Symposium Paper

Saccharin as a Sugar Surrogate Revisited

Proceedings of a Fest on 18 October 2001 at Columbia University, New York
Honouring the scientific career of George H. Collier, PhD

Guest Editor: Anthony Sclafani

Saccharin as a sugar surrogate revisited

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Two papers by George Collier are reviewed and replications and extensions of these data are presented. The first paper by Collier and Bolles (1968) reported the total caloric intake of rats during sucrose versus water preference tests. In addition, a pairwise comparison was made with each of a wide range of sucrose solutions. The latter experiment resulted in a re-thinking of "preference" in that it showed that although rats drank more of a middle-range concentration, they always consumed more of the higher concentrations in pairwise comparison tests. Many other behavioral studies have confirmed that the rat's attraction to the taste of sucrose is a direct function of sucrose concentration.

The second paper by Collier and Novell (1967) reported that saccharin was similar to sucrose in that intake increased and then decreased as concentration increased, although in direct choice tests, higher concentrations were preferred to lower ones except in one case. Subsequent studies using a wider range of saccharin concentrations and a variety of test measures revealed, however, that saccharin preference and acceptance decreases substantially as concentration exceeds 0.4% (19.5 mM). Furthermore, saccharin versus sucrose choice tests indicate that optimal saccharin solutions (0.2-0.4%) are "isoprefere" to only dilute sucrose solutions (0.4%). Thus, at best, saccharin is only a weak surrogate for sugar.

Introduction

During a long and productive scientific career, George Collier has made many important contributions to the study of ingestive behavior. Perhaps best known are his landmark studies on the ecological and economic determinants of foraging and meal patterns in rats and other species (Collier et al., 1972). This work is the subject of several contributions to this Festchrift and is not further discussed here. Prior to developing his laboratory-based model of foraging behavior, Collier initiated a series of important studies on the motivational and ingestive responses to sugar and saccharin which are revisited and updated in this paper.

Sucrose acceptance and preference

In the seminal paper on ingestion of sugars by rats, Richter and Campbell (1940) first reported the inverted U-function for sugar intake. In this study, rats were given two-bottle preference tests with various sugars (sucrose, glucose, maltose, galactose, lactose) vs. water at different concentrations in an effort to measure taste thresholds and maximum sugar preferences. In the case of sucrose, the rats drank little or no water (except at concentrations below 0.4% and above 30%) so that these tests were essentially not preference tests at all but measures of the absolute intake or "acceptability" of the various sucrose concentrations. The rats drank the most sucrose at an 8% concentration and drank less at lower and higher concentrations, resulting in the inverted

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U concentration-intake function. Richter and Campbell (1940) concluded that rats showed the maximum preference for sucrose at the 8\textsuperscript{th} (0.23 M) concentration. They also reported a preference threshold concentration for sucrose at 0.57\textsuperscript{th} (0.017 M). Their basic intake curve for sucrose as a function of concentration has been replicated in many other laboratories although the peak intake concentration varies somewhat from study to study (e.g., Cagan & Maller, 1974; Collier & Bolles, 1968; Sclafani & Nissenbaum, 1987; Smith & Rashotte, 1978; Young, 1949). By more sophisticated psychophysical methods, the absolute detection threshold for sucrose has been determined to be about one order of magnitude less than the preference threshold value reported by Richter and Campbell (e.g., Thaw & Smith, 1992).

