MOLECULAR STRUCTURE AND CONFORMATION IN TASTE PERCEPTION

by
William Jakinovich, Jr.

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Committee:

Professor J. E. Bardach, Chairman
Professor B. W. Agranoff

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I dedicate this work to my wife, Kitty, and my two children, Billy and Hellene, who, I know, are as happy as I at its completion.

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INTRODUCTION

Of all four taste qualities, the most puzzling are sweet and bitter. Sourness is generally thought to be caused by hydrogen ions, while "saltiness" is caused by ionized salts. For the other two qualities, however, no such specific agents have been identified. They are perplexing because there appears to be a complete lack of a common chemical mechanism in the compounds that produce them. In this paper the mechanism of "sweet" taste will be examined.

Theories Based on Behavioral Data

Until recently almost all theories concerning sweetness have relied heavily on behavioral data. Moncrief (1967), in an excellent review of gustatory stimulants which is based largely on human behavioral evidence, lists many categories of sweet compounds. Carbohydrates (e.g., glucose, glycols, sucrose), amino acids, chloroform, synthetic aromatic compounds, lead acetate and beryllium salts are all known to produce the sensation of sweetness in humans.

In an intensive study based on human experience, Oertly and Myers (1919) postulated that a sweet substance must contain a "glucophore" and an "auxogluc." A typical "glucophore" has the structure CH_2OH - CHOH for glycerol or $-CCl_3$ for chloroform. The "auxoglucs" for the two compounds are CH_2OH- and H- respectively. However, their model did not account for the sweetness of certain substances, especially saccharin. Schallenberger and Acree (1967), whose theory of sweet taste is remarkably similar to that of Oertly and Myers, could account for the sweetness of saccharin. They contended that a substance derives its properties of sweetness from an "AH, B" system. In this model "A" and "B" are electronegative

atoms separated by a distance greater than 2.5 Å but less than 4 Å. "H" is a hydrogen atom attached to one of the electronegative atoms by a covalent bond. Thus a sweet compound must have a slightly acidic proton. In order to account for the fact that many compounds that fit this model (especially carbohydrates and D-amino acids) are tasteless, Schallenberger et al. (1969) extended it to include certain stereochemical features of the receptor site. Using a very similar model, Dzendolet (1968) hypothesized that sweet compounds must include a proton-accepting atom (e.g., oxygen or nitrogen). In this model, substituent groups that prevent a molecule from approaching the receptor sites closely enough to remove a proton render it tasteless (e.g., N-methyl saccharin). Further evidence favoring this model comes from a study by Norris (1969) in which the beetle Scolytus multistriatus was tested with gustatory stimulants. In that study, it was concluded that stimulatory compounds must have hydroxyl groups that can be hydrogen-bonded.

Biochemical Approaches to the Problem of Sweetness

The nature of the initial interaction between sweet substances and taste receptors has been the subject of biochemical studies. The isolation of a sweet-sensitive protein at the receptor sites has been reported (Dastoli and Price, 1966; Dastoli et al., 1968; Dastoli, 1969). However, Price (1969) recently discounted these findings on the grounds that there was insufficient evidence that the protein isolated was related to taste. Recently an enzyme, α-glucosidase, has been proposed to play a role in insect sugar reception (Hansen, 1969). But Hansen was careful to point out that his enzyme hypothesis did not fit either the experimental or behavioral and electrophysiological results in studies with blowfly taste. The blowfly's sugar receptor is not

affected by conventional enzyme poisons or inhibitors nor does it respond to temperature conditions known to alter the action of enzymes.

Physiological and Behavioral Approaches to the Problem of Sweetness

While many of the taste theories attempt to account for the sweetness of all synthetic sweeteners, carbohydrates have been among the most intensively studied of the sweet-tasting compounds. Unlike the vertebrates, insects appear to respond behaviorally only to carbohydrates. Von Frisch (1935) and Dethier (1955), using the honey bee, Apis mellifera, and the blowfly, Phormia regina, respectively, were able to make only a few inferences about the structure of the sweet-tasting molecule. Among them were: (1) The size of the sapid molecule is important. Molecules smaller than pentoses are ineffective; only a few trisaccharides are effective. (2) Linear polyols such as D-glucitol are ineffective. (3) The ring oxygen is not required in hexoses (e.g., myo-inositol is sweet). (4) Compounds with α glycosidic linkages, such as sucrose and methyl α-D-glucopyranoside, are more effective than their β counterparts. However, attempts made to discover configurations that were common to the sweet sugars tested have for the most part been unsuccessful. Many electrophysiological experiments with mammals have attempted to relate the conducted impulse to the molecular configuration of carbohydrates. Andersen et al. (1963), using dogs, rated monosaccharides in order of stimulating ability: D-fructose > D-glucose > D-galactose. The corresponding rating for the disaccharides was sucrose > maltose > lactose. Experiments on human patients (Diamant et al., 1963) where the chorda tympani nerve was exposed during a routine surgery revealed the following order of effectiveness of sugars: sucrose > fructose >

mannose > lactose > glucose = maltose > sorbose = arabinose > galactose. Fujiya and Bardach (unpublished), recording from a multi-fiber preparation of the seventh cranial nerve, found similar results in the catfish, Ictularus natalis. Except for the fact that the carbohydrates tested fit the generalized taste theories, there have been no studies of conformational or configurational characteristics of stimulants that may shed light on the properties of the receptor site.

Electrophysiological studies on the taste receptor have been a source of quantitative information about its function. Using the integrated response of the chorda tympani nerve, Beidler (1954) was able to derive his well known equation for taste stimulation:

$$C/R = C(1/R_m) + (1/KR_m)$$

The relationship is based on the equations of Michaelis-Menten for enzymes. R and R_m are the response magnitude and its maximum respectively, K is the dissociation constant and C is the concentration of the stimulating substance. Morita (1969), comparing the values for K of Beidler's (1954) integrated response data and Kimura and Beidler's (1961) single cell receptor potential recordings, determined that there was too much variation between the reported values. He came to the conclusion that the relationships between receptor potentials and impulse frequency in vertebrate preparations are more complex than had initially been thought.

The difficulty of studying the vertebrate sweetness receptor was underscored by the study of Kimura and Beidler (1961) which showed that single cells respond to all four taste qualities. On the other hand electrical stimulation of single fungiform papillae in humans (von Békésy, 1964, 1966) also showed that individual papillae are responsive to specific taste qualities.

A Simple System

For many reasons, the sweetness-receptor site of insects would appear to be a more advantageous preparation for studies of receptor specificity than the sweetness receptors of vertebrates which are far too complex for these kinds of studies. The insect sweet receptor however may be highly specialized, rather than a primitive evolutionary precursor of that found in higher animals. More primitive organisms have receptors more complex than those of the fly. For example, chemotaxis studies with the bacteria E. coli revealed that there are at least four sugar and two amino acid receptor sites (Adler, 1969). In contrast, work by Evans (1961, 1963), Morita and Shiraishi (1968), Omand and Dethier (1969), as well as others has suggested that in some flies there is a single receptor cell with at least two receptor sites. One site is specific for glucose and the other for fructose. In flies, the sugar receptor cells are located in the tarsal and the labellar hairs. The kinds of receptor activity elicited by various sugars can be distinguished in flies according to the size of the action potential recorded extracellularly (Browne and Hodgson, 1962; Hodgson, 1956, 1957; Morita, 1968; Otter, 1967). Results correlate well with behavioral studies. There also appears to be a clear relationship between receptor potentials and impulse frequency in the fly labellar chemosensory neurons (Morita, 1969). Studies by Beidler and Smallman (1965) indicate that taste cells in mammals are constantly in the process of aging and being replaced by new cells. The receptors of the fly are not known to be replaced but the response of the Type 1 (salt) chemoreceptor of Phormia regina has been shown to change through aging during the first 25 days of adult life (Rees, 1970). However the age of the receptor cell can be

determined quite easily, so this is not an important drawback. It has long been known that sensitivity to "sweet" compounds also declines with age in humans. Temperature (Dethier and Arab, 1958) or pH (Shiraishi and Morita, 1969), within a reasonable range, have little effect on the activity of the fly's sugar receptor.

Because of the size of flies and the technique employed, volumes as small as two microliters are more than adequate. The fly receptor thus permits testing of rare substances.

Previous Work with Flies

Using the blowfly, Phormia regina Meigen, Evans (1963) attempted to correlate chemical structure and stimulation of the sweetness receptor. His main conclusion was that a molecule reacting to the glucose site must be in the pyranose form and that the hydroxyl groups of the C_3 and C_4 carbons are solely responsible for stimulation. This rule was based on studies with a limited number of substances.

Objective

The main objective of this investigation was: To measure the responses to a large number of sugars in the labellar sugar receptor of the fleshfly (Sarcophaga bullata Parker) using an electrophysiological technique, and to determine both the configurational and conformational requirements of the molecule binding to the glucose receptor site.

MATERIALS AND METHODS

Experimental Animals

The fleshfly, Sarcophaga bullata Parker*, was used throughout this work. The flies were raised at the Amphibian Research Facility of the University of Michigan and were available throughout the year. The larvae were grown on a controlled diet so that there was no variation in dietary conditions between flies. Evans (1961) found that the responses of adult flies to one another differed if the larvae had been reared in the presence of different sugars. Imagos between 2 and 5 days old were used in the experiments. During this period of life the chemoreceptor cells show the smallest amount of response change caused by aging (Rees, 1970) and the flies were food-deprived from emergence and were given tap-water ad libitum.

Recording Technique

Detailed physiological studies of single chemosensory structures of flies (Hodgson and Roeder, 1956; Wolbarsht, 1958; Wolbarsht and Dethier, 1958; Evans and Mellon, 1962) revealed that the hairs on the labellum of Phormia function as chemoreceptors (Fig. 1). Because of the small size of each hair, normal recording techniques had to be modified. The technique of recording the response of the sensory cell is similar to that of Hodgson and Roeder (1956). An unanesthetized fly was decapitated and its head was crushed with forceps. A small piece of platinum wire (0.007 inch diameter) with a sharpened point was forced gently down the intact proboscis, extending it. The wire

*Identification verified by Raymond J. Gagné, Systematic Entomology Laboratory, USDA, c/o U.S. National Museum, Washington, D.C. 20560.

