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Multiple Receptor Sites Mediate Sweetness: Evidence from Cross Adaptation[1]

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SCHIFFMAN, S. S., H. CAHN AND M. G. LINDLEY. *Multiple receptor sites mediate sweetness: Evidence from cross adaptation.* PHARMAC. BIOCHEM. BEHAV. 15(3) 377–388, 1981.—The method of cross adaptation was implemented to determine whether only one type of receptor site mediates the perception of sweetness, or whether more than one such type exists. Fourteen stimuli, seven artificial sweeteners varying widely in chemical structure as well as seven sugars, were cross adapted with one another. When a sugar was employed as the adapting stimulus, a consistent reduction in the intensity of the test solution's sweetness was found. However, the result of the cross adaptation when the adapting stimulus was an artificial sweetener was unpredictable; it led not only to a reduction but, in some cases, to an enhancement or no change in the test solution's intensity, depending on its identity. In previous investigations, enhancements have been explained through the existence of a water taste. Since this explanation is insufficient to account for the enhancement effects found in this study, it appears that cross adaptation does not always occur between sweet-tasting compounds. For this reason, it is concluded that more than one receptor mechanism may be responsible for the perception of the sweet quality.

Sweeteners Multidimensional scaling Cross adaptation Receptor sites

THE stimulus-receptor mechanisms responsible for the sweet taste are not fully understood at the present time. The most cogent explanation for the fact that compounds of widely varying chemical structure taste sweet is that the sweet taste is in part dependent upon the formation of two simultaneous hydrogen bonds separated by approximately 3 A° [17,18]. It has been proposed that a basic structural subunit, the AH-B system, of a "sweet" stimulus molecule interacts with a complementary AH-B site in the receptor membrane, where A and B are electronegative atoms and H is a hydrogen atom [17,18].

Additional sites of interaction of the stimulus molecules with the membrane may also be necessary to impart a sweet taste. The fact that the D-isomer of an amino acid can taste sweet while the L-enantiomer does not [16,19] implies a stereoselective receptor or at least three bonding sites. A three-dimensional receptor site for α amino acids has been proposed that consists of an AH-B system and a third site which is a spatial barrier. It has been observed that relative sweetness may depend upon a hydrophobic bonding area [4] which has led to proposal of a third so-called "dispersion" site to account for a potent sweet response [8].

Although the involvement of hydrogen bonding in the mediation of the sweet taste response is highly probable, there is no reason to assume (1) that there is only one type of AH-B system in the receptor membrane, (2) that the same AH-B site(s) are involved for all sweeteners, or (3) that the additional sites (such as hydrophobic bonding areas) are identical. Non-homogeneous variability of human sensitivities to a range of compounds varying in chemical structure [6,15] suggests that sweetness may be mediated by several receptor types. In addition, inhibition by pronase E and semi-alkaline protease [7] and gymnemic acid [2] is not uniform across sweeteners. Alloxan has not been found to suppress integrated neural responses to artificial sweeteners, such as sodium saccharin, although it selectively depresses sugars [20].

The psychophysical method of cross adaptation has also been used to investigate the possible number of gustatory receptor mechanisms for sweetness [10]. If adaptation to one stimulus results in a decreased sensory response to another stimulus, this may indicate that the two stimuli bind to a common receptor type. However, if adaptation to one stimulus does not decrease the sensation of another

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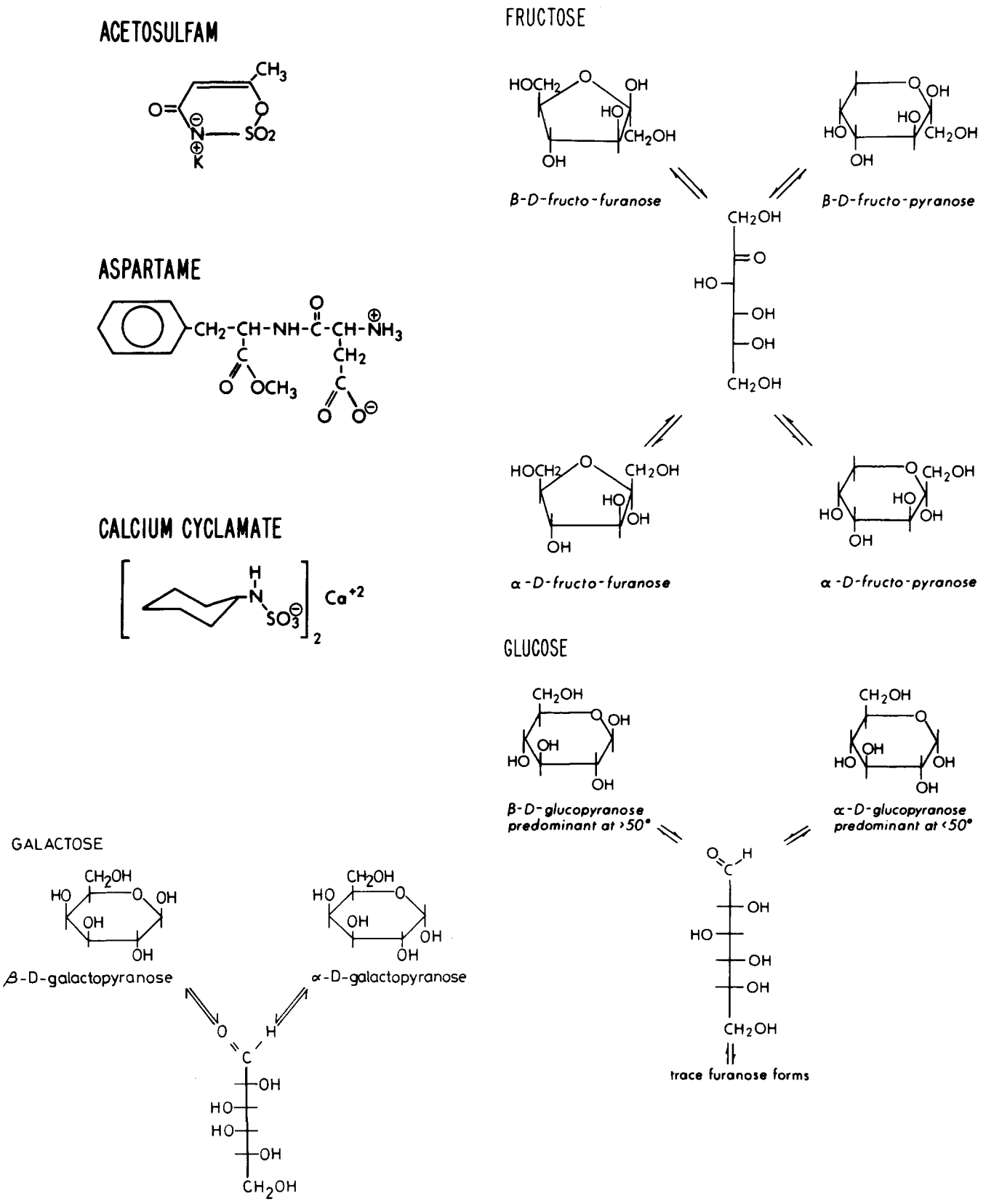
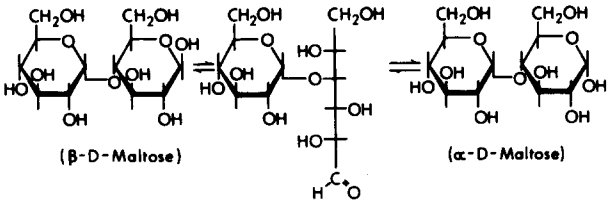
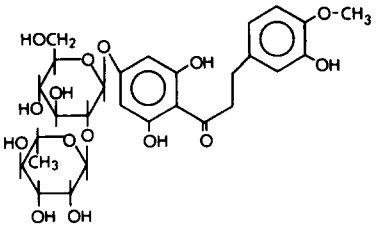