In a 1968 paper, George Collier and Robert Bolles significantly advanced our understanding of the determinants of sucrose intake. They noted that the relative intake of sugar solutions differing in concentration is typically taken as a measure of palatability, but that different test procedures yield different preference functions (e.g., Young, 1949). Prior investigators proposed that the satiating and caloric effects of the solution could explain the conflicting data, and Collier and Bolles (1968) introduced a third consideration: the contribution of the consumed sugar to the animal's total nutritional requirements. In their first experiment, they measured the rat's two-bottle intake of sucrose and water as a function of sugar concentration (4 \textsuperscript{th} to 1 M). In addition, they also measured caloric intakes and determined the proportion of energy consumed as sugar and as lab chow. In confirmation of Richter and Campbell (1940), Collier and Bolles (1968) reported that rats drank more of the 8\textsuperscript{th} sucrose solution than of less or more concentrated solutions. However, in contrast to Richter and Campbell (1940), they found that the amount of sucrose solution consumed increased with increasing concentration to 16\textsuperscript{th} and then leveled off. The intake of chow dropped as sugar concentration increased such that the proportion of total caloric intake based on sucrose reached about 60\textsuperscript{th} with concentrations of 16\textsuperscript{th} and higher. These findings indicated to Collier and Bolles (1968) that a major determinant of the amount of sucrose solution consumed in 24-h tests was the requirement to maintain a fixed proportion of total calories as sucrose. Note that Collier and Bolles (1968) observed that total caloric intake remained unaffected by the availability of sucrose solutions, but in subsequent studies from the Collier laboratory (Castonguay et al., 1981) as well as from many others (see Sclafani, 1987), rats increased their total energy intake by 20\textsuperscript{th} or more when given ad libitum access to chow and concentrated sugar solutions.

The laboratory of J. C. Smith has replicated these sucrose solution and solute results in many studies (Smith & Rashotte, 1978; Spector & Smith, 1984; Smith, 1988, 2000). For example, Smith and Rashotte (1978) reported sucrose solution intake peaked at a concentration of 0.2 M (7\textsuperscript{th}) and then declined, whereas sucrose solute intake reached an asymptote at 0.5 M (17\textsuperscript{th}) (Fig. 1A and B). Similar results were obtained from weaning to old age in Fischer 344 rats (Smith & Wilson, 1989). These data from Collier and Bolles and the Smith lab call into question Richter and Campbell's conclusion that an 8\textsuperscript{th} concentration represented the peak preference for sucrose. Rather these studies show that rats drink more of the middle range solutions, but consume more solute (sucrose) from the higher concentrations than from the lower ones.

![Figure 1](image-url)

**Figure 1.** Ingestive responses of rats to sucrose solutions as a function of concentration and behavioral test measure. (A) Sucrose solution intake during 24-h-day two-bottle tests with water at sucrose concentrations of 0.03-1.0 M (1-32\textsuperscript{th} molar) (from Smith & Rashotte, 1978). (B) Sucrose solute intake during 24-h-day two-bottle tests with water at sucrose concentrations of 0.03-1.0 M (from Smith & Rashotte, 1978). (C) Rate of licking during sucrose drinking bouts recorded during 24-h/day two-bottle tests with water at 0.03-1.0 M concentrations (from Smith, 2000). (D) Licks emitted during 30-s periods in the Davis Rig at sucrose concentrations of 0.03-1.0 M (Smith, unpublished data). (E) Sucrose solution intake during 30-min/day sham-feeding tests at concentrations of 0.03-0.94 M (1-32\textsuperscript{th}) (from Nissenbaum & Sclafani, 1987).
Collier and Bolles (1968) put the question of peak preference to rest in the last experiment of their study. Here they performed a paired-comparison analysis in which 4%, 8%, 16%, and 32% sucrose solutions were compared with each other in 24-h two-bottle tests. The results showed that the greater amount of solution was always consumed from the higher of the two concentrations. When studied with these real "preference" tests, the order of preference for sucrose solutions was 32% > 16% > 8% > 4%. Similar results were reported by Smith and Rashotte (1978). Thus, when given a direct choice, rats preferred the higher concentrations of sucrose, although in sucrose vs. water tests caloric factors limited the amount the animals could consume of the concentrated solutions.

Interestingly, Young (1949) reported in an early study that deprived rats preferred 30% sucrose to 4% sucrose in "immediate" choice tests that are very brief (less than 1 min). Yet, he concluded that in 24-h tests, rats preferred 4% to 50% sucrose based on the finding that they consumed more of the 4% solution than the 50% solution when given the choice of sucrose vs. water. Young and Greene (1953) studied a wider range of sucrose solutions using the immediate choice method and reported that the rats' order of preference was 70% > 36% > 18% > 9%. They also observed rats to prefer 36% to 9% sucrose when given the choice of these solutions in 1-h two-bottle tests, although the rats consumed more 9% sucrose than 36% sucrose in one-bottle tests. In their discussion, Young and Greene (1953) clearly distinguished between two-bottle tests which measure preference and one-bottle tests which measure the relative acceptability of solutions, and noted that one-bottle tests do not necessarily predict two-bottle preference. Collier and Bolles (1968) extended this analysis to 24-h tests and, most importantly, demonstrated that two-bottle sucrose vs. water and sucrose vs. sucrose tests also produce quite different results. As noted above, with highly preferred substances such as sucrose, two-bottle tests that involve water as the alternative choice are essentially the same as one-bottle acceptance tests.

