± 22	—	89 ± 13	89 ± 10	—
t_{18}	25	171 ± 16	62 ± 22	<0.01	81 ± 16	81 ± 10	—
t_{25}	25	149 ± 11	59 ± 30	<0.01	96 ± 15	91 ± 12	<0.02

acid 5 mg. Vitamins A and D were present in the commercial Norwegian cod liver oil in the potency of 1800 and 180 units per gram, respectively.

Twenty obese and twenty lean mice were used in each of the chronic experiments; each group was further subdivided into test and control animals and received the appropriate diet. The "control" diet contained no additions, while saccharin* in the amounts indicated was added to the "test" diet. As nearly as possible equal numbers of male and female mice were tested. Twenty test and twenty control rats were used, all individually caged. All animals were allowed to eat ad libitum during both experiments. Blood was taken from animals in the fed state.

Each experiment was run for the period of four weeks. Blood sugar was determined on each individual animal prior to and at designated intervals during the experiment. Weight (once a week) and food intakes (every other day, corrected for spilling) were determined routinely throughout the experiment.

* Saccharin Sodium (Soluble Powder U.S.P., Lot No. B96), supplied by Monsanto Chemical Company, St. Louis, Mo., was used throughout the experiment.

II. Acute Experiment

In the acute experiment both the mode of administration and the physiologic status of the animals was varied. In contrast to the chronic experiment both fed and fasted animals were used. Again obese and lean mice were tested, in addition to female Wistar rats. Administration of the saccharin solution was made both by intraperitoneal injection and by stomach tube. Each test rat received 100 mg saccharin, while each test mouse received 10 mg, and their control counterparts received an equal volume of distilled water and similar handling. Rats were fasted for 24 hours and mice for 15 hours. The 24-hour food intakes were determined immediately preceding and after the administration. Blood samples were taken prior to and 30 minutes after the administration. The blood sugar determination was identical to that of the chronic experiment.

RESULTS

I. Chronic Experiment

A. *Food Intake.* During the first experiment ("synthetic" high-carbohydrate diet), the test animals consumed neither more nor less than their controls—the rats 11 to 14 g,

TABLE III

Blood Glucose Levels (mg per 100 ml) of Obese-Hyperglycemic Mice and Their Lean Littermates in Chronic Experiment: Ground Purina Lab Chow with Saccharin Added

Day of experiment	Saccharin (g/kg diet)	Obese			Lean		
		Control (N = 10)	Test (N = 10)	p	Control (N = 10)	Test (N = 10)	p
t_0	—	164 ± 15	152 ± 18	<0.05	85 ± 17	87 ± 7	<0.05
t_7	25	149 ± 36	128 ± 16	<0.05	95 ± 18	77 ± 14	<0.05
t_{14}	25	161 ± 30	98 ± 5	<0.01	110 ± 14	101 ± 14	<0.05
t_{21}	25	155 ± 28	95 ± 46	<0.01	106 ± 22	73 ± 15	<0.01
t_{28}	25	166 ± 54	*82 ± 34	<0.01	98 ± 13	78 ± 15	<0.05

* Two test mice died.

TABLE IV

Weight of Female Adult Wistar Rats (grams) in Chronic Experiment: High Carbohydrate Diet with Saccharin Added in Amounts Indicated

Day of experiment	Saccharin (g/kg diet)	Test (N = 12)		Control (N = 12)		
		Weight	% Change	Weight	% Change	p
t_0	—	250 ± 16	0	252 ± 18	0	>0.05
t_7	5	246 ± 12	−1.6	261 ± 22	+3.6	>0.05
t_{14}	5	248 ± 12	−0.8	264 ± 23	+4.8	>0.05
t_8	25	249 ± 11	−0.4	276 ± 25	+9.5	<0.01
t_{25}	25	249 ± 15	−0.4	276 ± 28	+9.5	<0.01

the obese-hyperglycemic mice 5 to 7 g, and the lean mice 3 to 5 g daily. However, when ground Purina chow was the dietary medium, the obese test mice consumed significantly more than their controls; a less marked increase was noted in the case of the lean mice (Table I).