FIG. 1. Chemical structures of stimuli used in the study.

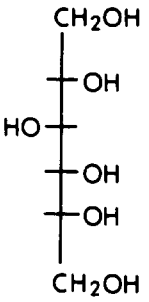
MALTOSE



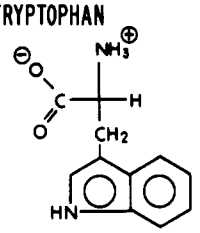
β-NEOHESPEREDIN DIHYDROCHALCONE



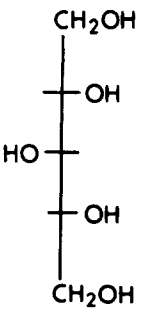
SORBITOL



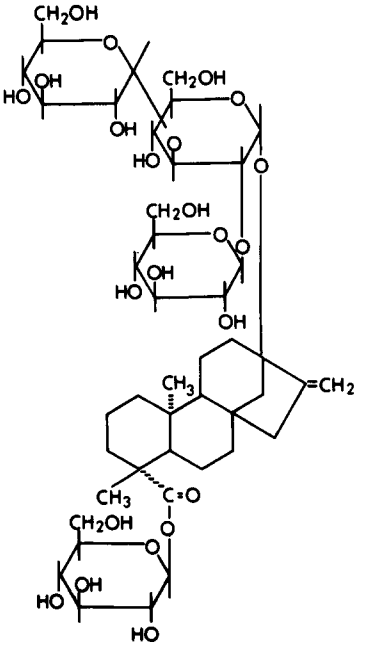
D-TRYPTOPHAN



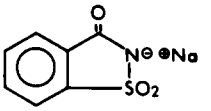
XYLITOL



REBAUDIOSIDE



SACCHARIN (Na SALT)



XYLOSE

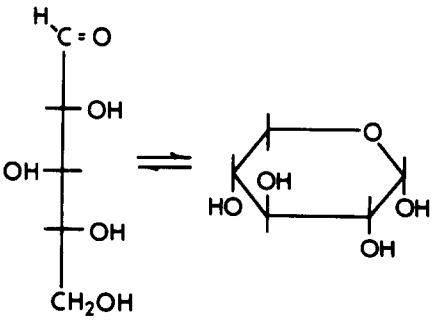


TABLE 1
STIMULI EMPLOYED IN THE STUDY, INCLUDING CLASSIFICATION, COMMERCIAL SOURCE, CONCENTRATION, AND USE IN THE STUDY

Compound	Classification	Source	Use in Study			Concentration Used
			Part 1	Part 2	Part 3	
Acetosulfam	Oxathiazinone dioxide (methyl derivative); 3,4 dihydro-6-methyl-1,2,3 oxathiazin-4-one-2,2-dioxide potassium salt	Hoechst (Germany)	X	X	X	0.35%
Aspartame	Dipeptide: L-aspartyl-L-phenylalanine methyl ester	Searle		X	X	0.25%
Ca cyclamate	Calcium cyclohexyl sulfamate	Monsanto	X	X	X	0.6%
Fructose	Monosaccharide ketohexose	Sigma	X			0.6 M
Galactose	Monosaccharide aldohexose	Sigma	X			1.0 M
Glucose	Monosaccharide aldohexose	Sigma	X	X	X	1.1 M
Maltose	Disaccharide	Sigma	X			1.2 M
Neohesperidin dihydrochalcone	Dihydrochalcone glycoside	California Aromatics		X	X	0.016%
Rebaudioside A	Diterpene glycoside	California Aromatics	X			0.07%
Na saccharin	0-sulfobenzimide: 1,2-benzothiazol-3(2H)-one-1,1-dioxide, Na^+ salt	Logica International	X	X	X	0.045%
Sorbitol	Polyhydric alcohol	Sigma	X			1.2 M
D-tryptophan	D-amino acid	Sigma		X	X	0.3%
Xylitol	Polyhydric alcohol	Sigma	X	X	X	1.0 M
Xylose	Monosaccharide aldopentose	Sigma	X			1.3 M

stimulus, a possible implication is that different receptors code these stimuli. In one cross adaptation study, sucrose adaptation, and to a lesser extent saccharin adaptation, reduced sweetness ratings in a series of 16 compounds leading to the hypothesis that there may be a single receptor type responsible for the sweet quality [27].

The purpose of this study was to extend the method of cross adaptation to a wider range of compounds with varying structures to examine further whether only one receptor type mediates sweetness as suggested previously [27] or whether the sweet taste is similar to the bitter taste, which has been found to be mediated by multiple receptor types [14].