A number of other behavioral measures indicate that the rat's attraction to sucrose is a direct function of concentration in both long- and short-term tests. In a study by Smith (2000) drinking patterns were recorded in animals given 24-h water vs. sucrose tests at sugar concentrations of 0.03 - 1.0 M. As in earlier work, sucrose solution intake increased as concentration increased to 0.25 M (~8%) and then decreased. However, the rate of licking within a bout of drinking increased as a direct function of sucrose concentration (Fig. 1C). This "integrated" lick rate was obtained by dividing the number of licks within bouts by the duration of that bout in minutes.

As first reported by Davis (1973), lick rates measured in brief access tests (0.5 - 3 min) also increase as a direct function of sugar concentration. Davis and Smith (1992) subsequently measured lick microstructure by recording the timing and distribution of individual licks within a drinking bout. They observed that licks occurred in bursts and clusters with a burst defined as a group of licks separated by pauses of 230 ms or longer, and a cluster defined as a group of bursts separated by pauses of 500 ms or longer. As the case of the integrated lick rate (licks per minute of drinking), the size of lick bursts and clusters increased as sucrose concentration increased.

Smith (unpublished study) obtained similar lick data using a brief access procedure that allowed all sugar concentrations to be examined within the same test session. This was accomplished using the "Davis Rig" (Smith, 2001). Briefly, eight bottles equipped with stainless steel drinking tubes were mounted on a motorized tray which moved under computer control so that any one of the tubes was aligned with a drinking port in the front of the test cage. Rats were initially trained to drink in this apparatus with all the tubes containing 0.25 M sucrose. They had access to each tube for 30 s beginning with the first lick, and there was a 30-s delay between tube presentations. When the rats readily licked on all eight presentations of 0.25 M sucrose, testing began under nondeprived conditions. For these tests, seven drinking tubes were used which contained water, 0.05, 0.06, 0.125, 0.25, 0.50 and 1.0 M sucrose. The tubes were presented for 30-s trials in an ascending order for four consecutive days. As shown in Fig. 1D, the number of licks emitted during the 30-s trials increased directly with sucrose concentration. Analysis of the lick microstructure during these short trials revealed that lick burst size increased as sucrose concentration increased to 0.25 M and then leveled off.

The value of brief access tests is that they provide a measure of the ingestive response to the taste of sugar with postigestive factors minimized by the limited consumption during the test. This is also accomplished in sham-feeding tests in which rats drink a sugar solution with an open esophageal or gastric fistula that allows the ingested solution to drain out the gastrointestinal tract with little or no sugar being absorbed. Several studies demonstrate that the amount of sucrose shunt-fed increases monotonically with sucrose concentration (Davis et al., 2000; Nissenbaum & Selafian, 1987; Weingarten & Watson, 1982); an example of this is presented in Fig. 1E. In contrast, when rats "real" feed sucrose, i.e. with a closed fistula, sucrose intake increases then decreases at higher concentrations as postigestive feedback limits intake. This is the same pattern observed in intact rats tested 24-h day or 30-min day.

**Saccharin acceptance and preference**

Using procedures parallel to those of the Collier and Bolles (1968) study, Collier and Novell (1967) investigated the rat's acceptance and preference for the non-nutritive sweetener sodium saccharin. The question of interest was whether saccharin intake, like sucrose intake, would increase and then decrease as saccharin concentration increased. Since saccharin is non-nutritive and hypotonic at the standard concentrations used, postigestive factors should not influence saccharin intake as a function of concentration. Yet, in 24-h two-bottle tests with water vs. saccharin at concentrations ranging from 0.011% to 2.7% (0.5-131.6 mM) Collier and Novell (1967) observed an inverted U-function. Saccharin intake increased as concentration increased to 0.1% and then decreased as concentration was raised to 0.3%, 0.9% and 2.7% (Fig. 2A). Saccharin solution intake also increased with concentration.
and then sharply decreased at the 2.7% concentration (Fig. 2B).

