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The effect of early gustatory experience upon taste preference in the mature albino rat.

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The Effect of Early Gustatory Experience Upon Taste Preference in the Mature Albino Rat

A Thesis Presented

By

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This study utilized a choice method to assess preference in adult rats who had been exposed to one of four treatments (Sucrose; Quinine; Water; and Handling) at an early age. The up and down (psychophysical) method (Dixon & Massey, 1957) was used to determine the mean concentration of sucrose, which when mixed with a set concentration of quinine, is isohedonic (equally preferred) to a set concentration of sucrose (standard). Responses clustered around a mean score (quinine-sucrose mixture) which is hypothetically equivalent (equally preferred) to a fixed concentration of sucrose (standard). Sensitivity to quinine was reflected by the response frequencies recorded since the rat will ingest the sucrose solution of greater concentration in a two choice situation (Young & Green, 1953).

It was found that only the Sucrose group (early experience with sucrose) differed (.05 confidence intervals) from the control groups at more than one standard. At the 4% standard, the Sucrose group differed from controls over the last 2 levels of quinine (0.01 & 0.1 of the first exposure and 0.001 & 0.01 of the replication). At the 16% standard, the Sucrose group differed from the control groups over all levels of quinine (one exception at the 0.01 quinine level of the first exposure). Also, at this standard level, it was observed that the Quinine group differed from the controls at the 0.00001 quinine level of the replication.

It is probable that early exposure to sucrose results in a change in adult preference. The Sucrose group becomes more sensitive to quinine and therefore requires more sucrose in combination with quinine to mask the resulting bitter component of a sucrose-quinine mixture. The Quinine group did not differ (.05 confidence intervals; one exception at the 0.00001 quinine level of the replication) from the control groups but showed a trend (similar to the Sucrose group at the 16% standard) which might manifest itself with the testing of more subjects. The replication of the first exposure to the quinine-sucrose mixture demonstrated the robustness of the data recorded. It is possible that the responding subject remembered the sucrose-quinine mixture or the response to the combination. Nevertheless, the present study is suggestive but by no means inclusive. There is an obvious need for a larger N and an analysis of variance with the appropriate correction formulas.

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Introduction

Palatibility is a term that refers to the acceptability of foodstuffs as determined by the gustatory characteristics of the food stimulus (Young, 1949). Richter (1943) contended that food acceptability is controlled by bodily need of the organism. It is now accepted that the condition of deprivation induces behavior designed to alleviate the need in question.

A second problem, however, is the control of the appetitive behavior of the non-deprived animal. In answer to this, Young (1959) proposed a theory of incentive motivation which is directly proportional to the strength of the effective arousal.

An arbitrary definition of preference threshold, according to Young, Burright, and Tromater (1963), is defined as that concentration of a solution (eg., sucrose) which is preferred by 75% of the responding subjects. Using this definition, Burright and Kappauf (1963) found that non-deprived rats, given a choice between sucrose and water, preferred 0.32/100cc. of sucrose solution. According to other experimenters, preference thresholds for sucrose have ranges from 0.45% to 0.57% solutions (wt./vol.) for non-deprived rats (Richter & Campbell, 1940a 1940b; Young, 1949; and Campbell, 1958). Koh and Teitelbaum (1961) reported that rats could discriminate 0.21% (wt./vol.) sucrose from an electrolytic tasteless standard (0.000002M NACl) when maintained at 80 to 90% of

ad libitum feeding weight and subjected to mild deprivation of water (one hour). Also, Koh and Teitelbaum observed a sucrose preference threshold of 0.47% for rats under the conditions of shock incentive. Shock was utilized as a punishment for the incorrect selection of a standard tasteless solution.

According to Young, Burright and Tromater (1963), aversion threshold can be defined as that concentration of a solution (eg., quinine) which is ingested by only 25% of the responding population. The aversion threshold for quinine hydrochloride was determined using lick contacts/30 minutes/per day for two days (non-deprived rats). Benjamin (1955) established the aversion threshold for quinine hydrochloride using two methods: 1. 24 hour intake in a choice situation (tap water vs. quinine solution) and 2. 1 hour intake of a single stimulus (quinine hydrochloride) after 16 hour fluid deprivation. The mean was 0.0012 gm./100 ml. for the 24 hour method and 0.0016 gm/100 ml. for the 24 hour method and 0.0016 gm./100 ml. for the single stimulus method. The range extended from 0.0001 to 0.007. Koh and Teitelbaum (1961) using the conditions mentioned previously, reported a mean of 0.0005 gm./ 100 ml. and a range from 0.0003 to 0.0006.

Obviously, there is marked variability across and within individual experiments and experimental conditions. This variability could be ascribed to any of the following factors: the nature of the comparison solution in a two solution choice; the deprivation level of

the animals; the testing procedure (single vs. multiple stimuli); the duration of testing; the use of ascending and/or descending series of concentration; the index of preference; etc.

One generalization that can be drawn, according to Kappauf et al. (1963), is that the subject's sensitivity to sucrose or quinine is measured more effectively in the presence of the other substance, quinine or sucrose. For example, the measurement of quinine sensitivity is more effective in the presence of sucrose, especially high sucrose concentrations (Kappauf, Burright and DeMarco reported sensitivity to 0.00003% quinine in contrast to the 0.0003% quinine of Koh & Teitelbaum).