METHOD

This study consisted of three parts.

Subjects

Part 1 (Pretesting): The subjects were two nonsmoking 20-year-old undergraduate males at Duke University. Both were aware of the purpose of the study.

Part 2: The subjects were 151 nonsmoking Duke University undergraduates, 65 male and 86 female. All ranged in age from 18–21 years and were naive as to the purpose of the investigation.

Part 3: The subjects were 74 nonsmoking undergraduates at Duke University, 29 male and 45 female, ranging in age from 18–21 years. None were aware of the purpose of the study.

Stimuli

The stimuli are listed in Table 1 along with classification, source, concentration, and use in the study. The structures are given in Fig. 1. All stimuli were dissolved in deionized water at concentrations found by Schiffman *et al.* [15] to be approximately equal in overall intensity. Fifteen milliliters of each stimulus were presented to subjects at room temperature (approximately 72°C) in small plastic cups.

Procedure

Part 1 (Pretesting): Each stimulus served as an adapting solution while the remaining stimuli were utilized as test solutions such that most of the possible combinations of the stimuli were investigated. Each combination of stimuli was presented in an ABA experimental design; the two subjects first tasted a test solution A after which they thoroughly rinsed their mouths with water. This part of the procedure provided the subjects with a preadaptation estimate of the test solution's sweetness intensity. Without delay, the subjects next held an adapting solution B in their mouths, frequently swirling the solution to ensure complete adaptation. After the sweet taste of the adapting solution disappeared (approximately 30–60 seconds), the subjects emptied the contents of their mouths and, without a water rinse, quickly retasted the test solution A, once again estimating the intensity of its sweetness. The subjects then judged whether this postadaptation sweetness intensity was greater than, less than, or equal to the preadaptation sweetness intensity.

Part 2: In a manner similar to Part 1, each stimulus served

GLUCOSE	ACETOSULFAM	XYLITOL	D-TRYPTOPHAN
3	6	9	12
ASPARTAME	XYLITOL	NA SACCHARIN	GLUCOSE
2	5	8	11
GLUCOSE	ACETOSULFAM	XYLITOL	D-TRYPTOPHAN
1	4	7	10

FIG. 2. An example of a stimulus arrangement for presentation to subjects in Part 2.

as an adapting solution while the rest of the stimuli were used as test solutions. However, while each combination was evaluated by two subjects in Part 1, in this case all the combinations were tasted by 10–12 subjects. In addition, each subject evaluated only four combinations in contrast to the pretesting, where the two subjects evaluated all but a few of the possible combinations.

The four different stimulus combinations presented to each group of subjects were assigned randomly. Each subject was presented with 12 cups arranged in a 3×4 matrix such that each of the four columns represented a different combination and the three cups or triad making up any one column were part of a single stimulus combination arranged in an ABA design. Thus, in any one column or triad the first and third cups contained the same solution, a test solution, while the second cup contained an adapting solution. An example of a stimulus arrangement is provided in Fig. 2.

The subjects were instructed to take the entire solution in the first cup into their mouths and immediately rate its sweetness on a 5½-inch line labeled "very sweet" at one extreme and "not sweet" at the other. In order to avoid adaptation, the subjects were instructed to hold the test solutions in their mouths for no more than a few seconds. After rinsing their mouths with water three times, the subjects waited four minutes before holding the solution contained in the second cup in their mouths for 60 seconds. Pretesting suggested that one minute was ample time for complete adaptation. At the end of the 60 seconds, the adapting solution was emptied from the mouth and the solution in the third cup was promptly tasted without an intervening water rinse. In a manner analogous to that described above, this solution's sweetness was rated again on a 5½-inch line. A water rinse (three times) and a four-minute intertriad interval then followed before testing the next series of solutions. This procedure was repeated for all four columns or triads.

Part 3: In this part of the study certain stimulus combinations already tested in Part 2 were retested, and, in addition, the contribution of the water taste to the sweetness intensity of the test solution after adaptation was examined. Each group of subjects, ranging in number from 8 to 20, was presented with only two combinations. In Part 2 of this study, by the fourth combination several subjects commented on fatigue. Thus, in an effort to alleviate this problem, only two combinations were presented to each subject in this part of the study. In addition, the interstimulus intervals were extended from four to five minutes, and the number of water rinses was increased from three to four.

The subjects were first presented with two triadic combinations in an ABA design similar to that already described for the second part of the procedure. Next, they were presented with the water taste part of the procedure. This involved adapting to one of the two solutions previously employed as an adapting solution in the ABA combinations. After 60 seconds, the subjects emptied the adapting solution from their mouths and, without a water rinse, tasted 15 ml of deionized water and rated the intensity of its sweetness. After rinsing their mouths with water four times and waiting five minutes, this procedure was repeated for the other previously employed adapting solution. An example of a stimulus arrangement that subjects were given is shown in Fig. 3.

XYLITOL	ASPARTAME	DEIONIZED WATER	DEIONIZED WATER
3	6	8	10
GLUCOSE	NA SACCHARIN	GLUCOSE	NA SACCHARIN
2	5	7	9
XYLITOL	ASPARTAME		
1	4		

FIG. 3. An example of a stimulus arrangement for presentation to subjects in Part 3.

RESULTS

The data were transcribed from 0 to 100 with "not sweet" corresponding to a rating of "0" and "very sweet" represented by a rating of "100."

Part 1 (Pretesting): Table 2 illustrates the effect adaptation to each stimulus had on the subsequent test stimulus for the combinations of the 11 compounds tested. Typically, there was a reduction in the sweetness of a test compound following adaptation to a sugar. Due to this consistent result, not all the possible combinations that involved sugars as adapting stimuli were evaluated. However, since the effect upon sweetness of the test solution following employment of an artificial sweetener as the adapting stimulus could not be predicted, all such possible combinations were tested.

A positive sign in the table corresponds to the situation where the postadaptation sweetness intensity of the test solution was perceived to be less than its preadaptation sweetness. When the test solution tasted sweeter after adaptation, the sign is negative. An equal sign indicates that the sweetness of the test solution was considered unaffected by the adaptation.

