Journal of Comparative and Physiological Psychology
1965, Vol. 60, No. 3, 443-446

POTENTIATION OF INSULIN COMA BY SACCHARIN[1]

ELLIOT S. VALENSTEIN AND MARGARET L. WEBER[2]
Fels Research Institute *Biology Department, Antioch College*

Cat 04
4 pages
Code 1
HC 1.00
MF .50

Rats receiving an injection of insulin which was lethal to approximately 50% of untreated animals were permitted to drink either a saccharin, water, or glucose solution. Saccharin potentiated the effect of insulin, as significantly more Ss of this group succumbed than did those in the water group; Ss that had glucose were protected. It may be an error to assume that saccharin is physiologically inert. Speculation concerning the question of why a nonnutritive substance such as saccharin may serve as an effective reward for animals is also presented.

Saccharin, a sweet-tasting but nonnutritive substance, has been used in numerous studies to test the validity of theories which emphasize the importance of a reduction of biological needs for learning. The essential features of the argument may be expressed as follows: Animals prefer a saccharin solution to water and a saccharin solution is an adequate reward in learning experiments (Hausmann, 1933; Sheffield & Roby, 1950) in spite of the fact that this substance is nonnutritive and passes through the body unchanged chemically. Sheffield and Roby (1950), e.g., regarded their saccharin studies as "essentially sham-feeding experiments in which the animal was innately stimulated to ingest a substance which did not change his state of hunger . . . [p. 481]." The observation that animals ingesting saccharin do not diminish their food intake has been viewed as providing further support for this position (Hausmann, 1933). In addition, the fact that a saccharin preference is maintained over repeated tests has led to the conclusion that this substance has not acquired reinforcing properties because of taste similarities with sweet, nutritive substances (Sheffield & Roby, 1950). A similar conclusion was reached as a result of a recent study on the ontogeny of saccharin preference with neonatal rats. Although rat milk contains 2.8% lactose, a saccharin solution is preferred by young rats over a lactose solution (Jacobs, 1964). The importance of a learning factor is also minimized by studies indicating that saccharin preference may be inherited (Nachman, 1959).

It has been concluded, therefore, that saccharin has no significant effect on biological needs as a result of either inherent or acquired properties. The effectiveness of saccharin as a reward, it is generally argued, depends either upon the ability of this substance to elicit a consumatory response which is claimed by some to be a sufficient condition for reinforcement (Sheffield & Roby, 1950; Sheffield, Roby, & Campbell, 1954) or upon inherent reinforcing value of the neural pattern resulting from the stimulation of taste receptors by saccharin (Pfaffmann, 1960).

We would submit that in much of the relevant literature there has been a tendency to assume that because saccharin is nonnutritive it is also physiologically inert. It hardly seems necessary to point out that a substance may pass through the body in an unchanged form without influencing hunger state but nevertheless change the state of the organism in numerous ways. It may be appropriate to recall the conclusion of a series of studies on the physiological action of saccharin completed by Carlson, Eldridge, Martin, and Foran (1923):

> The prevailing view that, except for its action on the organs of taste in the mouth, saccharin is an inert substance, having no action on organs and tissues, is not tenable. Saccharin acting in the mouth decreases appetite gastric secretion, acting in the stomach it increases gastric secretion, and decreases peptic digestion, acting in the small intestine it decreases absorption, acting on the erythrocytes it decreases hemolysis. These actions of saccharin cannot be explained by the osmotic factor.
>
> Saccharin in the blood, in proportion to its concentration, passes into the lymph, cerebrospinal fluid, saliva, tears and mammary secretion [p. 476].

With the above considerations in mind we attempted to determine if saccharin intake had any significant physiological effect. In view of the fact that the amount of saccharin ingested

[1] The authors wish to acknowledge the support of Research Grants MH-4529 from the National Institute of Mental Health, NsG-437 from the National Aeronautics and Space Administration and Career Development Award MH-K6-4947 from the National Institutes of Health.

[2] Now at Psychology Department, Queens College of the City University of New York.