Young(1955) noted that the possibility of making a choice is a prerequisite for a preference but it is not a guarantee of a resultant preferential discrimination. Changes in the frequency of a preference are indicative of the learning of a preferential discrimination habit in a choice situation. In addition, the growth rate of a preferential discrimination (choice test) is dependent on the difference in palatability between test fluids, the relative intensities of hedonic processes evoked by the test fluids and the number of repetitions of a choice (Young, Burright and Tromater, 1963). It should be noted that habit strength changing with practice does not imply a change in acceptability. Also, preference is incompatiable with a positional habit.

Preference implies the possibility of choice but it is apparent that the single stimulus method provides no such opportunity. Consequently, the relative acceptability as measured by the single stimulus method must

be different from the relative acceptability measured by choice. Young and Green (1953) experimented with both the choice and single stimulus methods, using rats in a one hour drinking test with 9% (wt./vol.) and 36% sucrose. They found that their rats ingested almost equivalent amounts of 36% sucrose during both methods but ingested 2.3 to 20.6 times as much 9% sucrose during the single stimulus method. It was concluded that the rate of ingestion and the quantity consumed are an erroneous criterion for preference. It is evident that both palatability and satiation of appetite are present and interacting during a one hour preference test. It was suggested that preference is best indicated by a short term choice method (Young & Madsen, 1963), since, the resulting preference is the product of only immediate stimulation of head receptors.

Objective testing of preference has resulted in the realization that there are mixtures of quinine and sucrose equivalent to solutions of sucrose. The term equivalence is used here to imply an equal acceptance of both solution stimuli. Guilford, as cited by Young and Trafton (1964), proposed that the term isohedon be employed to designate a contour line of equal affective value on a stimulus surface. The isohedon is a discrimination function dependent on the preference behavior of the experimental subject. Fig.-1 is an example of isohedonic contour map which might be found at the 1%, 4%, and 32% standards (Young & Schulte, 1963).

The vertical axis indicates the percentage concentration (wt./vol.) of sucrose in a sucrose quinine mixture. In addition, the darkened dot on the vertical axis indicates the % concentration of sucrose used as a