The compounds listed at the top of the table represent the adapting solutions and those on the left side of the table correspond to the test solutions. For example, both subjects agreed that adaptation to a glucose solution reduced the sweetness intensity of a solution of acetosulfam. The pres-

TABLE 2

RESULTS OF PART ONE (PRETESTING): DIFFERENCE IN PERCEIVED SWEETNESS AFTER ADAPTATION

	Adapting Solution*										
Test Solution	Galactose	Glucose	Fructose	Maltose	Sorbitol	Xylitol	Xylose	Acetosulfam	Ca cyclamate	Na saccharin	Rebaudioside
Galactose			++	+	++	++	++	−−	==	?	++
Glucose	+		+	?	+	++		−−	==	==	?
Fructose	++	++		++	++	++	++	−−	==	==	++
Maltose	++	+	++		++	++	+	−−	==	==	==
Sorbitol	++	+	++	++		++	+	−−	?	?	−−
Xylitol	++	++	++	++	++			−−	==	?	?
Xylose	++	+	++		+			?	++	−−	−−
Acetosulfam	++	++	++	++	++	++	++		==	++	++
Ca cyclamate	++		+	++	+	++	++	==		==	==
Na saccharin	+	++	++	?	?	++	+	++	==		==
Rebaudioside		++	++	+	+	++	?	−−	−−	==	

*+ indicates that the postadaptation sweetness intensity was perceived to be less than its preadaptation sweetness.

TABLE 3

COMBINED RESULTS OF PART TWO AND PART THREE: DIFFERENCE IN PERCEIVED SWEETNESS AFTER ADAPTATION

	Adapting Solution							
Test Solution	Acetosulfam	Aspartame	Ca cyclamate	Glucose	Neohesperidin dihydrochalcone	Na Saccharin	D-tryptophan	Xylitol
Acetosulfam		10.0 —	8.0 —	5.4 —	−18.6 —	43.8 0.001	30.6 0.01	25.2 0.02
Aspartame	4.0 —		−7.4 0.05	13.8 0.05	−15.4 0.05	−13.0 0.05	40.0 0.001	23.8 0.01
Ca cyclamate	−11.0 0.05	0.7 —		20.6 0.05	14.6 0.01	4.2 —	−7.8 —	6.2 —
Glucose	−12.0 0.20	−9.3 —	9.1 0.20		−6.6 —	−17.4 0.001	6.0 —	15.2 0.10
Neohesperidin dihydrochalcone	−11.5 0.20	6.6 —	−5.7 —	0.8 —		−21.6 0.001	15.8 0.10	1.7 —
Na saccharin	29.7 0.001	2.2 —	15.4 0.20	16.4 0.10	−14.3 —		10.2 0.20	2.0 —
D-tryptophan	−13.6 0.10	36.0 0.001	−14.0 0.10	27.8 0.05	−5.8 —	−5.6 —		22.0 0.05
Xylitol	−9.2 0.10	−1.4 —	9.2 0.20	10.5 0.20	−19.4 0.001	−3.7 —	9.8 —	

TABLE 4

WATER TASTE INDUCED THROUGH ADAPTATION TO THE STIMULI EMPLOYED IN THE STUDY

Adapting Stimulus	No. of Subjects	Arithmetic Mean Water Taste
Acetosulfam	20	7.4
Aspartame	8	7.0
Ca cyclamate	18	12.2
Glucose	27	3.0
Neohesperidin dihydrochalcone	20	9.0
Na saccharin	10	11.8
D-tryptophan	9	2.6
Xylitol	35	9.6

ence of only one sign indicates that only one of the two subjects evaluated that particular combination of stimuli.

There was agreement between both subjects on most combinations because the change in sweetness was generally large enough to allow a fairly confident response. However, the change for several combinations, noted with a question mark in the table, was either too small for the subjects to respond with confidence or the two subjects disagreed on the result. Overall, sugars were more effective adapting stimuli than artificial sweeteners.

Parts 2 and 3: The results from Part 2 were analyzed separately as well as pooled with the results from Part 3. The adaptation matrices for the two types of analyses were virtually identical and the pooled results are given in Table 3.

For each combination of the eight stimuli, a mean for the sweetness intensity of the test solution, both before and after adaptation, was calculated from the responses of all the subjects who tasted a particular combination. The differences between these means were then calculated and are presented in Table 3. A positive magnitude of change indicates a reduction in the test solution's sweetness following adaptation and a negative value corresponds to an enhancement in the sweetness of the test solution. The level of statistical significance of the difference as determined by t tests is given below the value for the difference of the means.

Table 4 shows the results of the procedure used to determine the water taste induced by each of the eight stimuli employed in the second and third parts of this study. The water taste for each stimulus was determined by calculating the mean of the responses the subjects made in rating the sweetness of water after having adapted to the stimulus. The water taste for each stimulus, in addition to the number of subjects used to determine this water taste, is listed in Table 4.

DISCUSSION

The results of this study suggest that the perception of sweetness is mediated by a process more complicated than that proposed by McBurney [10]. This is not surprising in lieu of the experimental evidence presently accruing on the complex nature of sweet-tasting compounds [6,15]. Especially troublesome in the analysis of results obtained from cross adaptation studies is the bitter component, which is quite salient in many artificial sweeteners.

In this study, cross adaptation was found to occur consistently when the adapting solution was a sugar (see Table 2). This might lend support to McBurney's hypothesis that there exists only one receptor type coding the sweet quality. However, when a synthetic sweetener was employed as the adapting stimulus, cross adaptation was not found in many instances. Rather, enhancement of the test solution's sweetness frequently resulted.

Were it not for the complicated nature of sweetness, this enhancement could readily lead to the conclusion that more than one receptor type must code sweetness. Enhancement would be incompatible with the assumption that only one type of receptor site is responsible for sweetness because cross adaptation would always be expected to occur.

However, due to the bitter component in many sweet-tasting compounds, this argument is not so straightforward. Adaptation to certain compounds is known to induce particular taste qualities in water. By the proper adaptations, McBurney and Shick [13] and Bartoshuk [1] were able to produce sweet, sour, salty, and bitter qualities in water. Furthermore, these studies revealed that water, following adaptation to a stimulus having a bitter component, acquires a sweet taste.