4 pages
Code 1
HC ____
MF ____

is correlated with hunger state (Bacon, Snyder, & Hulse, 1962; Sheffield & Roby, 1950; Smith & Duffy, 1957), we considered the possibility that this substance might influence some aspect of carbohydrate metabolism. It seemed at first that the most direct line of attack would be to examine glucose levels. A search of the literature revealed that while a number of investigators have reported blood glucose decreases (Althausen & Wever, 1937; Ciaccio & Racchuisa, 1927; Jorgensen, 1950; Kun & Horvath, 1947; Thompson & Mayer, 1959), other studies have shown increases (Syllaba, 1930) or no change (Bunde & Lackey, 1948) after saccharin administration. We therefore decided upon another approach.

Method

By means of exploratory tests we determined a dose of insulin (Iletin U-40; Eli Lilly) that resulted in lethal coma in approximately 50% of an injected rat population. Injections were administered subcutaneously into the middle of *S*'s back. Prior to insulin injection *Ss* were treated with either glucose, saccharin, or tap water. As the method of administering these solutions varied, this aspect of the procedure can be more efficiently described with the results. The percentage of *Ss* which succumbed and the time of their death was observed. The solutions used were .25% sodium saccharin and either 10% or 20% glucose. Preliminary testing had indicated that our *Ss* clearly preferred these concentrations to water. As the results with glucose were consistent with a large body of experimental and clinical evidence, we used this substance only in Tests 1 and 5.

Nine separate tests were run and a total of 164 rats were used. For the first two tests albino rats were obtained from the Fels Research Institute colony and for all remaining tests Holtzman albino rats were used. The *Ss* were housed in individual cages during all experimental stages. Purina lab chow, which was fed ad lib, was removed from the cage at the time of insulin injection. The *Ss* weighed 230–330 gm. Males and females were balanced in each group for the first three tests, but as no sex difference was observed, subsequent tests utilized only male animals. The insulin dose level and results are summarized by test in Table 1.

Results

On the first test, 15 *Ss* were divided randomly into three groups. One hour prior to insulin injection water bottles were removed and a glucose solution, a saccharin solution, or tap water was placed in the drinking bottles. The results indicated that although one *S* of the glucose group had a mild convulsion all *Ss* of this group survived. In contrast, 40% of both the saccharin and water groups succumbed following severe convulsions. The only difference between the water- and saccharin-treated *Ss* was in the elapsed time from insulin injection to death; the saccharin treated *Ss* succumbing earlier. As there was great variability in these figures only the average time of the first two *Ss* which succumbed was used in this comparison. This procedure appeared to be justified also by the possibility of individual differences in reaction to saccharin as reported by Williams (1946, p. 71–72).

The *Ss* in the first test had not been deprived of fluid and consequently drank relatively little of the test solutions. In order to encourage drinking, in subsequent tests saccharin, water, or glucose were made available for a 72-hr. pe-

TABLE 1

Reaction to Insulin Injections by Animals Receiving Different Solutions

Test	Insulin dose[a] (cc/kg)	Saccharin			Water			Glucose	
		Survivors	Deaths	Mean time of first two deaths (in min.)	Survivors	Deaths	Mean time of first two deaths (in min.)	Survivors	Deaths
1	.775	3	2	206.5	3	2	322.5	5	0
2	.775	0	6	217.5	2	4	222.5		
3	.600	0	7	194.0	0	7	231.0		
4	.400	4	0	—	4	0	—		
5	.500	1	7	182.0	3	5	219.0	5	0
6	.500	4	6	180.0	6	4	196.5		
7	.500	1	11	160.0	3	9	184.0		
8	.475	2	10	184.0	6	6	203.0		
9	.475	4	9	163.0	6	7	168.0		
Totals		19	58		33	44		10	0

[a] Iletin U-40 (Eli Lilly & Company). Injections were administered between 9:30 and 10:30 A.M.

ried. Then *Ss* were deprived of fluid for 24 hr. prior to receiving the insulin injection. The solutions were made available again 1 hr. after the insulin injection and remained available to *Ss* throughout the experiment. No significant difference in the amount of water or saccharin consumed was observed after the insulin injection.

In the second test, 100% of the saccharin-treated *Ss* succumbed to the insulin injection while only 66⅔% of the *Ss* receiving water succumbed. The difference in time of the first two deaths was in the same direction as noted in Test 1.