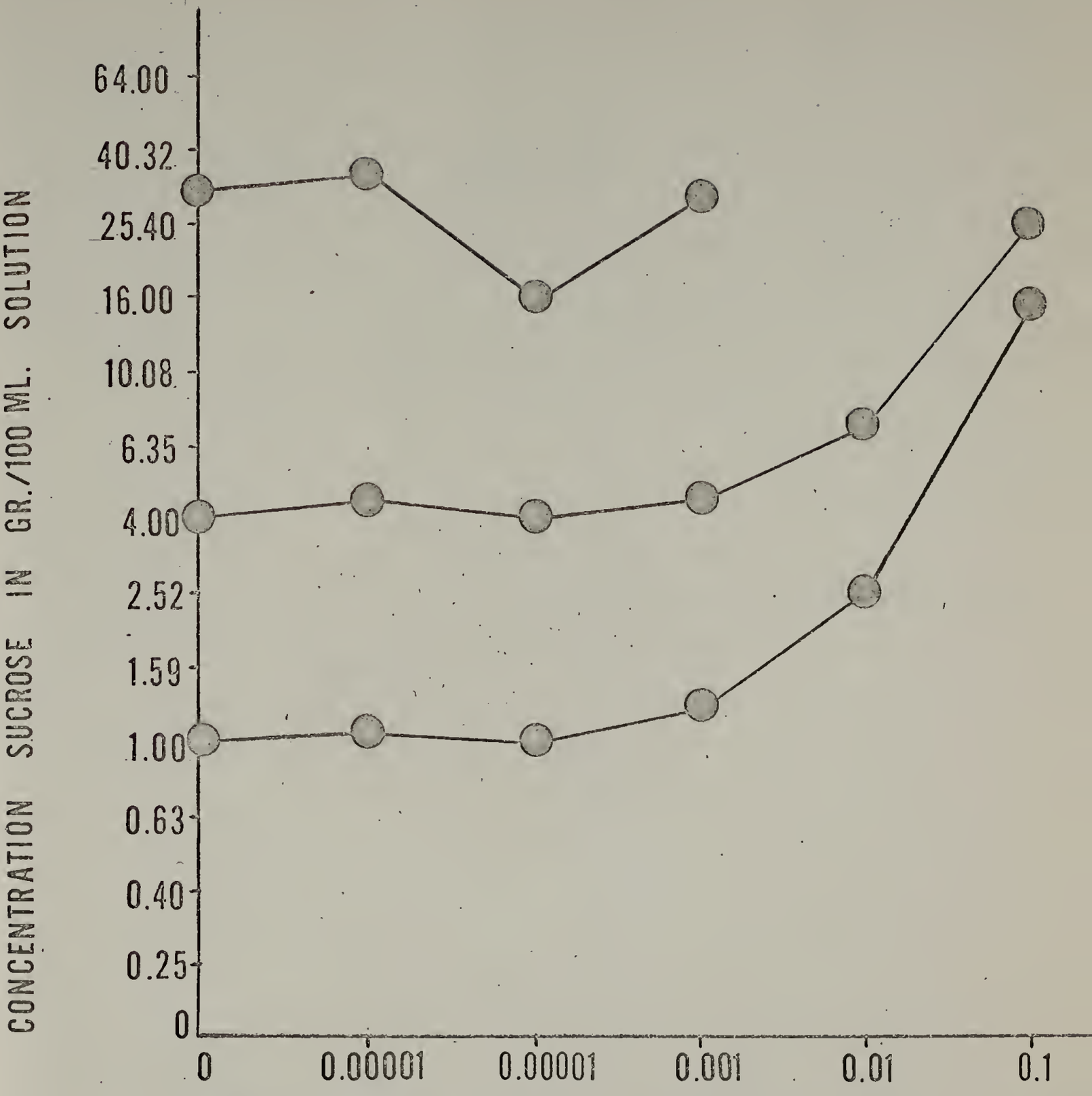


FIG.-1 ISOHEDONIC CONTOURS IN THE SUCROSE-QUININE HYDROCHLORIDE STIMULUS AREA

standard. The horizontal axis is segmented according to the different levels of quinine hydrosulfate used in the experiment. Each point on an isohedonic contour map represents a compound solution (eg., sucrose-quinine) which is hedonically equivalent to a standard solution (eg., sucrose) of fixed concentration.

In an exploration of these relationships, Kappauf, Burright and DeMarco (1963) and Young and Schulte (1963), reported that high levels of quinine always contribute negatively to acceptability of sucrose-quinine mixtures (regardless of sucrose concentration).

It was noted that certain low quinine levels in a quinine-sucrose mixture result in enhancement of acceptability when sucrose concentration is above a given criterion. A 1% sucrose concentration in the sucrose-quinine mixture is not enhanced by quinine additives.

Frequently, lick rate is used as the index of preference but experimentation (Young & Schulte, 1963; Young & Trafton, 1964) has raised many questions about this index. Young and Schulte (1963) reported that stimuli at different points in the isohedon vary in their potential to activate tongue movements. Therefore, the points along an isohedon represent standard and compound mixtures which are equal (S=C) but which do not represent equal activating potential. The subject doesn't respond to the standard solution with a constant rate of responding; rather, the subject responds to a pair of fluids as a gestalt or unit. This is an important criticism of the conclusions drawn by Young and Trafton (1964).

In response to this, Young and Trafton appropriately made the distinction between an isohedon and a isoacton. It was proposed that the term isoacton be applied to a contour line of equal activity on a stimulus surface. They concluded that isohedons based upon preference testing are separate and distinct curves from isoactons based on rates of licking. The quality of choice, revealed by preference testing, indicates the relative value and appraisal of the stimulus. In contrast, the rate of licking or ingestion of a solution is indicative of the degree of voracity or hungriness (eagerness to devour large quantities of food). It is obvious that the measurement of preference and voracity may be difficult if the experimenter fails to eliminate or minimize the interaction of palatability and satiety factors.

The literature contains a number of experiments which have contributed significantly to the study of taste preference in the mature, intact, albino rat. However, past experimentation has failed to provide adequate information about the effect of early experience with certain solutions upon taste preference and voracity later in life.

Deneberg (1962) reported that the age when handled and the number of days of handling are critical parameters affecting later behavior. Bloomquist and Candland (1965) using 15 hour deprived rats and the single stimulus method, found no differences in solution intake (water vs. sucrose vs. quinine) as a function of age. Wagner (1965) recorded consumption of 25% and 12 1/2% glucose solution for six hours over a period of 14 days. He found no differences in sugar preference attributable to age (prior early experience) or satiation. Wagner postulated that the rate of intake was probably the best index of preference since the effects of satiation

may alter preference over prolonged periods of time.

Warren and Pfaffmann (1958) observed the effect of early experience with sucrose octa-acetate (a non toxic bitter substance) upon preference behavior. A group of guinea pigs were separated from their mother and raised two days postpartum with $10^{-3}M$ SOA solutions as the only source of fluid during the first three weeks of life. Preference testing, after the experience, demonstrated an equivalent preference for water and SOA. However, three months on a normal diet and tap water resulted in no significant difference from controls. The authors concluded that exposure (duration, intensity, masking, etc.) may be crucial to the degree and persistance of SOA ingestion during testing.

In preliminary work[1.] done at the University of Massachusetts, results were obtained that suggested that differential taste experience in infancy (sucrose or quinine at the age of 4-20 days) led to differential adult preference as measured by intake. Three litters of newborn albino rats were subdivided into four groups. In Group S, each pup was hand fed a few drops of an 8% sucrose solution with a medicine dropper. In Group Q, each pup was hand fed a few drops of an 0.1% quinine solution. In Group W, each pup was hand fed a few drops of tap water, and in Group H, each pup was handled by the experimenter for a few minutes.

Experience with the designated solution began on the 5th day after birth and terminated on the 20th day (weaning). Using a single stimulus method, testing was initiated when the animals reached 90 days of age. The quantity of solution ingested was measured for three 24 hour periods.