Figure 1. a. Schematic drawing of the fleshfly's head. b. A single chemosensory hair showing the relationship of the sensory neuron to the sensory pore (after Dethier, 1955).

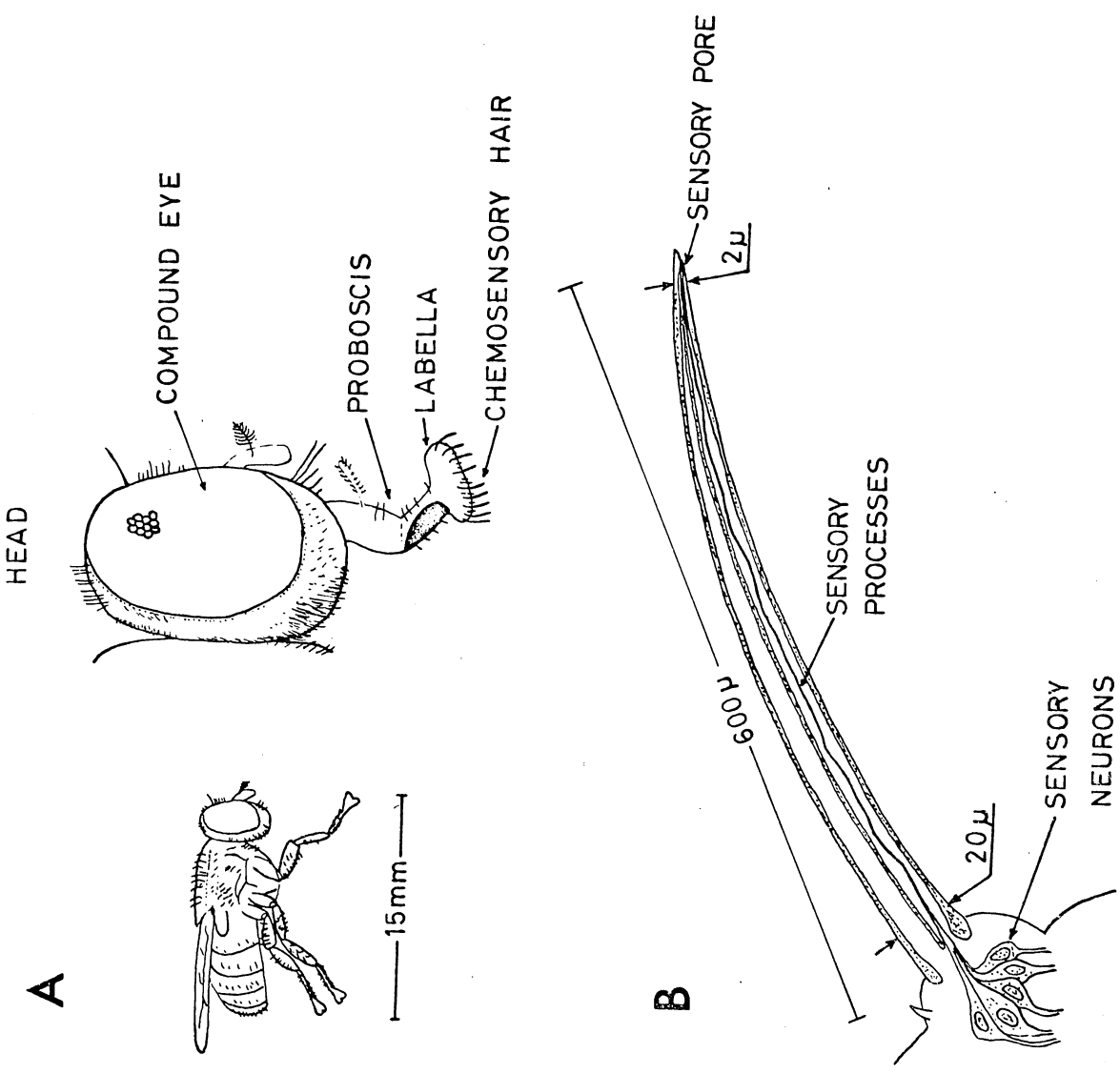

A
HEAD
COMPOUND EYE
PROBOSCIS
LABELLA
CHEMOSENSORY HAIR
15mm
B
SENSORY PORE
2μ
600μ
SENSORY PROCESSES
20μ
SENSORY NEURONS

Figure 1

was then clamped to a micromanipulator and positioned under a dissecting microscope. This wire, which acted as an indifferent electrode, was grounded. The active electrode, an identical piece of platinum wire, was then inserted into a piece of 1 mm (I.D.) glass capillary tubing 2 cm long and drawn down to about a 50 μ tip diameter (Fig. 2). This tubing was inserted into a microelectrode holder on a second micromanipulator. Electrical contact between the sensory cell and the active electrode was accomplished by means of a 0.05 M NaCl solution placed in the tubing. This solution, which contained the compound being tested, was transferred from a storage vial to the capillary tubing by means of a micropipet a few seconds before the test.

The active electrode was led through a unity gain negative capacitance preamplifier (Bioelectric Instruments type NF1), thence through a Tektronix 122 amplifier. This signal was filtered (Krohn-Hite variable band-pass filter) to remove the slow base line drift and high frequency noise and then displayed on an oscilloscope. Both unfiltered and filtered signals could be displayed simultaneously. No serious distortions were introduced by the filtering process. Activity was monitored from a loudspeaker. In most cases, activity was photographed directly from the oscilloscope by means of a Grass camera. Occasionally the activity was recorded on an Ampex FM tape recorder at 19.05 centimeters per second. The tape was subsequently played back on the oscilloscope for photographic recording.

Many of the previous papers dealing with chemosensory studies of this type failed to report which labellar hairs were studied. Hodgson (personal communication) felt that sensory hairs of different sizes had different sensitivities. All the experiments in this

Figure 2. Diagrammatic representation of recording apparatus.

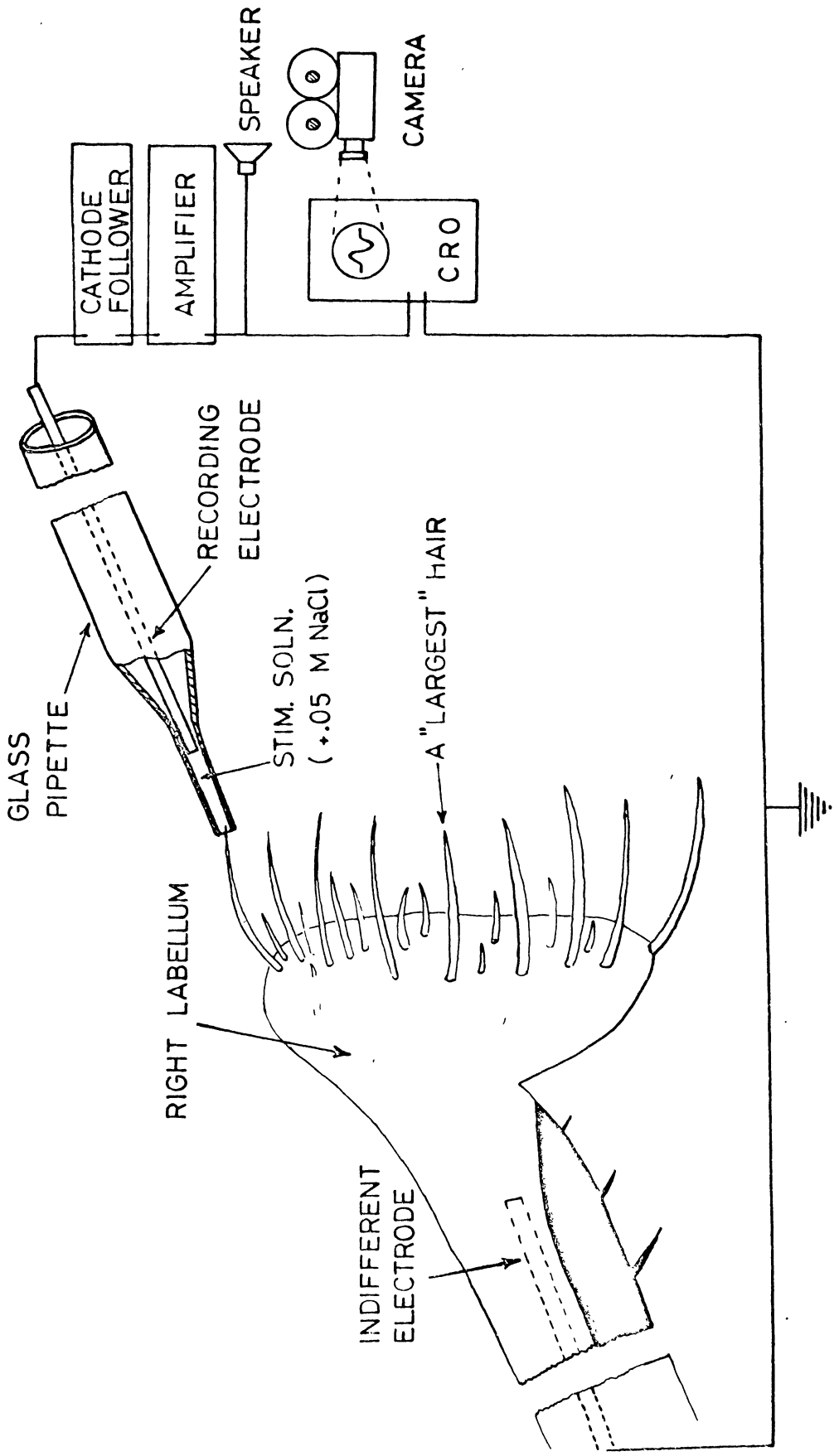

CATHODE FOLLOWER
AMPLIFIER
SPEAKER
CAMERA
CRO
RECORDING ELECTRODE
GLASS PIPETTE
STIM. SOLN. (+.05 M NaCl)
A "LARGEST" HAIR
RIGHT LABELLUM
INDIFFERENT ELECTRODE

Figure 2

investigation were conducted with the "largest" hairs (Wilczek, 1967). In Sarcophaga bullata, the "largest" hairs (see Fig. 2) numbered about 8-9 on each labellum, for a total of 16-18. Before the recording session, each hair was examined for obvious anomalies and prewashed with a 0.1 M NaCl solution. A recording session was begun by lowering the filled electrode onto the sensory tip of a single hair until it made contact with the surface film of the solution in the electrode tip. This procedure was repeated immediately with 3 to 7 more hairs randomly chosen. Evaporation of the solution in the electrode tip was minimized by the low temperature (22±2°C) and high humidity of the laboratory. Each hair was first tested with a control solution (0.05 M NaCl). Test solutions were always presented in order of increasing concentration. After each test solution the hairs were rinsed with 0.1 M NaCl and a 5 minute "rest" period was allowed between tests for disadaptation.