In the second experiment of this study, the relative preference for saccharin was directly determined by giving the rats the choice between all possible pairs of solutions at concentrations of 0.033%, 0.1%, 0.3%, and 0.9%. The rats chose to drink more of the higher than the lower concentration with the exception of the 0.9% saccharin solution which was never preferred. Collier and Novell (1967) concluded saccharin closely resembles sucrose because saccharin intake increased and then decreased with increasing concentration in two-bottle tests with water, and because the more concentrated saccharin solution was preferred to the less concentrated solution with the exception of the 0.9% saccharin. This accounts for the title of their paper: “Saccarin as a sugar surrogate.” Collier and Novell (1967) noted that the similarity between saccharin and sucrose intake presented a problem “since neither of the properties hypothesized to control short and long-term intake, hypertonicity and nutritive content, is present in saccharin.”

Subsequent research has revealed that this “problem” is more apparent than real. In a follow-up to Collier and Novell (1967), Smith and Rashotte (1978) measured saccharin preference in 24-hr two-bottle saccharin vs. saccharin tests over a wide range of concentrations. They obtained the following rank order of preference: 0.4% > 0.3% > 0.2% > 0.5% > 0.6% > 0.1% > 0.7% > 0.8% > 0.9% > 1.0% > 0.033%. Thus, unlike the case of sucrose, saccharin vs. water and saccharin vs. saccharin tests both indicate that saccharin acceptance and preference increases up to a peak concentration of about 0.4% and then declines sharply as concentration further increases. Smith and Rashotte (1978) concluded, therefore, that saccharin resembles sucrose only up to the peak intake.

Consistent with this characterization are more recent studies that investigated saccharin lick rates and sham-feeding intakes. In the Smith (2000) study cited above, saccharin vs. water intakes and drinking patterns were recorded at concentrations of 1.0, 6.6 mM (0.021 1.35%), Twenty-four-hour saccharin solution intake peaked at 8 mM (0.10%) and then declined as in prior studies. The new finding was that rate of licking within saccharin bouts also peaked at 8 mM and then sharply declined after 16 mM (Fig. 2C). This contrasts with the increasing lick rates recorded from the same animals when tested with sucrose (Fig. 1C). Smith (unpublished study) also measured saccharin licking as a function of concentration during 30-s trials in the Davis Rig. During each of four daily sessions rats were presented with water, 1, 2, 4, 8, 17, 33, and 60 mM saccharin in an ascending order. As illustrated in Fig. 2D, saccharin licking increased to a maximum at 4 and 8 mM and then sharply declined at higher concentrations. Analysis of the lick microstructure revealed that saccharin burst size also increased to a peak at 4 mM and declined at higher concentrations.

An analysis of the sham-feeding response to saccharin solutions also revealed an inverted U-shaped intake-concentration function (Sclafani & Nissenbaum, 1985). Rats tested 30 min day with saccharin at 0.05–0.8% (2.4–39 mM) concentrations, increased their sham-intake as concentration increased to 0.2% and 0.4%, but then sharply decreased their intake at the 0.8% concentration. At the most acceptable saccharin concentrations (0.2–0.4%), saccharin sham-intakes
were substantially less than that obtained with sucrose (14 ml vs. 65 ml; compare Figs 11: and 21). Other tests revealed that rats sham-fed only slightly more saccharin than they real-fed (16.6 ml vs. 12.0 ml at 0.2% concentration; Sclafani & Nissenbaum, 1985). This contrasts with their response to sugar solutions where sham intakes far exceed real intakes at mid to high concentrations. At low sugar concentrations (1 4%), however, sham intakes exceed real intake by much less if at all (Davis et al., 2000; Nissenbaum & Sclafani, 1987; Weingarten & Watson, 1982). These data suggest that saccharin, at optimal concentrations for stimulating intake, is only as attractive as dilute sugar solutions.