The results suggested the following: Group S and Group Q ingested larger amounts of sucrose and smaller amounts of quinine than did control groups, but Group S responded more negatively to quinine and Group Q responded more positively to sucrose. Group S and Group Q, when tested first with sucrose, ingested the largest amounts of sucrose and the smallest amounts of quinine, but when tested first with quinine, they consumed smaller amounts of sucrose and larger amounts of quinine. Group W showed a sensitivity to sucrose that was between the sensitivity displayed by Groups S and Q, and a sensitivity to quinine that approached that recorded for Groups S and Q. Group H, tested first with sucrose, was more sensitive to quinine and less sensitive to sucrose. Sensitivity to quinine equaled the sensitivity of Groups S and Q to quinine.

In summary, early experience with either sucrose or quinine resulted in increased sensitivity to both tastes. This finding could be consistent with the duplexity theory of taste which postulates a taste interaction between the qualities of bitter and sweet (Bekesy, G. V., 1964). If we assume that early experience with either quinine or sucrose, results in a permanent threshold change for the quality evoking substance used, then, it is probable that later testing would reveal a threshold change for the interacting quality since the resultant threshold change may modify thresholds for other qualities (bitter, sweet, and warm) through interconnecting neural pathways.

The purpose of this study was to expand these preliminary results under better controlled conditions. A choice method was used to assess preference in adult rats who had been exposed to one of four treatments

(Sucrose; Quinine; Water; and Handling) at an early age. The up and down method was used to determine the mean concentration of sucrose, which when mixed with a set concentration of quinine, is isohedonic (equally pleasing) to a set concentration of sucrose (standard). Responses clustered around a mean which is hypothetically equivalent to a fixed concentration of sucrose (standard) which is always presented in the choice trial. Sensitivity to quinine is reflected by the response frequencies recorded since the rat will ingest the sucrose solution of greater concentration in a two choice situation (Young & Green, 1953).

Method

Subjects

Eight female, albino rats (120 days of age; obtained through the Charles-River Breeding Laboratories in Wilmington, Mass.) were separated randomly into pairs and placed into double cages. A single, albino, male rat (300 days old; bred at the University of Mass. Colony) was placed into each of the 4 double cages for one week. At the conclusion of the first week, each male rat was relocated to a different double cage (containing two females) for one more week. At the termination of the mating period (2 weeks), the female, albino rats were separated into individual maternity cages. The first litter was born 24 days after the first mating of male and female rats. At this time, 2 females had died of a respiratory infection. The surviving females gave birth over a period of one week to a total of 63 baby, albino rats (6 litters). The babies were given continuous access to the mothers milk, and the maternal cage contained an ample supply of Purina lab chow pellets, tap water, and powdered Purina lab chow pellets mixed with

powdered milk. The 4th day after birth, each litter was subdivided into 4 groups. All subjects were housed (individually at 60 days of age) in a ventilated room under the conditions of continuous illumination.

Apparatus

The test unit consisted of a modified Wahmann small animal cage (Wahmann LC-126, Wahmann Manufacturing Corp., Baltimore, Maryland) equipped with two adjustable drinking tubes (opaque side). The Wahmann cage (galvanized) measured 9 in. wide (22.86 cm.), by 15 in. deep (38.10 cm.), by 9 in. high (22.86 cm.). Modification of the cage consisted of a rectangular hole 1 1/2 in. above the floor (3.81 cm.) measuring 1 in. high (2.54 cm.), by 2 in. wide (5.08 cm.). The rectangular hole was covered proportionally by a piece of masonite measuring 4 in. wide (10.16 cm.), by 4 in. high, attached to the cage by 1/4 in. metal screws. Two 9/16 in. diameter holes were cut into the masonite, 1 in. from the sides and 1 1/2 in. (3.81 cm.) from the top and bottom of the cage. Test solutions were presented through the holes cut into the masonite. The metal drinking spout (tip; 5/16 in. o.d. (8mm.); 1/4 in. i.d. (6 mm.); 1/8 in. i.d. (3mm.) at the orifice) was presented in such a manner that it was flush with the outside wall of the test chamber. Most of the subjects were unable to grasp or hold the drinking spout during testing.

Individual tongue contacts were monitored through commercial drinkometers (Grayson-Stadler E4690-A). The sensing heads were

attached to the metal drinking spouts and to the cage floor. The short circuit current of the drinkometer was less than 1 uA dc (manufactor's specification). The drinkometers were connected in series with electromechanical pulseformers and stepping switches to a bank of digital counters which recorded total tongue contacts with a test solution. The test unit was located in the same room which housed all the subjects to be tested. White noise from an audio generator (BRS (Foringer); AU-901) and the operational noise of an airconditioner masked extraneous sounds.

Solutions[2].

All solutions in this experiment were mixed as percent solutions employing the ratio of gram-weight of solute to the total gram-weight of solution. Tap water was always used as solvent.