McBurney and Bartoshuk [12] suggested that the cross enhancement phenomenon is not the result of actual interactions among the different taste qualities but, rather, a water taste, induced by the adaptation. Adaptation to one solution causes the water solvent of a subsequent solution to act as a taste stimulus. This additional taste stimulus adds to the usual taste of the solute dissolved in the subsequent solution. Similarly, McBurney and Shick [13] stated that when a test solution shows an increase or no change in intensity following adaptation to a different solution, the water taste induced by the adaptation is probably adding to the taste of the test solution. McBurney and Bartoshuk [11] concluded that following adaptation to one stimulus, the taste of a second stimulus is the sum of the taste produced by the second compound and the water taste induced by adaptation to the first stimulus minus any cross adaptation between the two stimuli.

Employing this reasoning, the enhancement effects found by McBurney [10] can be accounted for without disturbing the validity of his conclusion that there exists only one receptor type encoding sweetness. While saccharin, a sweet-tasting compound with a relatively large bitter component, was found to enhance the sweetness of 5 of the 16 compounds tested, this might be explained as follows. Although adaptation caused a reduction in the sweetness of the test solution through cross adaptation, there was a concurrent sweet water taste also resulting from the adaptation which added to the overall sweetness of the test solution. Not only was the magnitude of this water taste large enough to negate the diminished sweetness of the test solution due to the cross adaptation, but it was large enough to increase the solution's sweetness above its "normal" or unconfounded intensity.

It will be shown here than an extension of this logic to the present study, however, does not fully account for the degree of enhancement found, suggesting that more than one receptor type may indeed be responsible for encoding sweetness. If there exists only one type of receptor site responsible for coding sweetness, then adaptation to a particular stimulus should produce the same water taste regardless of the test stimulus that follows. Such an assumption seems valid since the water taste is the taste of only the water solvent in which the test compound is dissolved. Thus, the

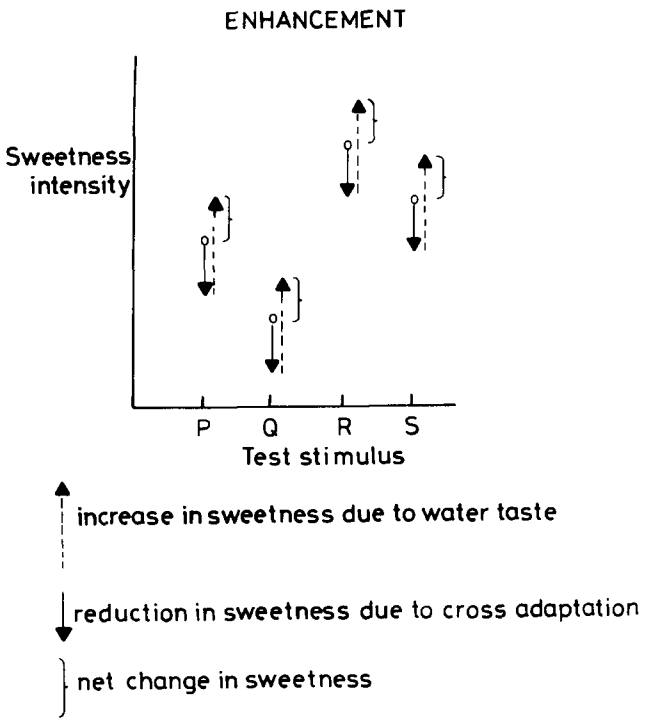


FIG. 4a. Enhancement.

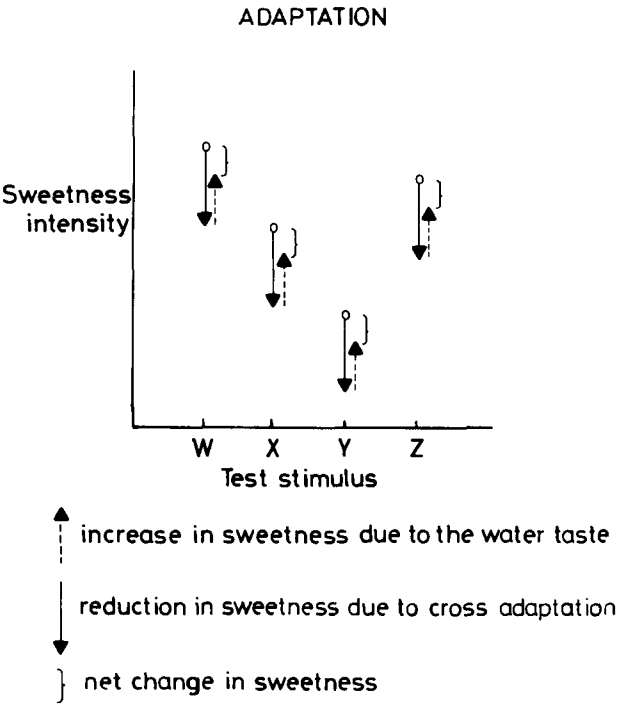


FIG. 4b. Adaptation.

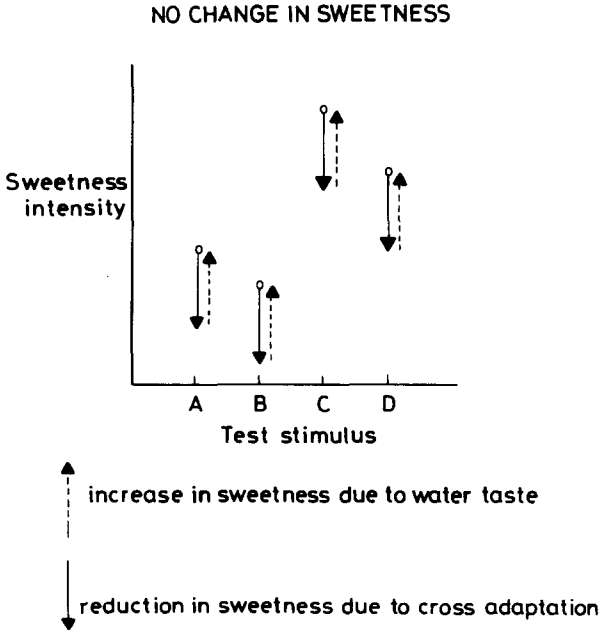


FIG. 4c. No change in sweetness.

water taste is a function only of the adapting compound and the water solvent of the test solution and has nothing to do with the solute dissolved in the test solution. Since the bitterness of a given adapting solution does not change from a trial with one test solution to a trial with a different test solution, the intensity of the water taste should remain constant for the different test solutions. While the water taste will change for different adapting solutions, when the adapting solution remains the same one would expect the component of sweetness added to the total sweetness of a test solution to be constant, regardless of the test solution.