This is exactly what was found in direct two-bottle tests of saccharin vs. sucrose. Using immediate choice tests, Young and Madsen (1963) compared 0.41 10% saccharin solutions to various sucrose solutions. They observed that the optimal saccharin solution tested was 0.2% and it was “isoprefered” to about a 3.5% sucrose solution. Note that Young and Madsen, on the basis of data interpolation, concluded that the optimal saccharin concentration was 0.5%. But Smith and Rashotte (1978) reported that rats preferred 0.4% to 0.5% saccharin in long-term tests. (Collier and Novell (1967) subsequently conducted 24-h two-bottle tests with saccharin vs. sucrose at concentrations of 0.03, 0.1%, and 2.16%, respectively. Their optimal saccharin solution was 0.3% and it was slightly preferred to 2% sucrose, but less preferred than 4% sucrose. The other saccharin solutions examined (0.01%, 0.1%, 0.9%) were all less preferred to sucrose at 2 16% concentrations.

Discussion

There is now considerable agreement from a variety of test methods that the attraction of rats to sucrose solutions increases directly as a function of concentration. This is assumed to represent their palatability evaluation of the sugar’s sweet taste. In contrast, sucrose acceptability, i.e., absolute intake, in tests lasting more than a few minutes is an inverted U-function of concentration which is explained by the postigestive intake-inhibitory effects of concentrated sucrose solutions. When these effects are eliminated in sham-feeding tests, then sucrose acceptance and preference both increase with concentration.

The situation with the artificial sweetener saccharin is quite different. The rat’s attraction to saccharin increases with concentration only to about 0.2-0.4% and then decreases sharply at higher concentrations. This inverted U-function is obtained in one- and two-bottle intake tests, short- and long-term lick rate tests, and sham-feeding tests. Furthermore, at optimal concentrations (0.2-0.4%), saccharin is isoprefered to only dilute sucrose solutions (2-4%). Thus, although Collier and Novell (1967) described saccharin as a “sugar surrogate,” at best saccharin is only a weak sugar surrogate. This presumably occurs because, although the sweet (i.e., sucrose-like) taste of saccharin increases with concentration, so does its bitter (i.e., quinine-like) taste (see Dess, 1995). The off-taste of concentrated sucrose solutions may not completely explain its limited palatability, and some data suggest that activation of different subsets of sweet taste receptors may contribute to the differential responses to sucrose and saccharin solutions (see Sclafani & Nissenbaum, 1985). Nevertheless, while saccharin may be only a weak sugar surrogate for rats, to date it remains the best artificial sweetener available since newer sweeteners such as aspartame are ineffective in rats (Sclafani & Abrams, 1986).

Finally, it should be noted that the above review has focused on the ingestive responses to sugar and saccharin as influenced by unconditioned taste and postigestive factors. There is an extensive literature documenting the important role of learning in modifying taste preferences. Best known are the conditioned taste aversions produced by pairing saccharin or sugar with toxic agents such as lithium chloride (Garcia et al., 1974). In addition, conditioned decreases and increases in sweet taste preference can be produced by the postigestive actions of sugar solutions. Thus, while rats may initially prefer a concentrated to a dilute sugar solution in 24-h day choice tests, over time they may reverse their preference as they learn to avoid the rapid satiating effects of concentrated solutions (Booth et al., 1972). On the other hand, rats may also learn to increase their intake of and preference for sugar solutions as they associate the taste with the positive postigestive actions of the sugar (Sclafani, 2001). Similarly, pairing a saccharin solution with gastric infusions of sugar can significantly enhance saccharin acceptance and preference (Sclafani, 2001).

For example, one recent study produced a conditioned increase in the rats’ preference for a flavored 0.05% saccharin solution to match that of a 16% fructose solution by pairing saccharin intake with intragastric glucose infusions (Myers & Sclafani, 2001). Thus, sweet taste preference begins with gustatory receptors in the mouth but is ultimately refined as the brain integrates gustatory and visceral sensory stimuli as well as, depending upon the species and situation, social, cultural, and cognitive factors.