Table-1 is a listing of the various sucrose concentrations (for the standards and sucrose-quinine mixtures used in this study), as specified by various convential methods.[3]

Early experience occurred with only 8% sucrose and 0.01% quinine hydrosulfate.[4] An 8% sucrose solution was prepared by dissolving 80 grams of commercial cane sugar (granulated; $C_{12}H_{22}O_{11}$; mol. wt. 342.30 grams) in 920 grams of tap water. A 0.01% quinine sulfate solution was prepared by dissolving 100 mg. of quinine sulfate ($(C_{20}H_{24}N_2O_2)_2.H_2SO_4O$; mol. wt. 782.97 grams) in about 900 grams of heated water. When the solutions returned to room temperature, more water was added to make a total weight of 1000 grams. All solutions

Table-1

The concentration of Sucrose and Sucrose-Quinine Solutions

Specified by Different Methods

A	B	C	D	E
by weight as ams of subst- ce in 100 gm. solution	Specific Gravity	% by weight/vol. as grams of sub- stance in 100 cc. of solution	Molar Concentration = Col. D x 10 / Mol. Weight	Molality no. of moles solute / no. of kg. of solvent
00.25	1.0010	00.25	0.0073	0.0070
00.40	1.0016	00.40	0.0117	0.0121
00.63	1.0025	00.63	0.0187	0.0181
01.00	1.0039	01.00	0.0292	0.0303
01.59	1.0062	0160	0.0467	0.0467
02.52	1.0098	02.54	0.0742	0.0759
04.00	1.0157	04.06	0.1186	0.1218
06.35	1.0251	06.51	0.1901	0.1976
10.08	1.0403	10.49	0.3064	0.3281
16.00	1.0653	17.04	0.4978	0.5595
25.40	1.1073	28.13	0.8218	0.9946
40.32	1.1802	47.59	1.3901	1,9738
64.00	1.3108	84.04	2.4500	5.1944

were kept under refrigeration when not in use and were dropped to room temperature prior to use.

All test solutions were prepared by the weight/weight method described by Pfaffman, Young, Dethier, Richter and Stellar (1954). A Solution is referred to an x% solution. Test solutions were prepared by dilution from stock solutions of relatively high concentrations.

The sucrose solutions which were used as standard solutions were: 1%, 4%, and 16%. The concentrations of quinine sulfate that were used were: 0.00001%, 0.0001%, 01001%, 0.01% and 0.1%. Comparison solutions were prepared using the indicated levels of quinine sulfate and the successive concentrations of sucrose that are separated by equal 0.2 log units (64, 40.32, 25.40, 16, 10.08, 6.35, 4, 2.52, 1.59, 1, 0.63, 0.4, and 0.25). Each quinine, that is, hydrosulfate solution, was mixed with a variety of differential sucrose solutions to form the comparison solutions. One comparison solution was always presented with a set standard during a given trial.

Procedure

Early experience. The pups were divided into four groups:

Group-S: Each pup was hand fed a few drops (2-3) of the 8% sucrose solution with a medicine dropper.

Group-Q: Each pup was hand-fed a few drops of 0.01% quinine sulfate solution.

Group-W: Each pup was fed a few drops of tap water.

Group-H Each pup was picked up and placed on the

same table surface for administering test solutions. After a few seconds, the pup was returned to the litter.

The experience with quinine and sucrose solutions began 4 days after each litter was whelped and lasted until the pups were weaned at about 20 days of age. At this time, the pups were separated according to sex and group (Group-S: 6 males & 9 females; Group-Q: 7 males & 8 females; Group-H: 7 males & 8 females; and Group-W: 4 males & 11 females), and placed in double cages until 45 days of age. All rats were maintained on an ad lib diet of Purina lab chow and tap water from the date of weaning to the termination of the experiment.

Pretraining. At 60 days of age, each rat was water deprived during alternate days for a period of two weeks and given training in the preference apparatus (described elsewhere) with tap water. The duration of deprivation decreased across alternate days according to the following sequence: 48 hr. depriv., water, 12 hr. depriv., water 8 hr. depriv., water, 4 hr. depriv., water, 2 hr. depriv., water, 1 hr. depriv., and water.

A 5 second exposure to tap water was given first on the right side of the testing cage at the end of each deprivation period. This was followed by a second 5 second exposure to tap water on the left side of the testing cage and so on in random order until a total of 180 seconds had elapsed on each day. At 90 days of age, each rat was individually tested for preference using the following method.

Preference testing. Each group was subdivided into 3 subgroups. Each subgroup

was permanently assigned to one of 3 standards. Each subject then experienced a set standard and a different quinine level on each day. The concentrations of quinine sulfate are here designated in the order of administration (corresponding to days): 0.00001; 0.01; 0.0001; 0.001; 0.1; 0.001; 0.0001; 0.01; and 0.00001. The strongest concentration of quinine (0.1) was tested last because it was feared that this level of quinine might inhibit ingestion. Following the completion of testing (5 days) the series was repeated in reverse order. Each concentration was tested on 2 days except for 0.1 quinine which was not retested.