Given that only one receptor type exists for the perception of sweetness, another possible hypothesis is that adaptation to any one stimulus might cause the same magnitude of reduction in the sweetness of any test solution through cross adaptation if they are equally intense. (It should be noted that this argument could be challenged here since the sweeteners were equated for overall intensity and not sweetness alone.) Let us assume that all the stimuli use the same receptor sites and that there may be an equal amount of competition for receptor sites between a given adapting solution's molecules and the molecules of the test solution, regardless of which test solution is used. This could imply an equal cross adaptation effect for any test stimulus and a given adapting stimulus.

Using this line of reasoning, two of the three components that contribute to the overall taste of a test solution following adaptation to a given stimulus should remain constant for all test solutions. Of the three components, the unconfounded taste of the test compound, the additional water taste, and the reduction in taste due to cross adaptation, only the unconfounded taste of the test compound should vary from one test solution to another for a given adapting solution.

This leads to an important consequence that is illustrated in Figs. 4a, 4b and 4c. Each figure represents the effect of adaptation to a given stimulus on four different test solutions. Solid arrows represent the reduction in a test solution's sweetness due to cross adaptation. As discussed

above, it is proposed that this component remains constant for a given adapting solution (though it would change for different adapting stimuli). Each figure assumes an arbitrary, though constant, magnitude for this component. The enhancement of a test solution's sweetness due to the water taste is represented by dashed arrows. Once again, for each figure, a constant magnitude is assumed for this component. Since each of the three figures represents adaptation to a different stimulus, the magnitudes of these two components are different for the three different figures. However, the magnitudes of these components remain constant for any one figure since each figure corresponds to only one adapting stimulus. The unconfounded sweetness intensity of the stimuli being tested, that would be expected to vary from one stimulus to the next and for different concentrations of the same stimulus, is represented by the small circles. Arbitrary values were assumed for each test stimulus. Figs. 4a, 4b and 4c illustrate that the constancy of the two components necessitates that the sweetness of the components tested should all increase, all decrease, or all remain unchanged following adaptation to one given stimulus. Depending on the relative magnitudes of the two constant components, it is possible to illustrate a situation where all the test stimuli should show an increase in sweetness (Fig. 4a), where all the test stimuli should be reduced in sweetness (Fig. 4b), or where the sweetness of all the test stimuli should remain unchanged (Fig. 4c) following adaptation to any one given compound. When the intensity of sweetness added by the water taste to the test solution's unconfounded sweetness is greater than the reduction in sweetness of the test solution due to cross adaptation, an enhancement effect will be observed. When the sweet water taste is less than the decrease in sweetness resulting from the cross adaptation, the test solution should show a reduction in sweetness following adaptation. Adaptation will produce no change in the sweetness of the test solution if the water taste sweetness exactly equals the reduction in sweetness caused by cross adaptation.

Since each column in Tables 2 and 3 represents possible stimulus combinations for a given adapting stimulus, any one column in these tables should show consistent results according to the above hypothesis. In other words, all test solutions should show the same result after adaptation to any one stimulus; the results in any one column should be all enhancements, all cross adaptations, or all no changes. Furthermore, ideally the magnitudes of change should all be equal in any one column. If this were the case, McBurney's [10] argument used to explain the enhancement effects would reasonably account for any enhancements or "no changes" found in this study. However, if any one column does not show consistent results, the assumption of a single receptor site type for sweetness would not hold.

Examination of Table 3 reveals fairly large, highly statistically significant inconsistencies in a given column that are not compatible with the existence of only one receptor type encoding sweetness according to the hypothesis presented here. For example, adaptation to sodium saccharin results in a reduction of the sweetness of acetosulfam by 43.8 units (on a 100 unit rating scale). This change is significant at the 0.001 level for a two-tailed t test. However, adaptation to sodium saccharin was shown to enhance the sweetness of neohesperidin dihydrochalcone by 21.6 units, also significant at the 0.001 level. Even if all the possible sources of error were taken into consideration, such a large and significant inconsistency is most likely incompatible with a single receptor type responsible for the perception of sweetness. While this is the most extreme example, there are other inconsistencies in Table 3 which, taken together, all lead to the conclusion that there most likely exists more than one receptor site type responsible for sweetness.

The degree of enhancement reported by McBurney [10], slight and statistically insignificant, is easily explained through the existence of a sweet water taste. On the other hand, large, and very statistically significant, enhancements were found to occur in this study; several enhancements around 20 units in magnitude were discovered to be significant at the 0.001 level. Thus, the water taste argument, which is reasonably capable of explaining the small, insignificant enhancements reported by McBurney, may be much less effective in accounting for the large, highly significant enhancements found here.

An evaluation of the effect of the water taste is helpful in assessing the controversy over the number of type of receptor sites responsible for the perception of sweetness. The means for the sweet water taste induced by adaptation to the stimuli used in Parts 2 and 3 of this study were given in Table 4. These water tastes were insufficient in numerous instances to account for the enhancement effects shown in Table 3. For example, adaptation to neohesperidin dihydrochalcone significantly ($p<0.001$) enhanced the sweetness of xylitol by a magnitude of 19.4 units. The water taste must not only account for the magnitude of the enhancement, but it must also negate the reduction in the test solution's sweetness due to cross adaptation. Assume for the sake of discussion that, in this case, the cross adaptation reduced the sweetness of the test solution by approximately 15 units. Not only must the water taste account for the enhancement effect of 19.4 units, but it must also compensate for the cross adaptation effect of 15 units. In other words, the water taste, which only had a magnitude of 9 units, must theoretically add a sweetness component of 34.4 units to the overall sweetness intensity of the test solution. Of course, it is quite possible to argue that the taste components contributing to the overall sweetness of the test solution do not necessarily add linearly as this simple example assumes. However, it still seems reasonable to conclude that the magnitude of this water taste is insufficiently large, through whatever process by which the sweet taste components add to give a net sweetness intensity, to account for the observed enhancement effect.

