# Biological effects of saccharin ingestion in rats: A preliminary report[1]

*N. R. REMLEY and E. H. HARRELL, Texas Christian University, Fort Worth, Tex. 76129*

*The purpose of this study was to investigate the biological effects of saccharin ingestion on serum FFA and blood glucose levels in rats. Analysis of the data revealed a statistically significant effect on serum FFA levels, but not on blood glucose levels. The results also indicate that the effect is influenced by previous experience and the degree of water deprivation.*

Many studies have been reported concerning the reinforcing effects of saccharin ingestion. Most authors have obviously assumed that because saccharin has no nutritive value, the ingestion of saccharin has no biological effect. Collier & Novell (1967) concluded that the regulation of saccharin ingestion cannot be accounted for on the basis of taste or osmotic factors alone. They suggested that the physiological consequences of saccharin ingestion mimic those of sucrose ingestion.

Valenstein & Weber (1965) reported that saccharin ingestion potentiated the occurrence of insulin coma in rats. These investigators suggested that saccharin ingestion results in an increase in insulin activity. Since other investigators had reported inconsistent changes in blood glucose levels following saccharin ingestion, Valenstein and Weber concluded that even though saccharin ingestion apparently altered carbohydrate metabolism, blood glucose levels would not be an appropriate physiological measure of the biological effects of saccharin ingestion.

Glucose, however, is not the only circulating metabolite that is influenced by changes in carbohydrate metabolism. Changes in the levels of the metabolically active forms of fat, free fatty acids (FFA), are known to be intimately related to changes in carbohydrate metabolism (Tepperman, 1962). Serum or plasma FFA levels have been shown to increase with food deprivation (Dole, 1956), exposure to cold (Mager & Iampietro, 1966), and excercise (Gollnick, 1967); FFA levels have been reported to decrease after eating,[2] as a result of increases in glucose utilization (Van Itallie & Hashim, 1960), and blood amino acid increases (Hashim & Van Itallie, 1959). As a matter of fact, Van Itallie & Hashim (1960) have suggested that FFA levels are more reliable indicators of glucose utilization than either blood glucose levels or arterio-venous glucose differences.

The purpose of the present study was an attempt to evaluate the possible biological effect of saccharin ingestion by measuring serum FFA and blood glucose levels after saccharin ingestion.

## SUBJECTS

A total of 100 male albino Holtzman rats were used in two experiments. The Ss weighed approximately 300 to 350 g at the beginning of the experiments.

## APPARATUS

The test solutions included a 10% sucrose solution, a 0.075% saccharin solution, and tap water. All solutions were presented in calibrated eudiometers. The Ss were tested in their home cages in the colony room.

## PROCEDURE

The procedures for the two experiments were identical except that in one experiment the Ss were deprived of water for 2 h prior to testing, and in the other experiment the Ss were deprived of water for 23½ h prior to testing. All Ss were deprived of water and then allowed access to the solutions for 30 min. The procedure was repeated the next day. This procedure was used because of the possibility that if saccharin ingestion did have a biological effect, the effect might have been a conditioned one. That is, it may have been dependent on the Ss having had previous experience with either a sweet nutritive or sweet nonnutritive substance. As a result, five groups of Ss were run in each experiment. Each group consisted of 10 Ss. The groups differed as to which solutions were presented in the test periods on the 2 days. One group received tap water on both days (W-W group). One group received tap water on the first day and the sucrose solution on the second day (W-Su group). One group received tap water on the first day and the saccharin solution on the second day (W-Sac group). One group received the sucrose solution on the first day and the saccharin solution on the second day (Su-Sac group). The remaining group received the saccharin solution on both days (Sac-Sac group).

Immediately after the end of the second test period, the Ss were anesthesized with sodium pentobarbital, and blood was withdrawn from the left ventricle of the heart. Serum FFA levels were determined by the methods of Duncombe (1963) as modified by Itaya & Ui (1965). Blood glucose levels were determined by the Somogyi-Nelson method.[3]

## RESULTS

The results of the serum FFA analyses not only support the conclusion that saccharin ingestion does have a biological effect, but also suggest that the magnitude of the effect is influenced by previous experience and the degree of water deprivation. These data are represented in Fig. 1. In the 2-h deprivation study, the effect is statistically significant ($p < .05$), with the effect being attributable to the W-Sac and the Sac-Sac groups. But in the 23½-h deprivation study, the effect is not statistically significant ($p > .10$). However, there does appear to be a replicable order effect, with the Ss consuming saccharin tending to have the higher serum FFA levels. The only exception is the Su-Sac group in the 2-h deprivation study.