For practical reasons it was necessary to use Holtzman strain rats for all subsequent tests. A pilot study revealed that these *Ss* were more sensitive to insulin than the Fels strain. Although we adjusted the dose level, Tests 3 and 4 were not sensitive as too much insulin was administered in Test 3 and too little in Test 4. All *Ss* succumbed in the former case and all survived in the latter. We have included these data in Table 1 to illustrate that the time of death in Test 3 was again shorter for the saccharin-treated *Ss*. For subsequent tests an intermediate dose level of insulin was used.

The same procedure was used for Tests 5–8 save that a smaller insulin dose level was used in Test 8 in order to decrease the percentage of deaths and in Test 5 a 10% glucose group was added to demonstrate its protective value against insulin coma with the Holtzman strain rats. To insure that *Ss* had at least a minimum of solution in their bodies, 1.5 and 2.5 hr. after the insulin injection, *Ss* received 1-cc injections (ip) of either the saccharin, physiological saline, or glucose solutions. Test 9 was identical to Test 8 except that the ip injections were omitted.

Tests 5–9 produced a consistently greater percentage of deaths for the saccharin group (Table 1). The difference in this percentage between the saccharin and water *Ss* ($N = 154$) in all 9 tests was statistically significant ($\chi^2 = 5.7$, $p < .02$) even though Tests 3 and 4 were insensitive. The results from Tests 2 and 9 in which ip injections of saccharin and saline were omitted suggest that this aspect of the procedure was not essential. Also to be noted is the fact that in every test the average time of the first two deaths was shorter for the saccharin treated *Ss*. This result is statistically significant (binomial expansion; $p < .01$).

Discussion

After completion of these experiments, work by Macallum and Sivertz (1942) came to our attention. Our results are in complete agreement with this earlier study. Macallum and Sivertz had shown that rabbits receiving 1.5 units of insulin/kg do not have convulsions and blood sugar level returns to normal in 4–6 hr. The same dose of insulin plus saccharin accelerates the decline of blood sugar and produces convulsions persisting 6–10 hr. In another related study Macallum (1948) showed that sulphones "increased sensitivity to insulin, both in rate of fall of blood sugar levels and maintenance of hypoglycaemia [p. 232]."

Although the ability of saccharin to potentiate the effect of insulin with rats and rabbits seems to be clear,[3] we can only speculate at this time about the physiological mechanisms which may be involved. The possibility that the sweet tasting saccharin reduces food intake and thereby glycogen storage is not supported by the observation that saccharin does not decrease the amount of food ingested (Hausmann, 1933; Smith & Duffy, 1957). Another possible hypothesis is that insulin is released from the pancreas in response to a saccharin stimulus, an effect which could explain the finding that exogenous insulin is more effective in these cases. An alternative hypothesis which has been advanced is that the sulfonylureas are inhibitors of an enzyme (insulinase) which destroys insulin. The effect, however, would be the same, namely an increase in available insulin, which would be expected to be accompanied by a decrease in blood sugar level. Although the majority of previous investigators have found such a decrease, as indicated above, blood glucose changes in all directions following saccharin administration have been reported as well. It is possible that a glycogenolytic response to an initial hypoglycemia could be concealing this relationship and a continuous monitoring of blood sugar change, rather than sampling, might resolve the conflict. We are exploring this possibility in our laboratory.

Whether this finding contributes to our understanding of why saccharin is reinforcing to animals must also remain speculative at this

[3] In a footnote to a paper, Smith and Capretta (1956) mentioned that saccharin appeared to protect mice from insulin coma. It is not clear to us why results with the mouse should have been opposite those obtained with the rat and rabbit. The mouse is especially sensitive to insulin, but this fact by itself would not account for this difference in reaction. A personal communication with the senior author revealed that these observations with the mouse were only exploratory and that great variability in reaction between animals was most striking.

time. We would like to suggest for further consideration the possibility that saccharin in the mouth or gastrointestinal tract may trigger a number of internal responses that duplicate in significant respects the pattern seen with sugar intake. Such visceral reactions as insulin secretion in response to sugar in the mouth and GI tract have been called "preparative metabolic reflexes." Recently, Elrick, Stimmler, Hlad, and Arai (1964) have shown that the plasma insulin response to oral and intravenous administration of glucose differs. The greater and more sustained increase in insulin with oral glucose was attributed to the additional stimulus to insulin secretion triggered by alimentary glucose. Such responses which may be equally triggered by saccharin could serve as either conditioned or unconditioned reinforcing stimuli. We would like to emphasize that the evidence presented here certainly does not preclude the possibility that the neural pattern resulting from the stimulation of taste receptors by saccharin may have motivating properties, but it suggests that it may be erroneous to assume that saccharin has no other physiological effects.