The moment when the electrode first made contact with the hair tip was signaled by an artifact producing a distinct "pop" over the loudspeaker. This was followed by a train of action potentials. After a short time (< 1 sec), the electrode was withdrawn and moved to the next hair to be tested. Occasionally the initial artifact was so large that it saturated the amplifier and blocked its response for 0.1 sec or more. This saturation effect was reduced by placing a capacitor in series with the active electrode. Furthermore, since it only occurred when the first hair was being tested, a smaller hair was usually stimulated prior to the first "largest" hair. This procedure generally eliminated any amplifier blockage.

Half of all the "largest" hairs were presented with a test sugar of various concentrations. As few as 7 hairs were tested with one

compound. The remaining hairs were tested with D-glucose and D-fructose standards. In addition, each hair that had been tested with one of the compounds under study was ultimately stimulated with D-glucose as a further control. Flies that did not respond to any of the control solutions (about 10%) were rejected. A recording session usually lasted from 1 to 1.5 hours although some preparations gave "normal" responses for as long as 3 hours.

Compounds Tested - Preparation and Sources

All compounds tested were dissolved shortly before a testing session in 0.05 M NaCl prepared from distilled deionized water. At this concentration, the salt has little effect on the response to sugars (Morita, 1967). To conserve rare substances (as little as 4.5 mg in a few instances), small storage vials (Kontes "microflex" tubes) were used. These vials have a tapered cone which facilitates retrieval. The sample can easily be seen, as it is magnified by the thick glass base. Immediately after a test session the solutions were frozen and stored at -23°C. Substances that were available in substantial amounts were freshly prepared whenever possible. Most of the glucose derivatives were provided by Dr. Irwin J. Goldstein (Department of Biological Chemistry, University of Michigan). Reagent grade D-glucose was purchased from the J. T. Baker Chemical Company. D-Fructose was purchased from the Eastman Kodak Company, Rochester, New York. The latter did not differ significantly in these studies from a sample of highly purified fructose. Myo-inositol was purchased from the Nutritional Biochemical Corporation, Cleveland, Ohio. Quebrachitol ((-)-inositol-methyl ether), (-)-inositol and (+)-inositol were purchased from Calbiochem of Los Angeles, California. The remainder of the cyclitols were provided by Dr. Bernard W. Agranoff (Mental

Health Research Institute, University of Michigan). Cyclohexanediols (1,2; 1,3; and 1,4) were purchased from Aldrich Chemical Co. of Milwaukee, Wisconsin.

RESULTS

Identification of Spikes

In the labellar chemosensory hair of the fleshfly (Sarcophaga bullata Parker), the spike height of the sugar, salt and water receptors differed sufficiently to allow classification of a particular spike as belonging to one of these receptors. Recently Larimer and Oakley (1968) reported the difference between the sugar and the salt spike in the same fly. A typical record (Fig. 3) shows that when 0.05 M NaCl is presented alone a very small spike occurs. When D-glucose is added a larger spike appears. The frequency of this spike increases as sugar concentration increases. This is more clearly seen in the frequency distribution of the absolute spike amplitude (Fig. 4). Occasionally, when a water spike and a sugar spike occur simultaneously they summate, giving rise to slightly larger spikes. Salt spikes which are much larger (1.0 - 1.5 mV) are seldom seen at this concentration of salt and their rare appearances are quickly noticed. Often the water spike is so small that it is barely distinguishable from the baseline noise. In these instances it is not necessary to make a frequency distribution diagram of spike heights.

Responses to Single Sugars

While the responses of the whole population of flies showed some variability, the responses of a single fly to repeated applications of a sugar were remarkably constant (Fig. 5). The receptors were stimulated repeatedly with 0.3 M D-glucose to determine the reproducibility of the response. The results in Figure 6 show that the order of effectiveness of the stimuli was sucrose > fructose > glucose below 0.18 M; the order changes to sucrose > glucose > fructose at higher

Figure 3. A typical recording from a single labellar chemosensory hair. The arrows in the top record are 400 msec apart. Note the "pop" artifact at the beginning of most of the records.

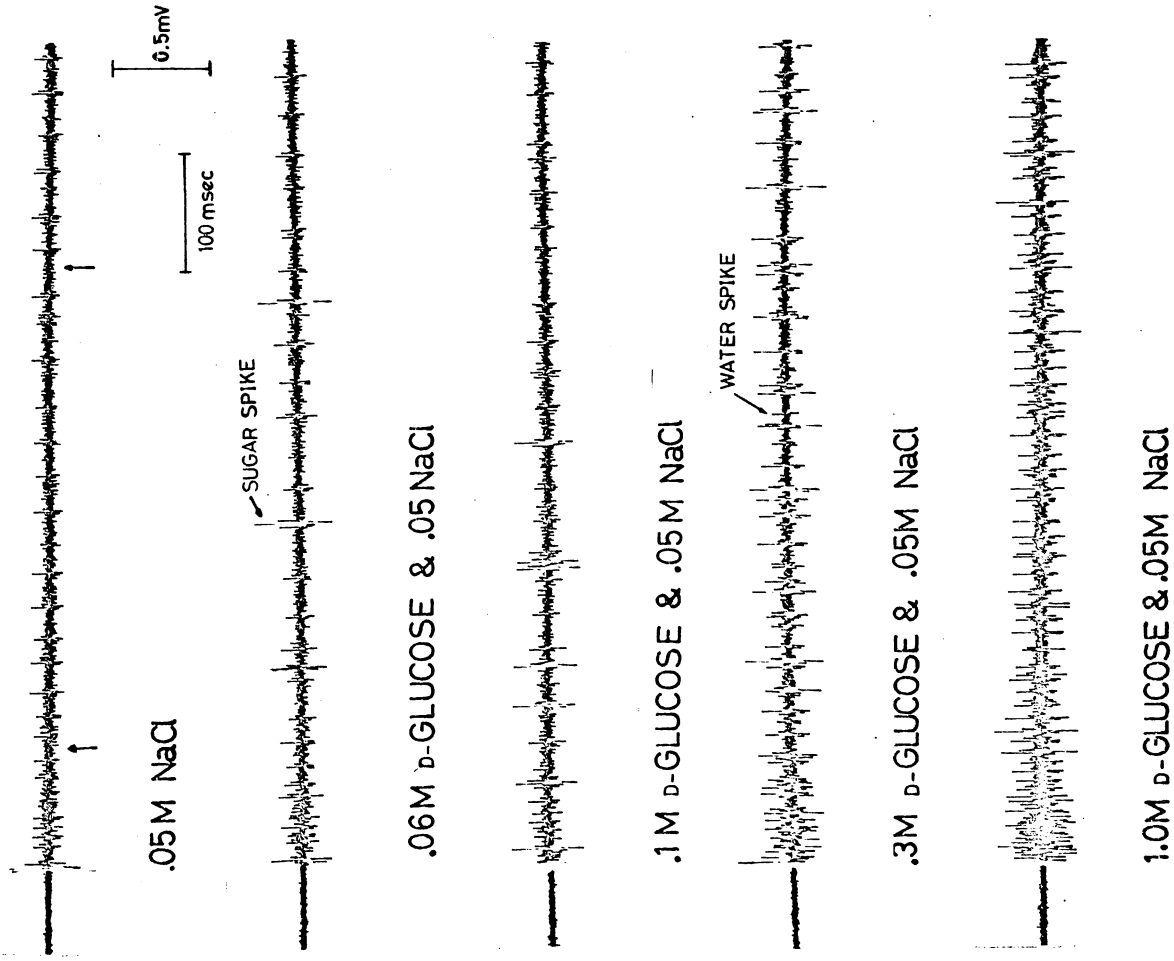

0.5mV
100 msec
.05 M NaCl
SUGAR SPIKE
.06 M D-GLUCOSE & .05 NaCl
.1 M D-GLUCOSE & .05 M NaCl
WATER SPIKE
.3M D-GLUCOSE & .05M NaCl
1.0M D-GLUCOSE & .05M NaCl

Figure 3

Figure 4. Histograms showing the frequency of a "sugar" spike increasing as the concentration of sugar in the 0.05 M NaCl solution increases. The shaded portion represents the sugar spike while the unshaded areas represent the water spike. Summated water and sugar spikes are represented as shaded areas.