The method of testing was similar to that used by Young and Madsen (1963). A simple sucrose solution (the standard) was always randomly placed in one tube while a mixture of quinine sulfate and sucrose (the comparison) was placed in the other tube. Each subject is subjected to the following specific routine during testing. The subject is first placed in an empty test cage with neither tube inserted. After a lapse of 5 seconds, the tube containing the standard is inserted on either the right or left side of the cage, until 5-10 licks are recorded or 7 seconds elapse. The tube is withdrawn and replaced immediately by the tube containing the comparison solution. After 5-10 licks are recorded or the lapse of 7 seconds, the tube is withdrawn and this sequence is repeated 3 times to insure adequate exposure. At the termination of forced sampling, a 5 second time lapse is interposed before both tubes are inserted simultaneously. A preference is recorded when the subject selects or licks one of the 2

solutions during simultaneous presentation. The entire procedure of forced sampling and simultaneous presentation is repeated 3 times at each concentration of sucrose and quinine. Preference is recorded as a percentage response frequency to the standard and comparison mixtures over the 3 simultaneous presentations. On each day of testing each subject is exposed to only one standard and only one level of quinine sulfate. The quinine-sucrose mixture is varied according to the up and down method (described later). Trials, including transport, sampling and testing required about 7 minutes. If a subject failed to complete the forced sampling series in less than 4 minutes, then that subject's data was eliminated and the next subject in random order is tested with the same pair of solutions. In addition, a minimum total of 5 licks over the 7 second testing period was required. Otherwise, the trial is eliminated and the next subject is tested with the same pair of solutions. The up and down method (described later) is used to determine hedonic equality.

Control was exercised over the following factors: the order of presenting standard and comparison solutions in preliminary sampling, the relative position of fluids attached to the test cage, ascending and descending arrangements of the comparison solution, the order of testing groups and individual subjects within a group.

The up and down method described by Dixon and Massey (1957) eliminates the effects of experience as a significant factor determining choice. The subject is tested only once with a given pair of fluids and is given no indication (anticipation) of a particular future preference test.

Typically, a test at a particular concentration of the standard begins with the presentation of a choice to the subject (usually a comparison mixture considered closest to the standard is presented). If the subject licks either more or only the S instead of the C, then the second subject is tested with the next more acceptable comparison mixture (C with the next higher CHO conc.). If the first subject licks either more or only the C instead of the S, then the 2nd subject is presented with a comparison solution containing the next lower concentration of CHO. And so on, up and down, the choice of the first subject determining the pair of test fluids to be offered to the next subject.

A test consisted of response frequencies to a single choice (at a particular standard) by all subjects (60; divided equally into 4 groups). The purpose is to determine the mean value of X in the following equation: K CHO = X CHO + K Q. The concentration of both the standard and the level of quinine sulfate is held constant while the concentration of CHO in the comparison fluid is varied by equal log steps. Hence, a particular standard is presented in every trial with one of five comparison solutions (usually). The number of subjects preferring S and C at each level of the variable component is recorded and transposed into frequency distributions. The mean concentration ($\bar{X}$) of the variable component (value which on the average makes C=S is computed from these distributions.[5]

Results and Discussion

Data was collected from all the subjects that were tested. Pro-
[illegible] of recording [illegible] results in the distribution

of any subject's data.

Figures 2-4 (based on the X values in Appendix I (A-F)) show the mean concentration of CHO, which when mixed with the designated level of quinine hydrosulfate, is hedonically equivalent to a set standard. Each graph represents a different standard and the responses of all the groups. 5 different levels of quinine (0.00001, 0.0001, 0.001, 0.01, and 0.1) were randomly administered on different days and replicated in reverse order, except for the strongest concentration, over the next four days.

Isohedonic concentrations (values of X) are plotted as points which are connected by straight lines to form the isohedons. Each point of each function represents the mean responses (X) of 5 subjects. The 5 levels of quinine are designated along the baseline and the 13 concentrations of sucrose along the vertical axis (separated by 0.2 log units). The darkened dot on the vertical axis designates the sucrose concentration used as a standard.

Appendix I (A-F) contains the means and standard deviations for each isohedon as computed by the appropriate formulas for the method used in this study. It is to be noted that the computed SD is considered a reliable estimate since $(NB-A^2)/N^2$ is always greater than 0.3 (Dixon & Massey, 1957).

Some of the characteristics of isohedonic contour maps (in the bitter-sweet area; Young & Schulte, 1963) are:

1. The curves in the bitter-sweet area follow a similar course and do not overlap.
2. Low concentrations of quinine are hedonically