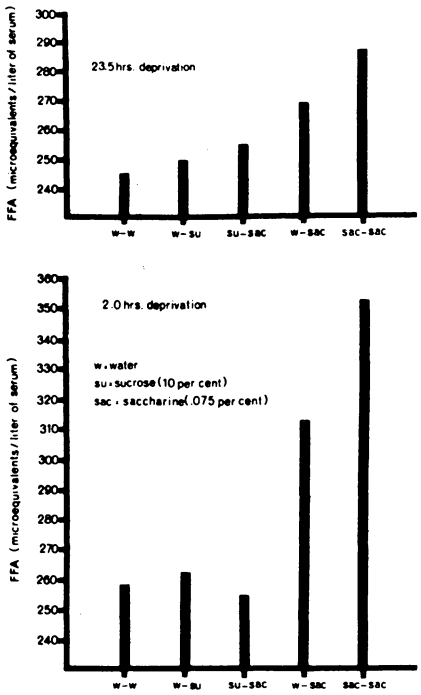


**Fig. 1. Serum FFA levels following the consumption of water (W), sucrose (Su), and saccharin (Sac). The letters designate the order in which the solutions were presented to each group on the 2 test days.**

The results of the blood glucose analyses, on the other hand, do not show as clearly the biological effect of saccharin ingestion (Fig. 2). The only statistically significant effect is associated with the W-Su group in the 2-h deprivation study. In both studies, the Ss consuming sucrose on the second test day had the highest blood glucose levels. The blood glucose levels for the Ss consuming saccharin on the second test day tended to be higher than the blood glucose levels for the Ss consuming water, but the differences were not statistically reliable. The blood glucose data appear to support the suggestion of Valenstein & Weber (1965) that blood glucose level is not an appropriate measure of the biological effects of saccharin ingestion. However, in this study,

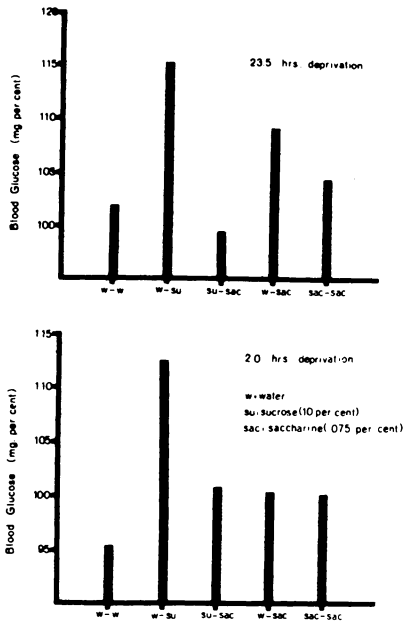


**Fig. 2. Blood glucose levels following the consumption of water (W), sucrose (Su), and saccharin (Sac). The letters designate the order in which the solutions were presented to each group on the 2 test days.**

blood glucose was determined by the Somogyi-Nelson method. This method is not glucose specific; it measures all reducing sugars. It is possible that glucose-specific methods may give more meaningful data.

## DISCUSSION

Our original hypothesis was that saccharin ingestion, if saccharin had a biological effect, would result in elevated serum FFA levels. This hypothesis was based on the assumption that sucrose ingestion would result in a decrease in glycogenolytic activity because of the availability of exogenous glucose. If this decrease in glycogenolytic activity could be triggered by oral or taste factors, then we assumed that saccharin ingestion, because of the similarity of taste quality to sucrose, would also trigger a similar decrease in glycogenolytic activity. But in this case, because there would be no exogenous glucose available for oxidation, we predicted that glucose utilization would decrease, thereby raising the serum FFA level.

Valenstein & Weber (1965), on the other hand, offered two alternative hypotheses. First, they offered the possibility that the oral response to saccharin stimulates the release of insulin from the pancreatic beta cells. Alternately, they suggested the possibility that saccharin inhibits insulinase, an enzyme that destroys insulin. Both of their hypotheses would result in the same effect, an increase in the availability of indogenous insulin. If either one of their hypotheses is correct, then one would expect both lower than normal blood glucose and serum FFA levels as a result of saccharin ingestion. This would be the case because insulin not only increases the rate of glucose utilization, but also stimulates the synthesis of triglycerides from FFA, thereby removing FFA from the blood. Our data do not appear to support either one of their hypotheses.