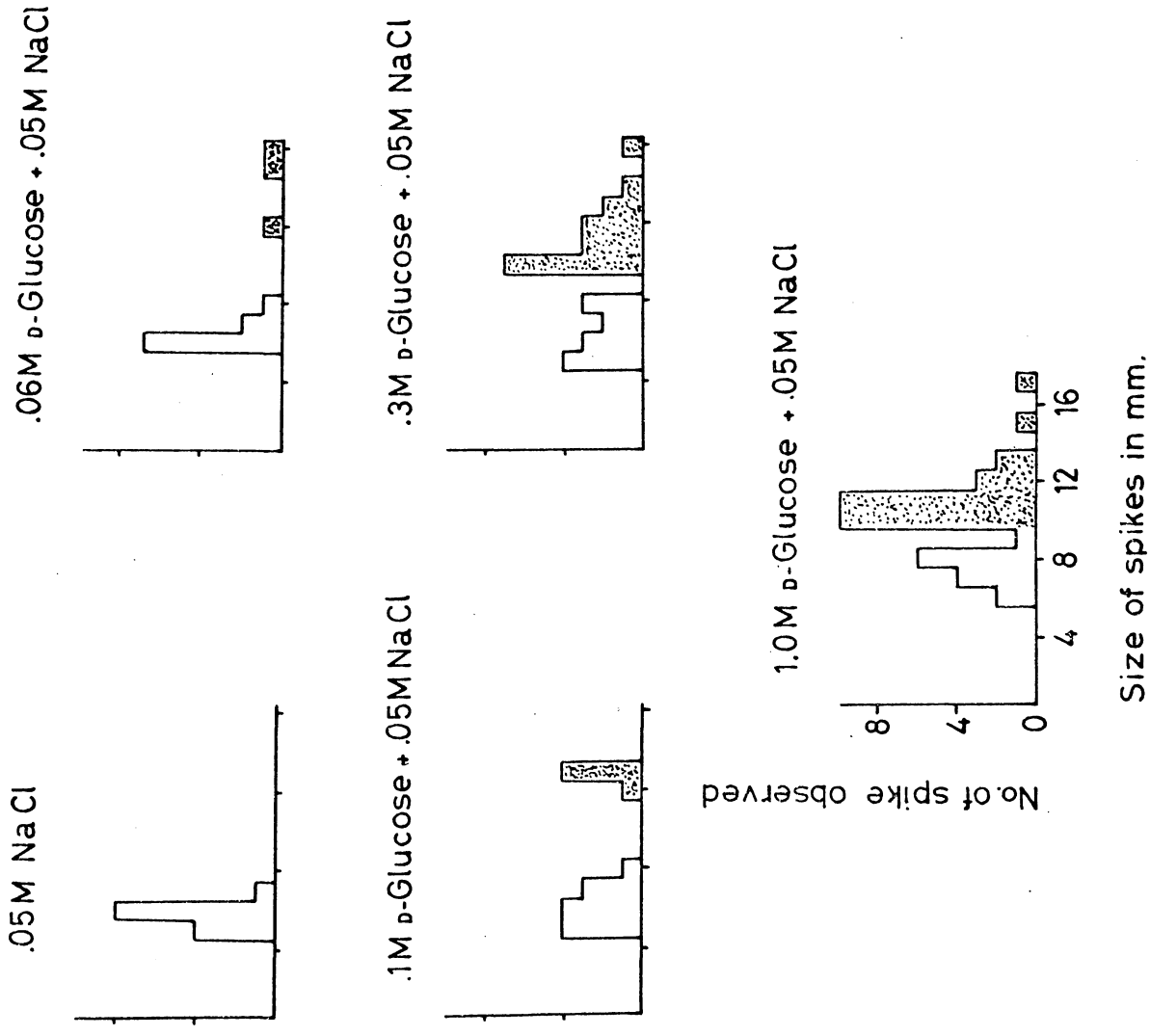

.05M NaCl
.06M D-Glucose + .05M NaCl
.1M D-Glucose + .05M NaCl
.3M D-Glucose + .05M NaCl
1.0M D-Glucose + .05M NaCl
No. of spike observed
0
4
8
4
8
12
16
Size of spikes in mm.

Figure 4

Figure 5. The response of a single hair to D-glucose. The number attached to each symbol indicates the order of stimulation. The symbol "x" is 0.3 M D-glucose given to test the reproducibility of response.

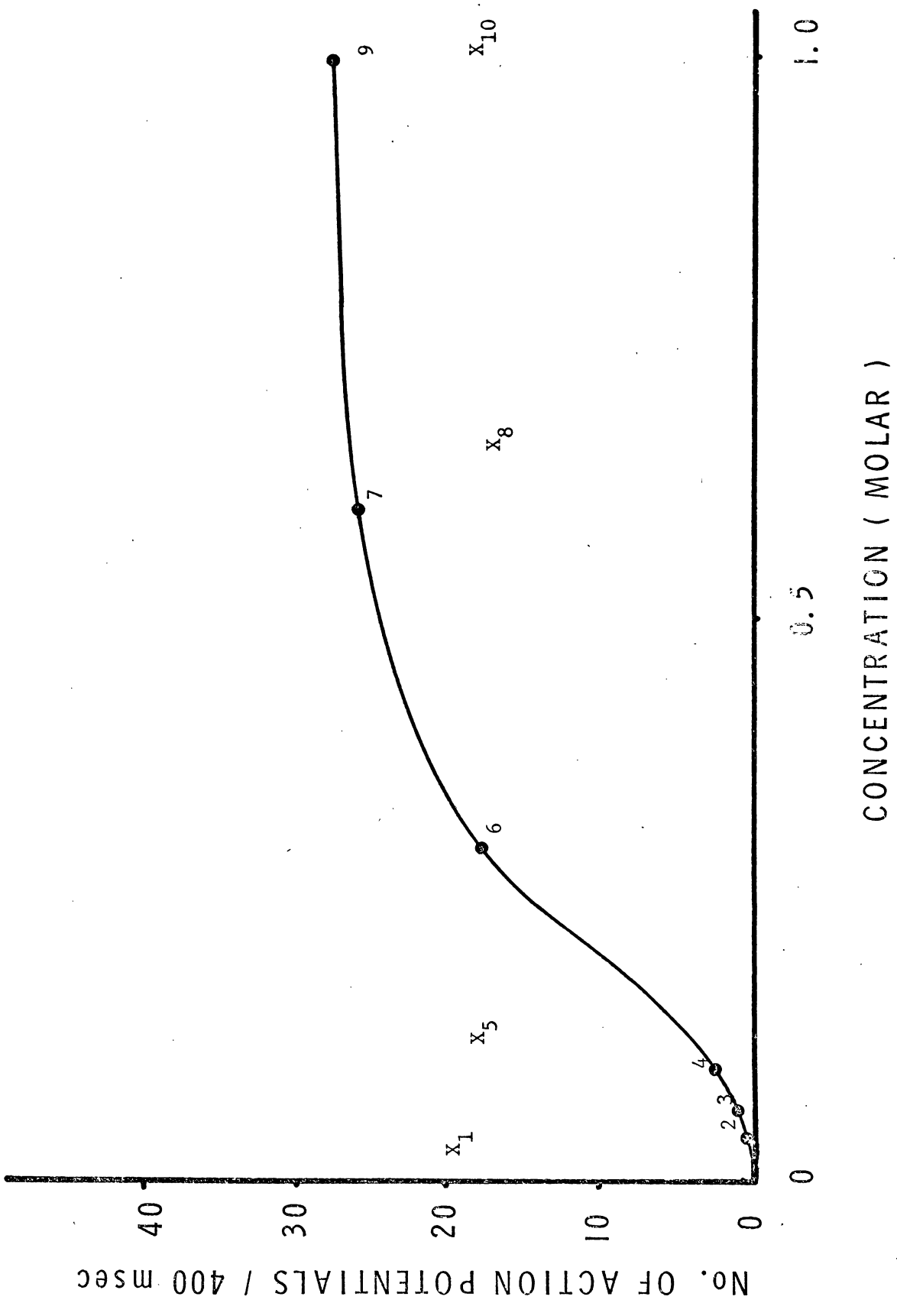

No. OF ACTION POTENTIALS / 400 msec
0
10
20
30
40
0
0.5
1.0
CONCENTRATION (MOLAR)
x_1
x_5
x_8
x_{10}
1
2
3
4
6
7
9

Figure 5

Figure 6. Comparison of response-concentration relation in stimulation by sucrose, glucose and fructose.