Table 5 shows the combined results of Parts 2 and 3 (i.e. Table 3) after correcting for the water taste. This correction made the magnitudes of the cross adaptations larger and the magnitudes of the enhancements smaller. In some cases, an enhancement became a cross adaptation after correcting for the water taste. For example, assume the water taste for a particular stimulus has a magnitude of 5 units. If adaptation to this stimulus reduced the sweetness of some test solution by 15 units, the corrected value for this reduction would be 20 units. An enhancement with a magnitude of 10 units (represented in the table by -10) would become an enhancement of 5 units after correcting for the water taste. If the enhancement had a magnitude of only 3 units, the result of the correction would be a cross adaptation of 2 units. Table 5 indicates that even after eliminating the effect of the water taste, some combinations of stimuli, especially those where acetosulfam, Na saccharin, and neohesperidin dihydrochalcone are the adapting stimuli, still show enhancement, further suggesting that more than one type of receptor site codes sweetness.

TABLE 5

COMBINED RESULTS OF PART TWO AND PART THREE AFTER CORRECTING FOR THE WATER TASTE

	Adapting Solution							
	Acetosulfam	Aspartame	Ca cyclamate	Glucose	Neohesperidin dihydrochalcone	Na saccharin	D-tryptophan	Xylitol
Test Solution								
Acetosulfam		17.0	20.2	8.4	−9.6	55.6	33.2	34.8
Aspartame	11.4		4.8	16.8	−6.4	−1.2	42.6	33.4
Ca cyclamate	−3.6	7.7		23.6	23.6	16.0	−5.6	15.8
Glucose	−4.6	−2.3	21.3		2.4	−5.6	8.6	24.8
Neohesperidin dihydrochalcone	−4.1	13.6	6.5	3.8		−9.8	18.4	11.3
Na saccharin	37.1	9.2	−3.2	19.4	−5.3		12.8	11.6
D-tryptophan	−6.2	43.0	−1.8	30.8	3.2	6.2		31.6
Xylitol	−1.8	5.6	21.4	13.5	−10.4	8.1	12.4	

Some interesting observations are revealed through further examination of Table 3. The results imply the existence of, at the very least, two different receptor mechanisms involved in the perception of the sweetness of the stimuli employed in this study. Sodium saccharin and acetosulfam appear to operate through a similar receptor site that is different than the receptor site shared by aspartame and D-tryptophan. Cross adaptation between a pair of stimuli is not evidence for a common receptor type unless the cross adaptation is reciprocal, i.e. adaptation results when either stimulus is employed as the adapting solution. Cross adaptation is reciprocal for sodium saccharin and acetosulfam, and also for D-tryptophan and aspartame. Adaptation to sodium saccharin significantly ($p<0.001$) reduced the sweetness of acetosulfam by a magnitude of 43.8 units while there was a significant degree of cross adaptation ($p<0.001$) with a magnitude of 29.7 units when acetosulfam served as the adapting stimulus. Similarly, D-tryptophan shows a significant reduction in sweetness by 36 units ($p<0.001$) following adaptation to aspartame while adaptation to D-tryptophan significantly reduced the sweetness of aspartame by 40 units ($p<0.001$). This suggests that D-tryptophan and aspartame employ similar receptor sites while acetosulfam and sodium saccharin also share common sites. However, the receptor type responsible for the perceived sweetness of acetosulfam and sodium saccharin appears to be different than the receptor type encoding the sweetness of D-tryptophan and aspartame since intergroup stimulus combinations do not show consistent, significant cross adaptation. It should be noted here, however, that the sites shared by acetosulfam and Na saccharin (also aspartame and D-tryptophan) are not identical because they are not consistent in their effects on all sweeteners.

The results of cross adaptation were also examined from the viewpoint of the possible AH-B units that may be involved in the perception of sweetness (see Table 6). First of all, it is striking that adaptation to xylitol and glucose result in a reduction of the perceived intensity of all test solutions. Thus, it would appear that at least some of the AH-B receptor sites complementary to the OH, OH stimulus systems are shared by the AH-B systems of the other stimuli. Acetosulfam and Na saccharin, which mutually cross adapt, have identical possible AH-B systems. D-tryptophan and aspartame share one possible system in common. Adaptation to Ca cyclamate, the only artificial sweetener with a single possible AH, B system, leads to a reduction in the perceived intensities of the sugars, xylitol and glucose. The reverse is also true, i.e. adaptation to glucose and xylitol lead to a reduction in the sweetness of Ca cyclamate. This suggests that at least some of the complementary AH-B systems of the receptors for Ca cyclamate, xylitol, and glucose are the same. A similar argument for the mutually adaptive triad of D-tryptophan, xylitol, and glucose holds as well.

Another approach to determine the actual number of type of receptor sites coding for the sweetness of the stimuli employed in this study is through multidimensional scaling analysis of the results. The greater the degree of cross adaptation between two stimuli, the closer they will be located in a multidimensional space. Thus, stimuli that tend to group together in space use similar receptors. After correcting for the water taste, the combined results of Part 2 and Part 3 (Table 5) were subjected to multidimensional scaling analysis using the KYST procedure (see Kruskal *et al.* [9]). The two-dimensional, metric solution is presented in Fig. 5. As expected, acetosulfam and sodium saccharin fall proximate to one another, indicating that they may share a common site type for stimulation. In addition, aspartame and D-tryptophan are also located near each other in the space,

TABLE 6
POSSIBLE AH, B SYSTEMS

Stimulus	Number	Type
Acetosulfam	2	S→O, NH and/or C=O, NH
Aspartame	2	C=O, NH - O and/or NH_3^+, COO^-
Ca cyclamate	1	S→O, NH
Glucose	1	OH, OH
Neohesperidin dihydrochalcone	3	OH, OH and/or OH, ⬡O and/or OH, OCH_3 (sugar units)
Saccharin (sodium salt)	2	S→O, NH and/or C=O, NH
D-tryptophan	2	NH_3^+, COO^- and/or NH, ⬡O
Xylitol	1	OH,OH

*S→O refers to the fact that the S is an electron donating atom. It does not form a true covalent bond with the O but donates electrons, making the O electronegative and indicating that it has an unshared pair of electrons (Ö).