REFERENCES

ALTHAUSEN, T. L., & WEVER, G. K. Effect of saccharin and of galactose on the blood-sugar. *Proc. Soc. Exp. Biol. Med.*, 1937, **35,** 517–519.

BACON, W. E., SNYDER, H. L., & HULSE, S. H. Saccharin preference in satiated and deprived rats. *J. comp. physiol. Psychol.*, 1962, **55,** 112–114.

BUNDE, C. A., & LACKEY, R. W. Failure of oral saccharine to influence blood sugar. *Proc. Soc. Exp. Biol. Med.*, 1948, **68,** 581–582.

CARLSON, A. J., ELDRIDGE, C. J., MARTIN, H. P., & FORAN, F. L. Studies on the physiological action of saccharin. *J. met. Res.*, 1923, **3,** 451–477.

CIACCIO, C., & RACCHUISA, S. The action of saccharin in various doses in hyperglycemia by dextrose. *Boll. Soc. Ital. Biol. sper.*, 1927, **2,** 323.

ELRICK, H., STIMMLER, L., HLAD, C. J., & ARAI, Y. Plasma insulin response to oral and intravenous glucose administration. *J. clin. endocrinol. Metab.*, 1964, **24,** 1076–1082.

HAUSMANN, M. F. The behavior of albino rats in choosing foods. II. Differentiation between sugar and saccharin. *J. comp. Psychol.*, 1933, **15,** 419–428.

JACOBS, H. L. Observations on the ontogeny of saccharine preference in the neonate rat. *Psychon. Sci.*, 1964, **1,** 105–106.

JORGENSEN, H. The influence of saccharin on the blood-sugar. *Acta physiol. Scand.*, 1950, **20,** 33–37.

KUN, E., & HORVATH, I. The influence of oral saccharin on blood sugar. *Proc. Soc. Exp. Biol. Med.*, 1947, **66,** 175–177.

MACALLUM, A. B. The potentiation of insulin by sulphones. *Canad. J. Res.*, 1948, **26,** 232–238.

MACALLUM, A. B., & SIVERTZ, C. The potentiation of insulin by sulfones. *Canad. Chem. Process Indust.*, 1942, **26,** 569.

NACHMAN, M. The inheritance of saccharin preference. *J. comp. physiol. Psychol.*, 1959, **52,** 451–457.

PFAFFMANN, C. The pleasures of sensation. *Psychol. Rev.*, 1960, **67,** 253–268.

SHEFFIELD, F. D., & ROBY, T. B. Reward value of a non-nutritive sweet taste. *J. comp. physiol. Psychol.*, 1950, **43,** 471–481.

SHEFFIELD, F. D., ROBY, T. B., & CAMPBELL, B. A. Drive reduction versus consummatory behavior as determinants of reinforcement. *J. comp. physiol. Psychol.*, 1954, **47,** 349–354.

SMITH, M. H., & DUFFY, M. Consumption of sucrose and saccharine by hungry and satiated rats. *J. comp. physiol. Psychol.*, 1957, **50,** 65–69.

SMITH, M. P., & CAPRETTA, P. J. Effects of drive level and experience on the reward value of saccharine solutions. *J. comp. physiol. Psychol.*, 1956, **49,** 553–557.

SYLLABA, G. The influence of saccharine on the respiratory exchange and on the blood-sugar level—An example of the conditioned reflex. *Guy's Hosp. Rep.*, 1930, **80,** 230–233.

THOMPSON, M. M., & MAYER, J. Hypoglycemic effects of saccharin in experimental animals. *Amer. J. clin. Nutr.*, 1959, **7,** 80–85.

WILLIAMS, R. J. *The human frontier.* New York: Harcourt, Brace, 1946.

(Received January 4, 1965)