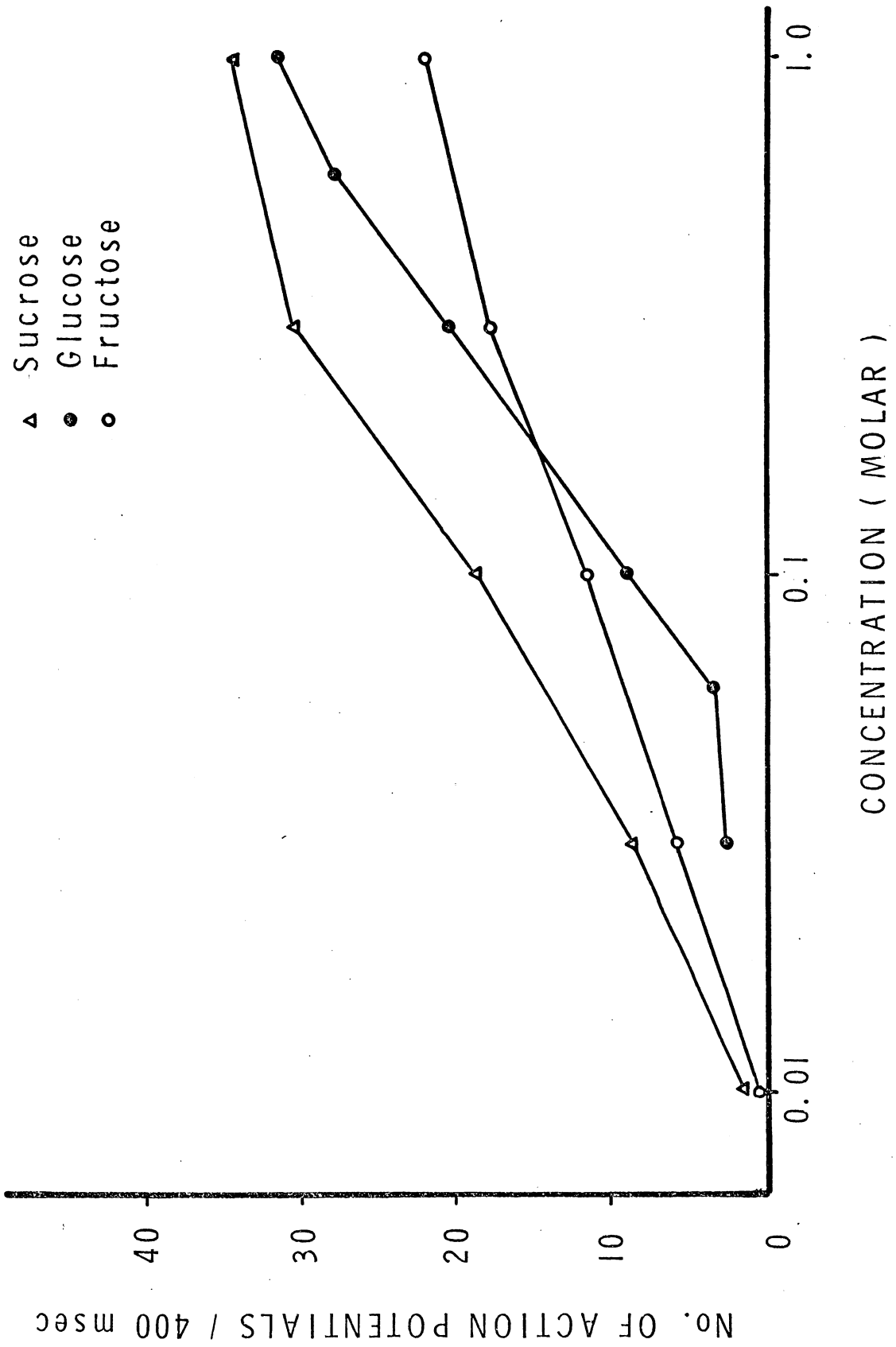

Sucrose
Glucose
Fructose
0
10
20
30
40
No. OF ACTION POTENTIALS / 400 msec
0.01
0.1
1.0
CONCENTRATION (MOLAR)

Figure 6

molarities. Although the concentration at which it occurred varied from animal to animal, crossing of the glucose and fructose curves was always seen. The crossing of curves was not observed between varying concentrations of D-glucose or any of the glucose derivatives tested.

The results of tests with different sugars are shown in Table I. The maximal responses are values obtained experimentally from response concentration curves similar to that in Figure 5 when the curve showed a nearly horizontal line. These values are computed relative to D-glucose. The maximal responses to sucrose, glucose and fructose compare closely to the values obtained by Morita and Shiraishi (1968) for the fleshfly, Boettcherisca peregrina.

Configuration and Conformation Requirements

As stated earlier, Evans (1963) concluded that the glucose receptor site of Phormia regina was responsive only to the C_3 and C_4 hydroxyl groups of the molecule stimulating it. This author suspected the same would hold for the fleshfly, Sarcophaga bullata. Subsequent experiments with cyclohexanediols showed that this was not the case. In fact, when diols were mixed with either D-glucose or D-fructose, they inhibited the response to these sugars. This result indicates that the molecule interaction with the receptor required more than just two hydroxyl groups to be effective.

Experiments with various glucose derivatives (Fig. 7) were done on the assumption that more than two hydroxyl groups are necessary to stimulate the receptor.

The C_1 Position

The results of this experiment are consistent with the view that the most favored position about C_1 for sweet receptor is the axial

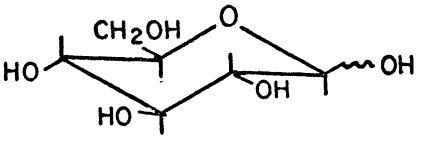
Table I. Maximum receptor activity of single labellar hairs evoked by the stimulation with various sugars

Compound	Maximum relative effectiveness (to D-glucose)	No. of hairs tested electrophysiologically
D-glucose (equilibrium mixture)	1.00	169
Methyl α-D-glucopyranoside	1.10	15
Sucrose (β-D-fructofuranosyl-α-D-glucopyranoside	1.43	18
1,5-Anhydro-D-glucitol	0.90	7
Methyl β-D-glucopyranoside	0.30	21
L-Glucose	0.70	15
Methyl α-D-mannopyranoside	0	18
2-Deoxy-D-arabino-hexopyranose	0.23	14
2-O-Methyl-D-glucose	0.53	8
Methyl 2-amino-2-deoxy-α-D-glucopyranoside	0.63	17
Methyl 2-acetamide-2-deoxy-α-D-glucopyranoside	0	12
Methyl β-D-fructopyranoside	0	24
D-Allose	0.33	14
3-Deoxy-D-ribo-hexopyranose	0	21
3-O-Methyl-D-glucose	0.17	8
Methyl α-D-galactopyranoside	0	10
4-O-Methyl-D-glucose	0	16

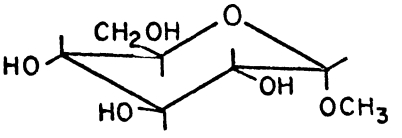
Table I (Continued)

Compound	Maximum relative effectiveness (to D-glucose)	No. of hairs tested electrophysiologically
Methyl α-L-sorbopyranoside	0	16
Methyl α-L-glucopyranoside	0	8
Methyl α-D-xylopyranoside	0.50	8
6-Deoxy-D-glucose	0.50	13
6-O-Methyl-D-glucose	0.73	8
D-Fructose	0.77	128
Methyl α-D-fructopyranoside	0	17
Methyl α-D-fructofuranoside	0	15
Methyl β-D-fructofuranoside	0	19
3-O-Methyl-D-fructose	0	13

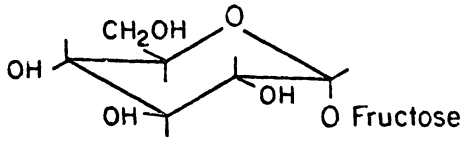
Figure 7. Schematic representation of the glucose derivatives used in this study.



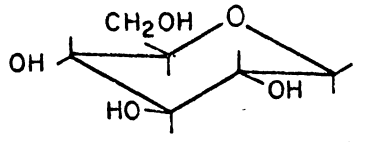
D-Glucose



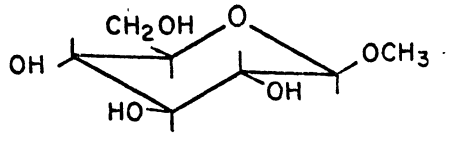
Methyl α-*D*-glucopyranoside



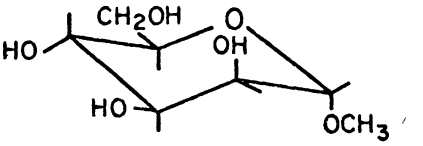
Sucrose



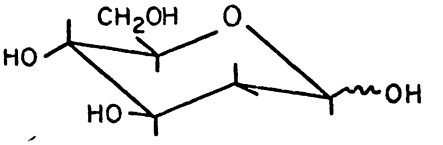
1,5-Anhydro-*D*-glucitol



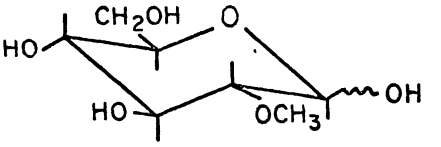
Methyl β-*D*-glucopyranoside



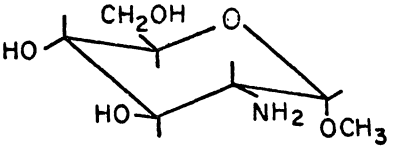
Methyl α-*D*-mannopyranoside



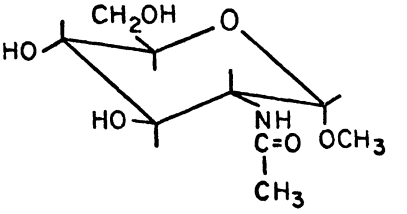
2-Deoxy-*D*-*arabino*-hexopyranose



2-*O*-Methyl-*D*-glucose

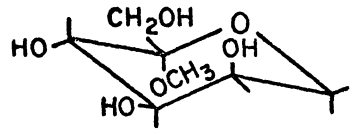


Methyl-2-amino-2-deoxy-
α-*D*-glucopyranoside

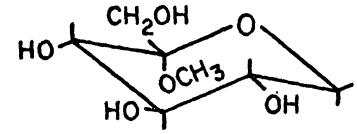


Methyl 2-acetamide-2-deoxy
α-*D*-glucopyranoside

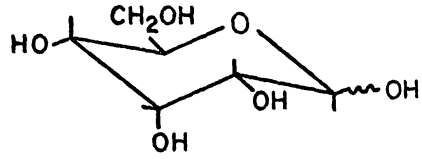
Figure 7



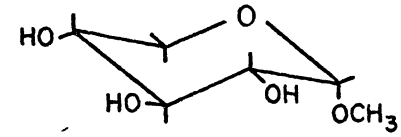
Methyl - β - *D* - fructopyranoside



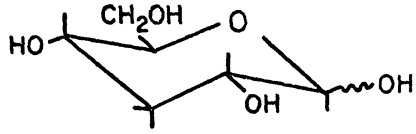
Methyl α - *L* - sorbopyranoside



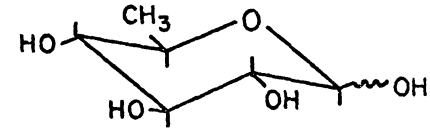
D - Allose



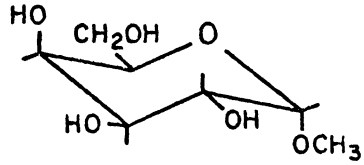
Methyl α - *D* - xylopyranoside



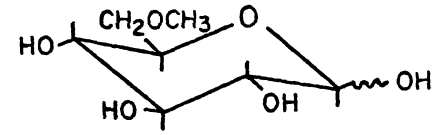
3 - Deoxy - *D* - *ribo* - hexopyranose



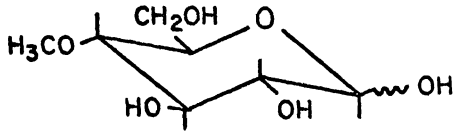
6 - Deoxy - *D* - glucose



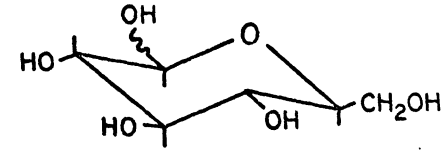
Methyl α - *D* - galactopyranoside



6 - *O* - Methyl - *D* - glucose



4 - *O* - Methyl - *D* - glucose



L - Glucose

Figure 7 (continued)

(Evans, 1963). Methyl β-D-glucopyranoside, which has the C_1 oxygen in the equatorial position, was much reduced in effectiveness. The effectiveness of β-D-glucose, which has an equatorial hydroxyl group, increased with time between its solution and the test. At low concentrations (≤ 0.3 M), β-D-glucose was only 15% as effective as D-glucose when tested within a few minutes after preparation of the solution. However at high concentrations (0.6 - 1.0 M), β-D-glucose was as effective as D-glucose as a stimulant. 1,5-Anhydro-D-glucitol, which lacks a C_1 hydroxyl, was nearly as effective as D-glucose, which indicates steric hindrance in the equatorial position rather than the absence of a reactive axial group. The α-substituted derivative, methyl α-D-glucopyranoside, showed a response slightly greater than that of D-glucose.* An even greater response was evoked by sucrose (β-D-fructofuranosyl-α-D-glucopyranoside). Evans (1961) claimed that sucrose acted primarily at a fructose receptor site. If this were true, methyl β-D-fructofuranoside might be expected to be an excitatory compound. However, it neither excited nor inhibited the receptor.