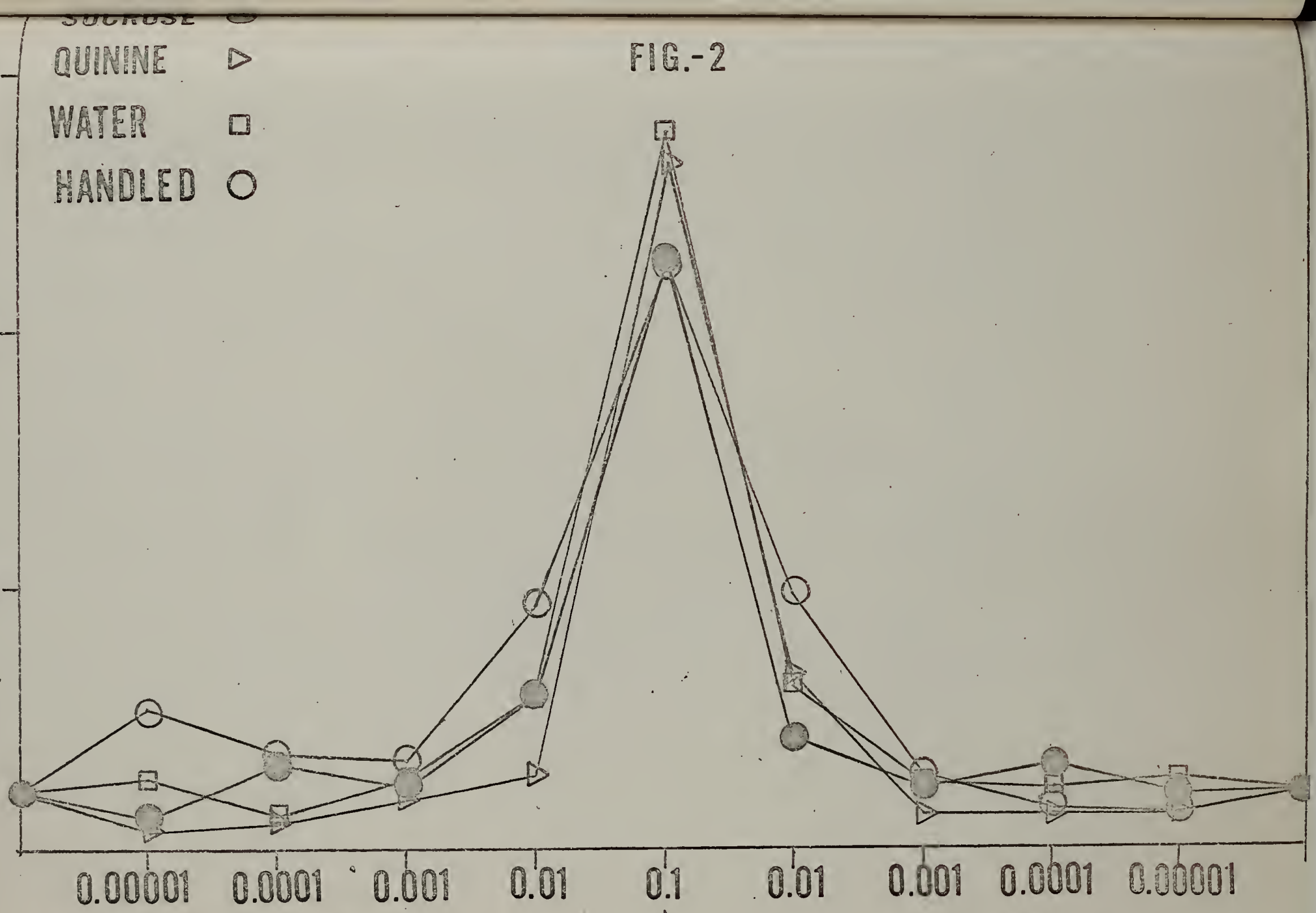
Figure Legends

Figure-2 Isohedonic contours at the 1% standard in the sucrose-quinine hydrosulfate stimulus area.

Figure-3 Isohedonic countours at the 4% standard in the sucrose-quinine hydrosulfate stimulus area.

Figure-4 Isohedonic contours at the 16% standard in the sucrose-quinine hydrosulfate stimulus area.

FIG.-2


SUCROSE
QUININE
WATER
HANDLED
CONCENTRATION SUCROSE IN W/W SOLUTION
15
10
5
0.00001
0.0001
0.001
0.01
0.1
0.01
0.001
0.0001
0.00001
FIRST EXPOSURE TO QUININE SULFATE IN W/W SOLUTION
REPLICATION