B. *Blood Sugar Levels.* The blood sugar values for the obese and the lean mice receiving the high-carbohydrate diet are shown in Table II according to concentration of saccharin in the test diet. Saccharin had a hypoglycemic effect in the obese-hyperglycemic mice only, and only in the concentration of 25 g/kg diet.

Saccharin in the concentrations used had no appreciable effect on the blood sugar levels of normal rats. In the high-carbohydrate experiment, saccharin up to 25 g/kg diet was added; the blood sugar of both test and control animals were in the range of 80 ± 3 mg per 100 ml glucose ($p > 0.05$).

A hypoglycemic effect was also seen when ground Purina chow is the basal diet (Table III). Again the decrease in the obese mice was of greater magnitude than that in the lean littermates; the percentage decrease was also greater.

C. *Weight.* There did not appear to be a weight loss in either of the two types of mice when maintained on the test diet (Table IV). There was a weight decrease in the test rats, however, which was especially marked when the animals were on a diet containing 2.5% saccharin ($p < 0.01$). This observation supports that of Fitzhugh *et al.*[1] that rats on 5% saccharin were smaller than control ($p < 0.05$).

In an attempt to relate this data to previous reports, the amount of saccharin ingested daily by an "average" animal in each of the three

TABLE V

Calculated Amount of Saccharin (mg) Consumed Daily by the Three Types of Animals: Chronic Experiment with Two Dietary Media

Species	High carbohydrate diet			Ground Purina chow	
	Daily consumption (g)	Saccharin 0.5%	Saccharin 2.5%	Daily consumption (g)	Saccharin 2.5%
Obese mice	5–7	25–35	125–175	6–9	150–225
Lean mice	3–5	15–25	75–125	4–5	100–125
Rats	11–14	55–70	275–650	—	—

TABLE VI

Blood Glucose Levels of Obese-Hyperglycemic Animals, Their Lean Littermates, and Normal Female Adult Wistar Rats in Chronic Experiment: Ground Purina Lab Chow; Administration of Saccharin Solution by Method Indicated; Tail Blood Taken Before and 30 Minutes After Administration

Animal		No.	Weight (g)	mg % glucose t_0	mg % glucose t_{30}	mg % glucose Diff.	p
Fasted, intraperitoneal saccharin							
Obese	Control	9	58 ± 6	95 ± 23	91 ± 19	− 4	>0.05
	Test	9	51 ± 5	103 ± 43	98 ± 38	− 5	>0.05
Lean	Control	10	26 ± 3	74 ± 14	84 ± 10	+10	>0.05
	Test	10	25 ± 3	84 ± 18	75 ± 20	− 9	>0.05
Rats	Control	6	284 ± 15	74 ± 16	65 ± 13	− 9	>0.05
	Test	6	236 ± 19	62 ± 6	59 ± 5	− 3	>0.05
Fed, intraperitoneal saccharin							
Obese	Control	15	48 ± 7	191 ± 28	180 ± 13	−11	>0.02
	Test	14	54 ± 5	184 ± 19	113 ± 32	−71	<0.01
Lean	Control	11	26 ± 5	108 ± 10	109 ± 10	+ 1	>0.05
	Test	12	23 ± 4	107 ± 18	68 ± 20	−41	<0.01
Rats	Control	18	276 ± 26	85 ± 9	83 ± 10	− 2	>0.05
	Test	18	285 ± 30	85 ± 8	70 ± 14	−15	<0.01
Fed, saccharin by stomach tube							
Obese	Control	10	52 ± 5	160 ± 25	169 ± 22	+ 9	<0.05
	Test	10	55 ± 5	175 ± 25	183 ± 33	+ 8	<0.05
Lean	Control	10	30 ± 9	105 ± 10	109 ± 21	+ 4	>0.05
	Test	10	27 ± 4	114 ± 6	114 ± 16	0	>0.50
Rats	Control	12	271 ± 23	85 ± 7	78 ± 9	− 7	>0.05
	Test	11	278 ± 19	85 ± 13	87 ± 7	+ 2	>0.05

groups was calculated (Table V). It is apparent that the maximum doses administered chronically were well in excess of the 20 to 50 mg of saccharin indicated in many of the references cited.