The C_2 Position

Removal or methylation of the C_2 oxygen dramatically changes the effectiveness of the sugar compared to D-glucose. 2-Deoxy-D-arabino-hexopyranose and 2-O-methyl-D-glucose show about the same degree of reduced effectiveness. It was concluded that the first compound was less effective because of its loss of the oxygen and the second for steric reasons. Replacement of the hydroxyl group with an amino group

*The equilibrium mixture of α- and β-D-glucose will be referred to as D-glucose in the rest of the paper.

(methyl 2-amino-2-deoxy-α-D-glucopyranoside) restored much of the effectiveness of the molecule. The compound was rendered totally ineffective by steric hindrance, as shown by methyl 2-acetamide-2-deoxy-α-D-glucopyranoside. If the C_2 hydroxyl group is in the axial position, as in methyl α-D-mannopyranoside, the molecule was also ineffective.

The C_3 Position

The requirement for a C_3 hydroxyl is suggested by the ineffectiveness of 3-deoxy-D-ribo-hexopyranose. Methylation of the hydroxyl group as in 3-O-methyl-D-glucose has the same effect. In D-allose the C_3 hydroxyl is axial and the molecule was not as effective in stimulation as D-glucose.

The C_4 Position

Even though a glucose derivative that lacks the C_4 hydroxyl was not available, methylation of the oxygen resulted in an inert compound (4-O-methyl-D-glucose). Methyl α-D-galactopyranoside, which has its C_4 hydroxyl in the axial position, was also inert.

The C_5 Position

Methyl α-L-sorbopyranoside, which is identical to 1,5-anhydro-D-glucitol except that it has a $-OCH_3$ group in the C_5 axial position, was ineffective. Removal of both the axial $-OCH_3$ and the equatorial $-CH_2OH$ groups, as in methyl α-D-xylopyranoside, makes the molecule slightly effective.

The C_6 Position

The C_6 hydroxyl group appears to be important because the maximum effectiveness of 6-deoxy-D-glucose was the same as that of methyl α-D-xylopyranoside. Methylation of it (6-O-methyl-D-glucose) reduces the

effectiveness, but not as much as for the hydroxyl groups of the other C positions.

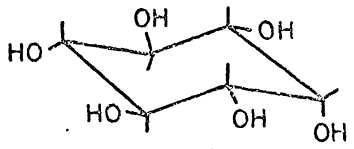
The Cyclitols

The importance of the ring oxygen was tested using the cyclitols (Fig. 8), which closely resemble the pyranose sugars. Myo-inositol, similar to α-D-glucose except for the substitution of a -CHOH group for the ring oxygen, was the most effective of these compounds (Table II). (+)-Inositol, which has an axial C_6 hydroxyl, was less effective than myo-inositol. (+)-Quercitol which lacks the hydroxyl at the C_2 position was inert. The importance of the C_2 hydroxyl in the axial position is indicated by the ineffectiveness of (-)-inositol which resembles α-D-mannose. Muco- and epi-inositol were ineffective for the same reason. Scyllo-inositol, which resembles β-D-glucose, was also ineffective. Allo-inositol failed to stimulate the receptor for the same reason that made D-allose only slightly effective. It both exhibits steric hindrance at the C_3 position and has an effectiveness-reducing factor with a C_6 axial hydroxyl. Cis- and neo-inositol were not available but it is predicted that they would be ineffective for steric reasons. Quebrachitol which is the methyl ether of (-)-inositol was as effective as (+)-inositol.

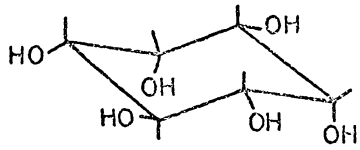
L-Sugars

All the compounds tested are generally accepted to occur in the CI chair for D-sugars as designated by Reeves (1949, 1950, 1951). LeFevre and Marshal (1958) have given evidence that the transport of sugars by the red blood cell is most favorable when they are in the CI conformation. However it has been shown that Phormia regina responds to L-fucose and L-xylose, which are expected to occur in the IC conformation

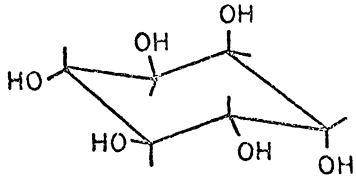
Figure 8. The cyclitols (modified from Posternak, 1965).



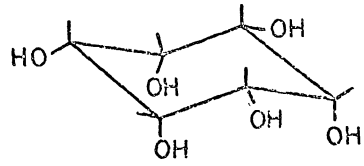
Myo - Inositol



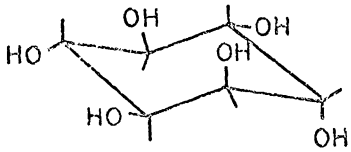
Epi - Inositol



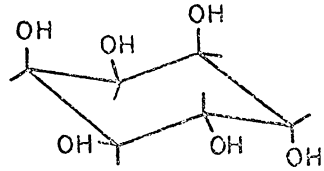
(+) - Inositol



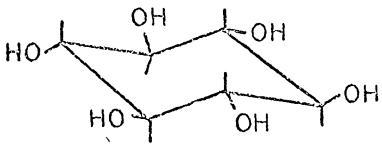
Cis - Inositol



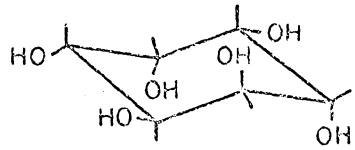
(−) - Inositol



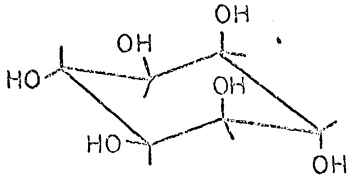
Allo - Inositol



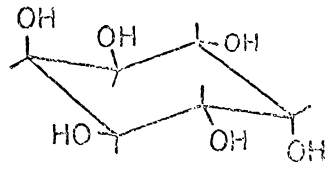
Scyllitol



Allo - Inositol



Muco - Inositol



Neo - Inositol

Figure 8

Table II. Maximum receptor activity of single labellar hairs evoked by the stimulation with various cyclitols.

Compound	Relative maximum effectiveness[a]	No. of hairs tested electrophysiologically
Myo-inositol	0.50	19
(+)-inositol	0.17	14
Scyllitol	0	9
(+)-Quercitol	0	13
(-)-inositol	0	27
Muco-inositol	0	16
Epi-inositol	0	8
Quebrachitol	0.23	17
Cis-inositol	Did not test	
Allo-inositol	0	8
Neo-inositol	Did not test	

[a] To D-glucose.

(Hasset et al., 1950). Caspary and Crane (1968) recently pointed out that the two chair forms are superimposable and the D- and L-glucose differ only in the interchange of two substituents: -OH and $-CH_2OH$. They further pointed out that in the active sugar transport system of the hamster small intestine, the $-CH_2OH$ at C_6 of glucose is not an absolute requirement and large substituents at C_1 permit transport. This is consistent with our finding that L-glucose was not as effective as D-glucose, which it closely resembles (see Fig. 7), because of steric hindrance at C_1 and C_5. The ineffectiveness of methyl α-L-glucopyranoside further supports this finding.

DISCUSSION

It appears that the sugar receptor of the fly is highly specific and unequally sensitive to various carbohydrates. With electrophysiological methods it has been possible to work out some of the details of its specificities.

The results are consistent with previous taste theories of sweet taste (Oertly and Myers, 1919; Schallenberger and Acree, 1967; Schallenberger et al., 1969; Dzendolet, 1968) in that there must be an electronegative atom in order for a molecule to be effective. The effectiveness of a hydroxyl group or amino substitution on the C_2 position of methyl-2-deoxy-2-amino-α-D-glucopyranoside is evidence to support these theories. Removal or methylation of either of these groups renders the molecule ineffective or greatly reduces its ability to stimulate the receptor. Oxygen is favored over nitrogen because of its greater electronegativity. Active transport in mammalian intestine of D-galactose and its 6-deoxy-6 halogen derivatives suggest that hydrogen bonding is significantly affected by the electronegativity of the attracting atom (Jarvis et al., 1967).

The results have shown that the structural configuration of a molecule is its most important determinant as an effective stimulus for the receptor. Evans' (1963) conclusion that only the hydroxyl groups on the C_3 and C_4 carbons are necessary for a sugar to be effective cannot be applied to Sarcophaga bullata. The source of difference is that the receptor site specificities between Phormia regina and Sarcophaga bullata are different. Frings and Cox (1954) observed that Phormia regina had a lower tarsal threshold for sucrose than Sarcophaga bullata.

The results of this study support the view that the hydroxyl groups on C_3 and C_4 are necessary. In fact these two hydroxyl groups appear to be the most important if a sugar molecule is to be an effective stimulant. In other biological systems, such as intestine sugar transport studies, where configurational and conformational analyses have proven to be very important for an understanding of the mechanisms, the other hydroxyl groups contribute to the effectiveness of the compound. The C_2 and C_6 hydroxyls are the most important in this respect (Table 1). Barnett et al. (1968) showed that a decrease in active transport in the intestine of the rat and the hamster occurs when replacements are made in positions 1, 2, 3, 4 or 6 of the sugar molecule, which suggests that five-point contact between the sugar and the membrane is necessary for transport.