Goldfine, Ryan, & Schwartz (1969), using human Ss, measured blood glucose and insulin levels after the Ss had consumed a diet cola containing saccharin. They reported no change in either blood glucose or insulin levels. These data question the validity of either one of the hypotheses put forth by Valenstein & Weber (1965) for the mechanism of the physiological effect of saccharin ingestion.

On the other hand, the data presented in Fig. 1 suggest that our hypothesis for the physiological effects of saccharin ingestion may also be questionable. In the first place, the FFA levels recorded were not abnormally high. In fact, if anything, the FFA levels of most of the groups are below normal. In our laboratory, the mean FFA level in normal, nondeprived animals is 307 microequivalents/L of serum. The low serum FFA levels recorded in this study could possibly be accounted for by insulin-induced inhibition of lipolysis. However, the most probable explanation is that the low serum FFA levels are the result of the recovery of water deprivation. Previous research has shown that the serum FFA levels in food-deprived rats do drop below the normal range after the consumption of food.[2] Therefore, the data represented in Fig. 1 are probably the result of an interaction of the effects of saccharin ingestion and the effects of recovery from water deprivation. The fact that the greatest effect occurred after only 2 h of deprivation supports this possibility.

When the present data and those of Valenstein & Weber (1965) are considered together, it appears that saccharin ingestion does have a biological effect. However, the data presented in this study suggest that the effect is not a simple one. At least four factors would appear to influence the quality and/or quantity of the effect. First, the effect appears to be a function of the degree, and possibly the type, of deprivation. When studying the physiological effects of an ingested substance, it is probable that a deprived organism will physiologically respond differently than a nondeprived organism. It is known that the almost total decrease in lipogenesis in a food-deprived organism cannot be instantly repaired by the immediate availability of glucose (Tepperman, 1962). Apparently, enzyme suppression occurs during deprivation, and the resynthesis of the enzymes necessary for the lipogenic process is time consuming.

Second, the magnitude of the effect appears to be a function of previous experience. Valenstein & Weber (1965) reported the greatest effect when animals were allowed access to saccharin for 72 h prior to receiving insulin injections. The data in Fig. 1 show that those Ss with previous experience with saccharin have the highest serum FFA levels. The data also indicate that those Ss that consumed saccharin with prior experience with sucrose had serum FFA levels similar to those Ss consuming sucrose.

Third, it is probable that an organism's physiological response to saccharin ingestion varies according to how long he continues to drink a saccharin solution. Valenstein & Weber (1965) suggested that the effect may be cyclic in nature. Data concerning the time course of the effect is obviously needed.

And fourth, a dose-response curve of the biological effects of different concentrations of saccharin also needs to be known. In the present study, a very low saccharin concentration was used (0.075%). Valenstein and Weber reported much more dramatic effects using a 0.25% solution.

More research is obviously needed before the exact mechanism of the biological effect of saccharin ingestion can be specified. The present authors would agree with Valenstein & Weber (1965) that blood glucose levels are not the appropriate response measure in this situation.

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NOTES

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2. Walker, D. W., & Remley, N. R. The relationships among percentage body weight loss, circulating free fatty acids, and consummatory behavior in rats, in preparation.

3. Sigma Chemical Co. Technical Bulletin No. 14.

# Behavioral selectivity in tropical fish

*H. A. CROSS,[1] L. J. LAUX, J. C. WRIGHT, V. J. PEZOLDT, J. J. LOWENSTEIN, T. D. VINCENT, and N. W. KING, Wittenberg University, Springfield, Ohio 45501*

*Three strains of tropical fish belonging to the same genus,* Xiphophorus, *were given the opportunity to view stimulus fish of the same or differing strains in a free-choice preference situation. The test Ss spent significantly more time with their own kind, indicating a behavioral selectivity which is consistent with previously observed biological selectivity.*

Although tropical platyfishes and swordtails belong to the same genus, *Xiphophorus*, and will interbreed when restricted, they never, or almost never, cohabit in their natural habitat (Clark, Aronson, & Gordon, 1964). Kallman (n.d.) reports that not a single natural hybrid has ever been discovered. This has been the case even when more than 50,000 fish have been collected together. The question must naturally arise as to the mechanisms which support this selectivity. A number of possible factors have been elaborated, and include: different preferences with respect to ecological niche; finer differentiation within the micro environment; small differences in the shape and size of the female genital papillae; and possible differences in behavior patterns (Kallman, n.d.).