II. Acute Experiment

Table VI contains a summary of the data obtained in the acute saccharin experiment. The method of administration of the saccharin and the physiologic status of the animals are defined for each group. It is evident from the data presented here that a hypoglycemic effect was not observed when the saccharin was administered by stomach tube to fed animals nor when animals were in the fasted state.

DISCUSSION

The data presented in this paper indicate that saccharin produces a hypoglycemic effect under certain defined conditions. The hypoglycemic effect is seen in fed normal rats given intraperitoneal injection of 100 mg saccharin, in obese hyperglycemic mice after chronic administration of 150 ± 25 mg saccharin per day, and in fed obese mice and their lean littermates after intraperitoneal injection of 10 mg saccharin.

Furthermore, under no conditions does the administration of saccharin cause an increase in blood sugar greater in magnitude than that observed in their controls, which received water and similar handling. In our opinion the hyperglycemic response reported by Syllaba[12] after administration by stomach tube (per os) may very possibly be due to the stress produced by the trauma of intubation. No controls were run in his experiment to demonstrate the effect of intubation per se.

To our knowledge there is no report in the literature on the effect of a chronic administration of saccharin on the blood sugar. The observation made in this paper—that in this situation hypoglycemic response is elicited in the obese-hyperglycemic mice only—is interesting when one considers the previously men-

tioned findings of Macallum and Sivertz[13] and the fact that the obese-hyperglycemic mice have been shown to have not only an increased pancreatic insulin but also a greater amount of circulating insulin than their non-obese controls.[18] Whether chronic administration would have produced a hypoglycemic effect in the lean mice if the duration of the experiment had been lengthened is not known at present.

It is difficult to explain this phenomenon. The action of saccharin may depend upon a property peculiar to saccharin, or it may act in a mechanism analogous to that of the group of oral hypoglycemic sulfonylureas being studied as possible "insulin substitutes." Several theories have been mentioned:

(1) Saccharin causes a reflex release of insulin mediated by the gustatory nerves. This theory has been proposed by Kun and Hormath[8] and by Jorgenson,[5] and is supported by their observation that the hypoglycemia is produced as readily after washing the mouth with saccharin as by drinking the saccharin solution. It is the "sweet taste" of saccharin which is responsible for its hypoglycemic effect, a property not common to the sulfonamides as a group. The effectiveness of saccharin after intraperitoneal injection would appear to refute this theory.

(2) Proposed mechanisms of the hypoglycemic effect of the sulfonylureas and sulfonamides in general: (a) they effect a release of insulin from the pancreas—i.e. "pancreatropic effect;"[18] (b) they reduce insulin degradation, inhibit "insulinase activity."[19] This theory has been refuted by Vaughan[20] and by Berson *et al.*;[21] (c) they may act as "alpha cell cytotoxins," destroying the cells which produce glucagon and thereby producing a relative excess of insulin;[22] (d) the hypoglycemic agents may reduce the response to epinephrine and/or glucagon or act directly on the phosphoglucomutase or phosphokinase system;[20] (e) there may be a "selective interference with hepatic gluconeogenesis;"[23] and (f) they inhibit glucose-6-phosphatase activity.[24,25]

Against the theory that a mode of action common to saccharin and to the commonly used oral hypoglycemic agents is the fact that it has been demonstrated in this laboratory that carbutamide is ineffective in reducing the blood sugar of the obese-hyperglycemic mice,[17] so that it appears more likely that saccharin may act by a selective and unique mechanism. An explanation of this action must also await further experiments.

SUMMARY

A study was made of both chronic and acute administration of saccharin to obese-hyperglycemic mice, their lean littermates, and adult female Wistar rats. Hypoglycemia was produced in obese-hyperglycemic mice following chronic administration of 125–175 mg saccharin daily; no change in blood sugar was seen in the other animals.

Acute intraperitoneal injection of 100 mg and 10 mg saccharin to fed rats and to the fed mice, respectively, was also effective in lowering the blood sugar. There was no reduction when the same doses were injected into fasted animals or when administration was by stomach tube.

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