α-Glucosides have long been known to be the most effective sweet taste stimulants (e.g., sucrose and maltose). It was once thought that sucrose acted at a fructose site (Evans, 1961). However, since methyl β-D-fructofuranoside was ineffective it must be concluded that sucrose interacts primarily at the glucose site. Morita and Shiraishi (1968) came to the same conclusion by more indirect means with the fly, Boettcherisca peregrina. Molecules with β substituent groups are rendered less effective by steric hindrance while the α counterparts are possibly enhanced by additional interaction between the substituent and the cell membrane as can be inferred for a comparison of sucrose and methyl α-D-glucopyranoside.

Even though the mechanism of stimulation is unknown the most effective compounds have all of the necessary hydroxyl groups in an equatorial position relative to the plane of the molecule. This is not at all surprising since this is the most favorable situation for the

sugar molecular stability and many of the sugars that have the highest affinity in sugar transport systems (e.g., human erythrocyte and intestine) have their important substituent groups equatorial (see Figure 9).

The finding that L-glucose was stimulating was surprising. In other biological systems such as sugar transport, L-sugars have been more thoroughly studied. LeFevre and Marshal (1958) presented evidence that transport of sugars in the red blood cells has the simple requirement of the C1 conformation. However Caspary and Crane (1968) pointed out that differences in effectiveness of the D and L enantiomorphs should be viewed as a configurational problem, in intestinal sugar transport, related to substituent groups rather than as a conformational problem related to the chair form. Viewed in this light one can readily see that the C_1 position of L-glucose now becomes the C_5 position of D-glucose. One can also see that β-L-glucose which is a favorable stimulating compound resembles methyl β-D-glucopyranoside. However this disagreement is remedied by examining space-filling atomic models which reveal that C_6 hydroxyl of the L-sugar is in a more favorable position to interact with the receptor site. This interaction, such as an additional binding, may be very similar to that which enhances the stimulating effectiveness of sucrose and methyl α-D-glucopyranoside.

At the outset of this study it appeared that the cyclitols would be advantageous compounds to study with respect to the stereospecificity of this receptor. The responses of the cyclitols are extremely important in a study of sweet taste stimulation because their structures are known and, unlike many sugars, they do not undergo mutarotation and therefore do not exist as an equilibrium mixture of isomers. Even

Figure 9. Schematic drawing of a sugar molecule showing the required conformation and configuration of the substituent groups for a maximally effective compound. Doubly underlined groups are absolutely necessary and singly underlined groups are secondarily important. Substituent groups at C_1, while not important, add to the effectiveness of the molecule.

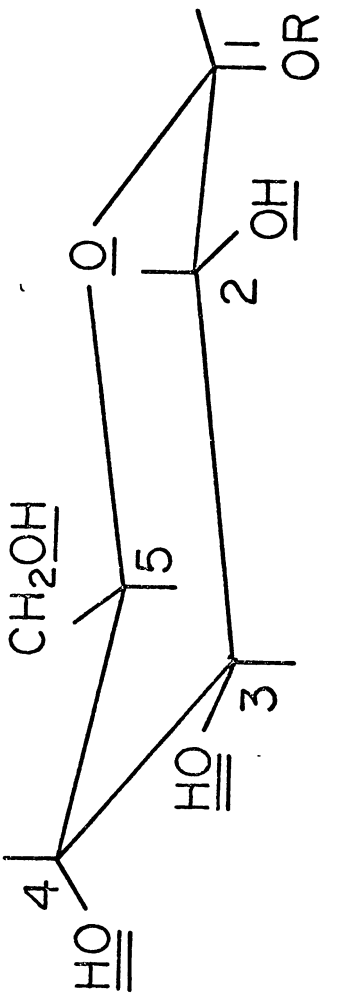


Figure 9

though many of them are rare compounds they can be useful tools in studying other similar receptor systems that are more sensitive because some of them are not very soluble at the concentrations used. When available they largely support the evidence obtained with the glucose derivatives, especially pointing out the importance of the ring oxygen. Evans (1963) dismissed the need for this atom because myo-inositol was 0.7 as effective as glucose in his preparation. Myo-inositol which only differs from (+)-inositol in having its C_6 hydroxyl in an equatorial position was much more effective than the latter in stimulating the receptor. So it appears that the oxygen is in the most favorable position for hydrogen bonding when it is part of the pyranose ring, less when equatorial and poorest when axial.

The results show that quebrachitol was as effective as (+)-inositol. There are two possible answers to this unusual result. It is possible that this compound is affecting another receptor site, either the fructose one, or one that has not yet been discovered. Or it is conceivable that the large substituent group affects the normal chair conformation in such a way that the molecule takes on a boat form. In this new conformation the molecular configuration is consistent with the other data. The preferred conformation for the maximally effective molecules appears to be the chair. Even though most of the compounds tested are generally believed to be in such a conformation, this does not mean that some form of a boat would be unfavorable.

The formation of a monomolecular complex between the stimulating molecules and the receptor site as described by Beidler (1954) does not apply to this preparation (Fig. 10). The resulting straight line

indicates that there is a monomolecular interaction between the receptor and the stimulating molecule. Figure 10 is a plot resulting from the application of Beidler's equation. A straight-line relationship was obtained for sucrose as observed by Morita and Shiraishi (1968) using the fleshfly Boettcherisca peregrina, but (as they also observed) not for D-glucose or most of its other derivatives. This means that the response to these compounds is not proportional to the number of monomolecular complexes formed between the molecule and the receptor. A similar result was observed by Tateda (1967) for glucose and fructose in the rat. To account for this phenomenon, Tateda developed a taste equation that was similar to the Hill equation for combination of hemoglobin with oxygen. In this equation:

$$\log \frac{R}{R'_m - R} = n \log C + \log K'$$

R'_m is the maximum response obtained in high concentration of stimulation, K' is the equilibrium constant and n is a whole number. R is the response at concentration C. In the plot $\log \frac{R}{R'_m - R}$ against log C, a linear relationship should be obtained. The slope n is the number of sugar molecules interacting with the receptor site. Figure 11 is a plot resulting from the application of Tateda's equation to a few of the sugars tested. Table III lists the n and K' values for all the compounds tested. However it appears that Tateda's (1967) method of determining the size of the complex formation may also be inadequate. The size of the complex varied from 0.85:1 for 2-O-methyl-D-glucose to 2.1:1 for methyl α-D-xylopyranoside. The fact that the complex sizes are not represented by whole numbers and that they vary in magnitude may be due to the ability of the molecule to form a complex,

Figure 10. Beidler's plot of stimulation by various sugars.

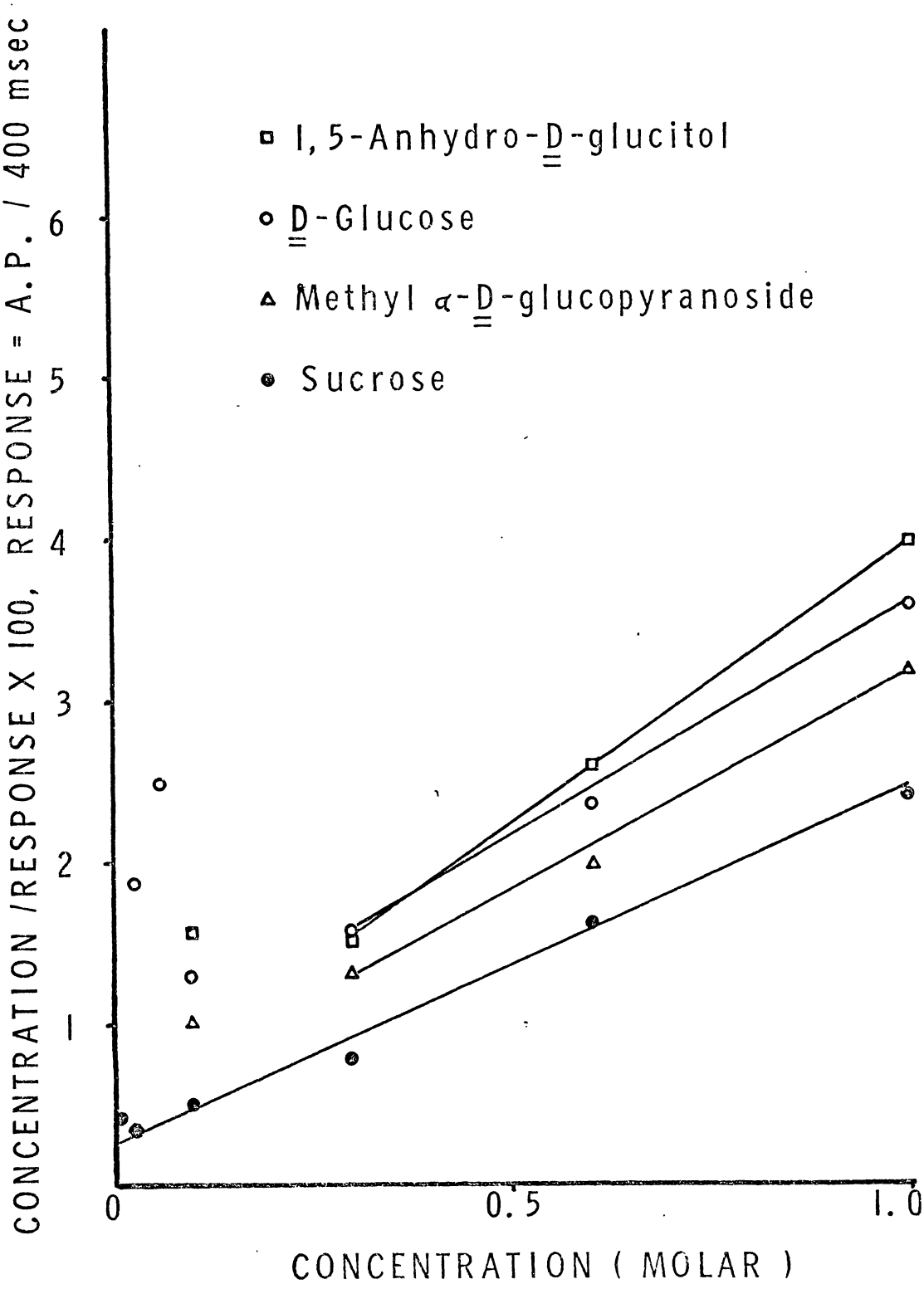

CONCENTRATION /RESPONSE X 100, RESPONSE = A.P. / 400 msec
1
2
3
4
5
6
0
0.5
1.0
CONCENTRATION (MOLAR)
1, 5-Anhydro-D-glucitol
D-Glucose
Methyl α-D-glucopyranoside
Sucrose

Figure 10

Figure 11. Tateda's plot of stimulation by various sugars. For the meaning of $\frac{R}{R'_m - R}$ see text. R = impulse/400 msec.