This study was designed to find out if the sexual selectivity observed by the biologist could be translated into a behavioral discrimination in a situation in which a fish was afforded an opportunity to justapose himself alongside a stimulus S of his own strain or fish of the same genus but of different strain. The free-choice preference method (Sackett, 1966; Cross, Halcomb, & Matter, 1967) was employed throughout. If the previously described exclusion is apparent in the behavioral situation, refined tests become possible. In short, a methodology will then be available which, potentially at least, could provide evidence concerning the relative effect upon preference behavior of a number of physical and behavioral variables, including: coloration, odor, physical shape and size, bodily orientation, and movement.

## EXPERIMENT 1

### Method

The test (T) Ss were 36 tropical fish: 12 *Xiphophorus maculatus* (Gold crescent moon); 12 *Xiphophorus maculatus* (Red calico moon); and 12 *Xiphophorus hellerii* (Green swordtail). Half of the Ss of each strain were male and half were female. Three males and three females of each strain were housed in individual 3-gal aquaria, making a total of 18 "isolated" fish. The remaining 18 fish were "social" Ss and were housed as follows: one fish of each strain in a 3-gal tank, such that half of the tanks contained one male and two females, and half contained one female and two males. Two isolated Ss, one female crescent and one male swordtail, died during the testing, and the subsequent statistical analysis involved only 34 fish. In addition to the T Ss, there were 12 male fish, 4 of each strain, who served as stimulus or object (O) Ss. These O Ss were housed socially, with each strain represented once in the home tank.

There were two identical test tanks, each consisting of a 10-gal aquarium measuring 20½ x 10½ x 11¾ in. The test tanks were divided in half by length with wire screen. One side was further divided into four equal compartments, each measuring 5 x 4¾ in. These smaller compartments served as stimulus areas and housed the male O fish. The other side comprised the T S's compartment and measured 19¼ x 4¾ in. The stimulus compartments were divided by opaque glass which extended through the screen 2½ in. into the T S's area. Balsa-wood inserts completely covered the interior of the 10½ x 11¾ in. sides, but the front side was open to E for easy observation of T fish. The water of all tanks was maintained between 72 and 78 deg F. A fluorescent tube was affixed 8 in. above the stimulus compartment of both test tanks. Each apparatus was accompanied by a recording console, consisting of four electric clocks that were activated by a four-position selector switch, manually operated by E.

On a given test, a male of each strain was selected from the O Ss according to a random schedule. The three O Ss were placed in one of the four stimulus compartments according to a random, but balanced, schedule. One compartment was empty and was the "start" compartment. A given set of O Ss served nine T Ss on a given day. All 12 O Ss were used each day of testing. A trial started immediately when a T S was placed in the test tank opposite the empty or "start" compartment and continued for 8 min. The measure of stimulus preference used was the total time a T S was in each stimulus area. The S was judged in a given area when two-thirds of its body was within the compartment.

The experimental design was a five-factor analysis of variance, involving sex, strain, and isolation condition of the T Ss as nesting variables and strain of the O Ss and blocks as repeated-measure variables. Thus, a given T S represented one of three strains, was male or female, and was isolated or social in his home tank. Test trials were continued over a 3-week period with all T Ss tested the first 4 evenings of each week. After 4 test days, one block, there was a 3-day period of no testing. This continued until all T Ss had completed 12 trials.

### Results and Discussion

An unequal-n analysis of variance, using total time scores for each block, was completed. Table 1 shows the mean daily time scores (in seconds), collapsed over blocks, that the test strains spent with the various O strains. An $\alpha$ level of .025 was imposed, with the result that the expected interaction of T by O Ss was significant ($F = 3.51$, $df = 4/44$, $p < .025$). The T Ss by O Ss by Block interaction was also significant ($F = 2.49$, $df = 8/88$, $p < .025$) but did not present a clear picture. No other F tests were significant.

The results were somewhat as predicted, but only the calico Ss consistently selected against Ss of the "outgroup." Experiment 1 gave an opportunity to observe a number of things. It became apparent that the fish kept in isolation were not being strictly deprived

Table 1
Mean Daily Total Time (Seconds)

| | Object Fish | | |
|---|---|---|---|
| Test Fish | Crescent | Calico | Swordtails |
| Crescent | 105.68 | 128.12 | 99.54 |
| Calico | 111.75 | 120.52 | 104.31 |
| Swordtails | 136.77 | 105.36 | 138.76 |