†Strictly speaking, the N in acetosulfam and sodium saccharin should be N^- because it is the salt form (Na^+ for saccharin, K^+ for acetosulfam) that is normally tasted. However, the "non-salt" form (NH) is also sweet.

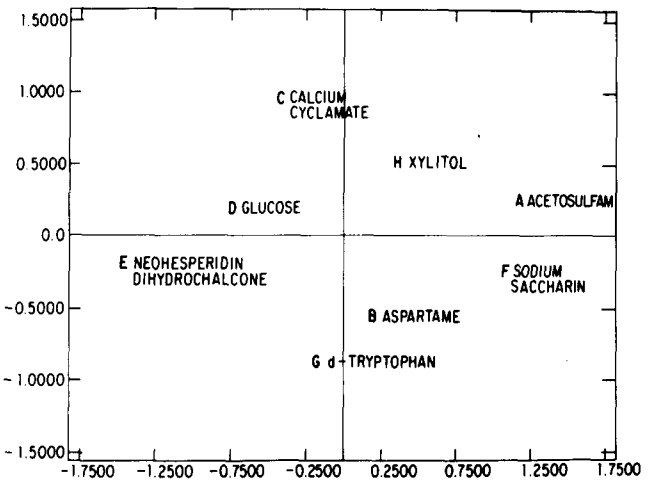


FIG. 5. Two-dimensional arrangement derived by KYST [9] based on cross adaptation results in Table 5. Stimuli found proximate to one another in the space are assumed to use similar receptor mechanisms.

suggesting that they may also be perceived through a common site.

This study indicates that cross adaptation may be a helpful procedure for determining common receptor sites. Application of multidimensional scaling procedures to cross adaptation results corrected for the sweet water taste may reveal groupings of stimuli which share receptor sites. This approach can be contrasted with that employed by Schiffman *et al.* [15] where two sweeteners, even though they may be using a common sweet receptor, are located distant from each other in a multidimensional space based strictly on quality because one compound has a strong bitter taste and the other sweetener does not. Schiffman *et al.* [16] reported that aspartame and D-tryptophan were located distant from one another in a three-dimensional space based on overall quality. However, when only sweetness was examined in this cross adaptation study, aspartame and D-tryptophan were found to share a common receptor site. Comparison of the structural similarities between sweeteners that are determined to group together in a multidimensional space based on cross adaptation procedures may be preferable for revealing the actual mechanisms mediating the perception of sweetness to groupings based on overall quality.

In summary, the results of this investigation support the conclusion that the perception of sweetness is a more complex process than that suggested by McBurney [10]. The existence of a single type of receptor mediating the sweet quality does not seem sufficient to account for the results of this cross adaptation study.

REFERENCES

1. Bartoshuk, L. M. Water taste in man. *Percept. Psychophys.* **3:** 69–72, 1968.
2. Bartoshuk, L. M., G. P. Dateo, D. J. Vandenbelt, R. L. Buttrick and L. Long. Effects of *Gymnema Sylvestre* and *Synsepalum Dulcificum* on taste in man. In: *Olfaction and Taste,* edited by C. Pfaffmann. New York: Rockefeller University Press, 1969, pp. 436–444.
3. Birch, G. G. and A. R. Mylvaganam. Evidence for the proximity of sweet and bitter receptor sites. *Nature* **260:** 632–634, 1976.
4. Deutsch, E. W. and C. Hansch. Dependence of relative sweetness on hydrophobic bonding. *Nature* **211:** 75, 1966.
5. Diamant, H., B. Oakley, L. Strom, C. Wells and Y. Zotterman. A comparison of neural and psychophysical responses to taste stimuli in man. *Acta physiol. scand.* **64:** 67–74, 1965.
6. Faurion, A., S. Saito and P. MacLeod. Sweet taste involves several distinct receptor mechanisms. *Chem. Senses* **5:** 107–121, 1980.
7. Hiji, Y. Selective elimination of taste responses to sugars by proteolytic enzymes. *Nature* **256:** 427–429, 1975.
8. Kier, L. B. A molecular theory of sweet taste. *J. Pharm. Sci.* **61:** 1394–1397, 1972.
9. Kruskal, J. B., F. W. Young and J. B. Seery. How to use Kyst-2A, a very flexible program to do multidimensional scaling and unfolding. Murray Hill, NJ: Bell Laboratories, 1978.
10. McBurney, D. Gustatory cross adaptation between sweet-tasting compounds. *Percept. Psychophys.* **11:** 225–227, 1972.
11. McBurney, D. H. and L. M. Bartoshuk. Water taste in mammals. Paper presented at the Fourth International Symposium on Olfaction and Taste, Munich, 1971.
12. McBurney, D. H. and L. M. Bartoshuk. Interactions between stimuli with different taste qualities. *Physiol. Behav.* **10:** 1101–1106, 1973.
13. McBurney, D. H. and T. R. Shick. Taste and water taste of twenty-six compounds for man. *Percept. Psychophys.* **10:** 249–252, 1971.
14. McBurney, D. H., D. V. Smith and T. R. Shick. Gustatory cross adaptation: Sourness and bitterness. *Percept. Psychophys.* **11:** 228–232, 1972.
15. Schiffman, S. S., D. A. Reilly and T. B. Clark. Qualitative differences among sweeteners. *Physiol. Behav.* **23:** 1–9, 1979.
16. Schiffman, S. S., K. Sennewald and J. Gagnon. Comparison of taste qualities and thresholds of D- and L-amino acids. *Physiol. Behav.,* in press.
17. Shallenberger, R. S. and T. E. Acree. Molecular theory of sweet taste. *Nature* **216:** 480–482, 1967.
18. Shallenberger, R. S. and T. E. Acree. Chemical structure of compounds and their sweet and bitter taste. In: *Handbook of Sensory Physiology, Vol. IV, Chemical Senses. Part 2. Taste,* edited by L. M. Beidler. Berlin: Springer-Verlag, 1971.
19. Solms, J. The taste of amino acids, peptides, and proteins. *J. agric. Fd Chem.* **17:** 686–688, 1969.
20. Zawalich, W. S. Comparison of taste and pancreatic beta cell receptor systems. In: *Olfaction and Taste IV,* edited by D. Schneider. Stuttgart: Wissenschaftliche Verlagsgesellschaft MBH, 1972.