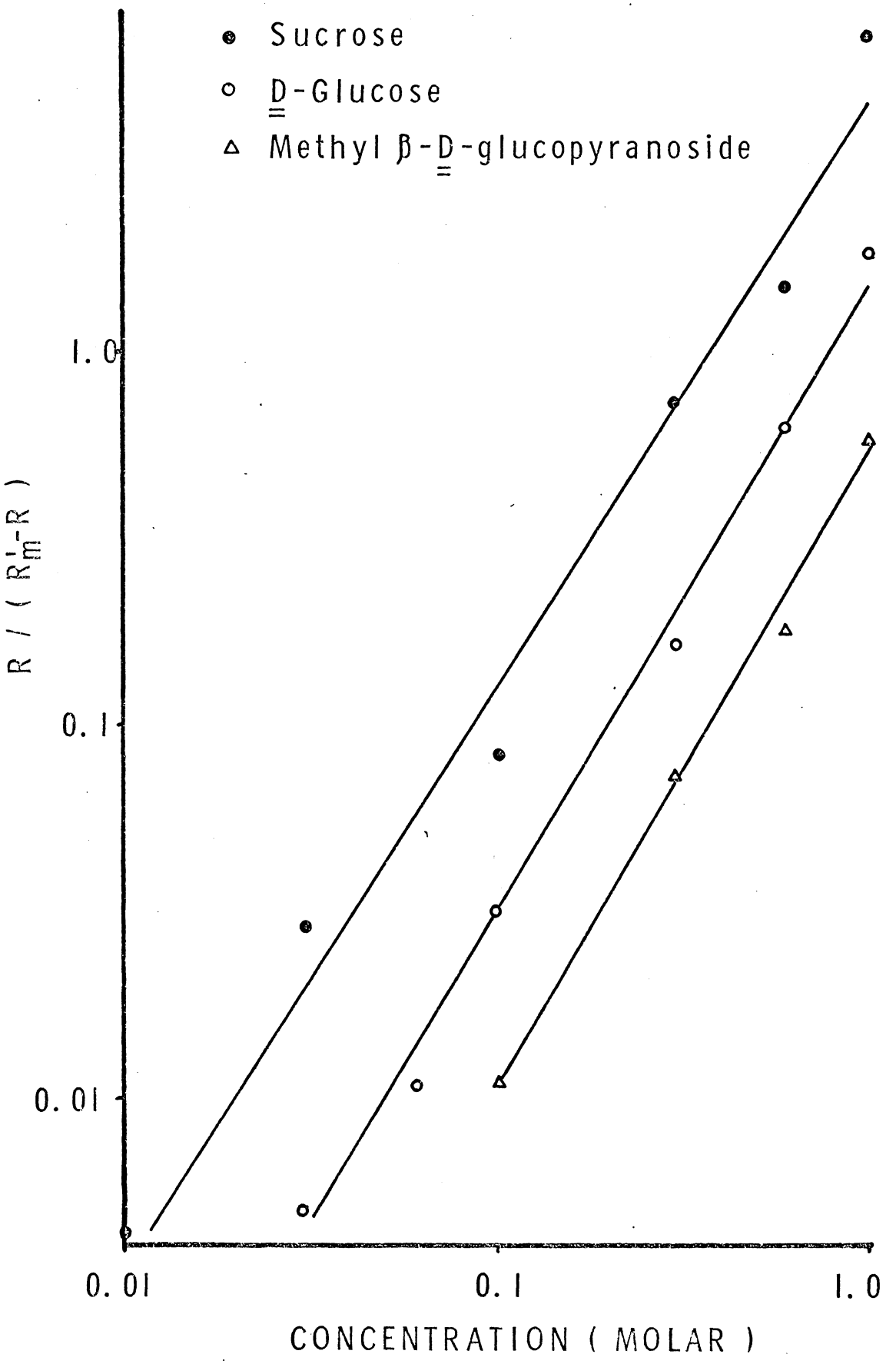

Sucrose
D-Glucose
Methyl β-D-glucopyranoside
1.0
0.1
0.01
R / (R'm-R)
0.01
0.1
1.0
CONCENTRATION (MOLAR)

Figure 11

Table III. n and K' values for the sugars to complex with the receptor site.

	n	K' (Molar)
Sucrose	1.5±0.3	0.10±0.07
D-Glucose	1.7±0.3	0.23±0.11
Methyl α-D-glucopyranoside	1.6±0.6	0.21±0.14
Methyl β-D-glucopyranoside	1.6±0.4	0.41±0.19
1,5-Anhydro-D-glucitol	1.6±0.5	0.23±0.15
L-Glucose	1.7±0.4	0.27±0.10
2-Deoxy-D-arabino-hexopyranose	1.2±0.2	0.26±0.09
2-O-Methyl-D-glucose	0.85±0.3	1.97±1.5
Methyl 2-amino-2-deoxy-α-D-glucopyranoside	1.8±0.5	0.33±0.13
D-Allose	1.6±0.8	0.41±0.35
3-O-Methyl-D-glucose	1.2±1.0	10.9±10.8
Methyl-α-D-xylopyranoside	2.1±0.2	0.44±0.08
6-Deoxy-D-glucose	1.9±0.8	0.51±0.26
6-O-Methyl-D-glucose	2.0±0.4	0.23±0.08
D-Fructose	1.1±0.2	0.14±0.09
Myo-inositol	1.9±0.8	0.37±0.11
(+)-inositol	1.8±0.3	0.53±0.17
Quebrachitol	1.6±0.5	0.49±0.09

For the meaning of n and K' see the text.

Confidence intervals of 95% are indicated.

as is certainly true for the C_2 and C_3 derivatives. Or, as Tateda (1967) observed when glycine had a 4.4 complex size in the rat, it may be due to the fact that when the receptor sites combine with the sugar molecules they make a whole series of different complexes. When such an analysis is used to characterize the behavior of "regulatable" enzymes the slope (n) is often nonlinear (White et al., 1968). Such nonlinearity reflects both a changing n and a changing strength of interaction among sites. The variation in n as indicated by the confidence limits and the variation in K' certainly suggest that a similar phenomenon is occurring at the receptor site of this fly. Furthermore, Morita and Shiraishi (1968) were able to show that the nonlinearity of the glucose response in Boettcherisca peregrina when plotted by Beidler's (1954) method could be explained by both a multimolecular complex or an allosteric transition at the receptor site. However they felt that they had insufficient data to decide which one was really correct.

The findings of this study, which examined the configurational and conformational requirements of the stimulating molecule, are incomplete. Whenever new sugars become available they should be tested. The possibility that other receptor sites exist has not been studied adequately. Evans (1963) postulated that there are at least two types of sugar combining sites on the membrane of the sugar receptor of the fly, one for glucose and the other for fructose. He and others (Omand and Dethier, 1969 and Morita and Shiraishi, 1968) were able to demonstrate that the response to fructose was inhibited by D-mannose. To complete the picture mixtures of the various sugars used in this study should be tested. Also attempts should be made to determine the conformational and configurational molecular requirements

for fructose. Unfortunately fructose derivatives used in this investigation failed to reveal any information concerning these requirements. Ultimately the final goal would be to discover the molecular structure of the receptor site. From these studies would emerge answers to the question, "How do uncharged molecules produce conformational changes in the cell membrane which allows the exchange of ions to occur?"

SUMMARY

1. The electrophysiological technique of Hodgson and Roeder was used to study the stereospecificity of the glucose receptor site on the labellar "sugar" cell of the fleshfly, Sarcophaga bullata Parker.
2. Response to individual sugars was well reproducible. It was observed that at low concentrations fructose was more stimulating than glucose which was the most effective stimulant at higher concentrations.
3. α-Glucosides were more effective than their β-counterparts. This phenomenon suggesting steric hindrance is consistent with the reported literature and appears to occur in several cases. Sucrose which is an α-glucoside was the most effective intimating that the fructofuranosyl moiety provides an additional binding site. However it has been demonstrated that these sites are separate from the fructose site.
4. Using various glucose derivatives and cyclitol isomers, it was found that the most critical points for binding the molecule to the receptor are at carbon -2, -3, -4 and to a lesser degree, -6.
5. Both the cyclitols and glucose epimers have demonstrated that the hydroxyl groups of the critical positions must be equatorial to the plane of the molecule.
6. Evidence derived from deoxy sugars and a single amino-substituted derivative suggest that the molecule is bound to the receptor site by hydrogen bonding.
7. The stimulating effectiveness of the D- and L-enantiomorphs of glucose was resolved on a configurational rather than a confor-

mational basis. From this and other evidence it is suggestive that the shape of the sweet tasting molecule should be a pyranose ring in the chair form. The absolute necessity for this requirement has not been tested.

8. The use of cyclitols has demonstrated the need for the ring oxygen. It has been proposed that the pyranose ring oxygen is involved in additional hydrogen bonding.
9. Application of various taste equations suggest that the response of the receptor to various sugars is not proportional to the number of monomolecular complexes formed. The results could either be explained by a formation of multimolecular complex or by an allosteric transition at the receptor site.

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