SUCROSE ○

QUININE ▷

WATER □

HANDLED ○

CONCENTRATION SUCROSE IN W/W SOLUTION

40

30

20

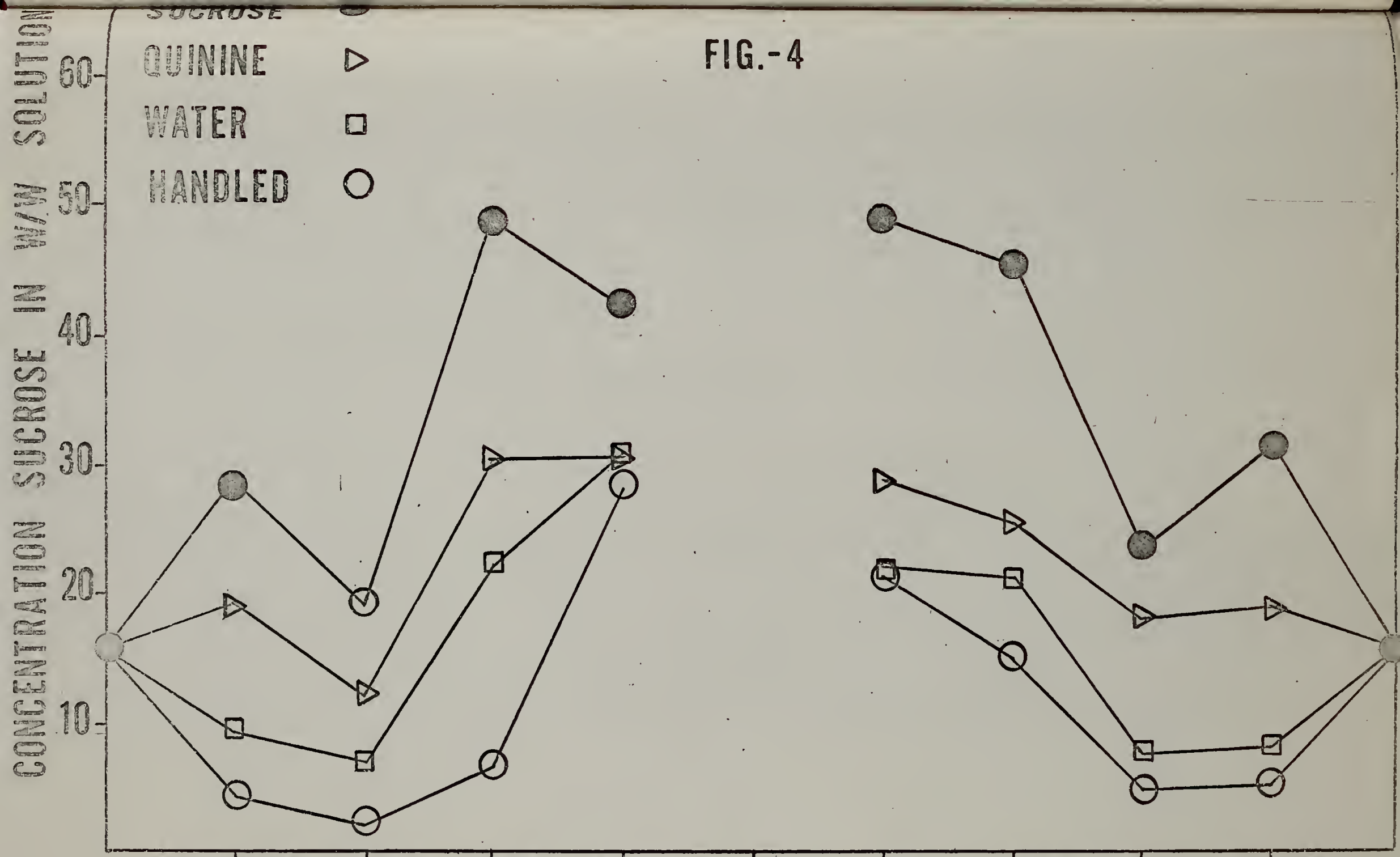
10

0.00001 0.0001 0.001 0.01 0.1 0.01 0.001 0.0001 0.00001

FIRST EXPOSURE TO QUININE SULFATE IN W/W SOLUTION

REPLICATION

FIG.-4


SUCROSE
QUININE
WATER
HANDLED
CONCENTRATION OF SUCROSE IN W/W SOLUTION
60
50
40
30
20
10
0.00001 0.0001 0.001 0.01
QUININE SULFATE IN W/W SOLUTION
FIRST EXPOSURE
0.01 0.001 0.0001 0.00001
QUININE SULFATE IN W/W SOLUTION
REPLICATION

negative. However, there is a point of interaction at 32% sucrose.

3. The inhibition resulting from high quinine concentrations is counteracted by increasing sucrose concentration within the compound solution.
4. There are indifferent concentrations (quinine) which neither add to or subtract from acceptability.
5. There is an implied upper limit at which it is impossible to make a compound mixture acceptable by adding more sucrose.

Visual inspection of Figures 2-4 finds general support for the isohedonic characteristics listed by Young and Schulte (1963); etc. It is apparent that the shape of the isohedon for controls and to a lesser extent for the experimental groups, is remarkably similar to the graphs cited by other experimenters. Figure-1 (page 5.), taken from Young and Schulte (1963), is an illustration of the correspondence of the data found in this study (Figure 2-4) with the data recorded by others (Young & Schulte, 1963; Kappauf, W. E., Burright, R. G., and DeMarco, W., 1963; etc.). The Replication of the isohedonic points of an isohedon in this study demonstrates the consistency of this data. There appears to be a robustness of the isohedon which may be attributed to the up and down method.

Since, the rat has a consistent preference for the more concentrated of two sucrose solutions (Young & Green, 1953), the relative amount of sucrose in a sucrose standard and its isohedonic mixture (sucrose-quinine),

must be a measure of the contribution which quinine makes to the acceptability of the mixture. Young et al. have consistently suggested that the rat is sensitive to the lower levels of quinine (0.00001, & 0.0001) and responds in a characteristic manner to these levels. In fact, it has been suggested by these authors that quinine makes positive contributions to acceptability when the quinine level is low and the sucrose concentration is high. Therefore, the isohedonic contour map is divided into regions where the non-sucrose variable has positive, neutral, and negative influences.

Visual inspection of Figure-2 revealed frequent overlapping of all groups across all the levels of quinine. Consequently, this data was not subjected to closer inspection. Figure-3 showed a separation (replicated) of isohedonic points between the Sucrose group and all other groups (frequent overlapping). The noticeable separation occurred across the last 3 levels of quinine, increasing with increased concentration of quinine. Figure-4 showed a greater separation (replicated) of isohedonic points for the Sucrose group opposed to all other groups across all levels of quinine. The separation is greatest between the Sucrose group and the control groups. The Quinine group was also consistently above (isohedon) the control groups but to a lesser degree (less separation). Consequently, the data recorded at the 4% standards was regraphed for closer inspection. Figures 5-8 show a comparison of the Sucrose, Quinine, Water, and Handled groups. The concentration of sucrose, which is mixed with quinine and tap water, is given on the vertical axis. In addition, a darkened dot on the vertical axis is used

Figure Legends

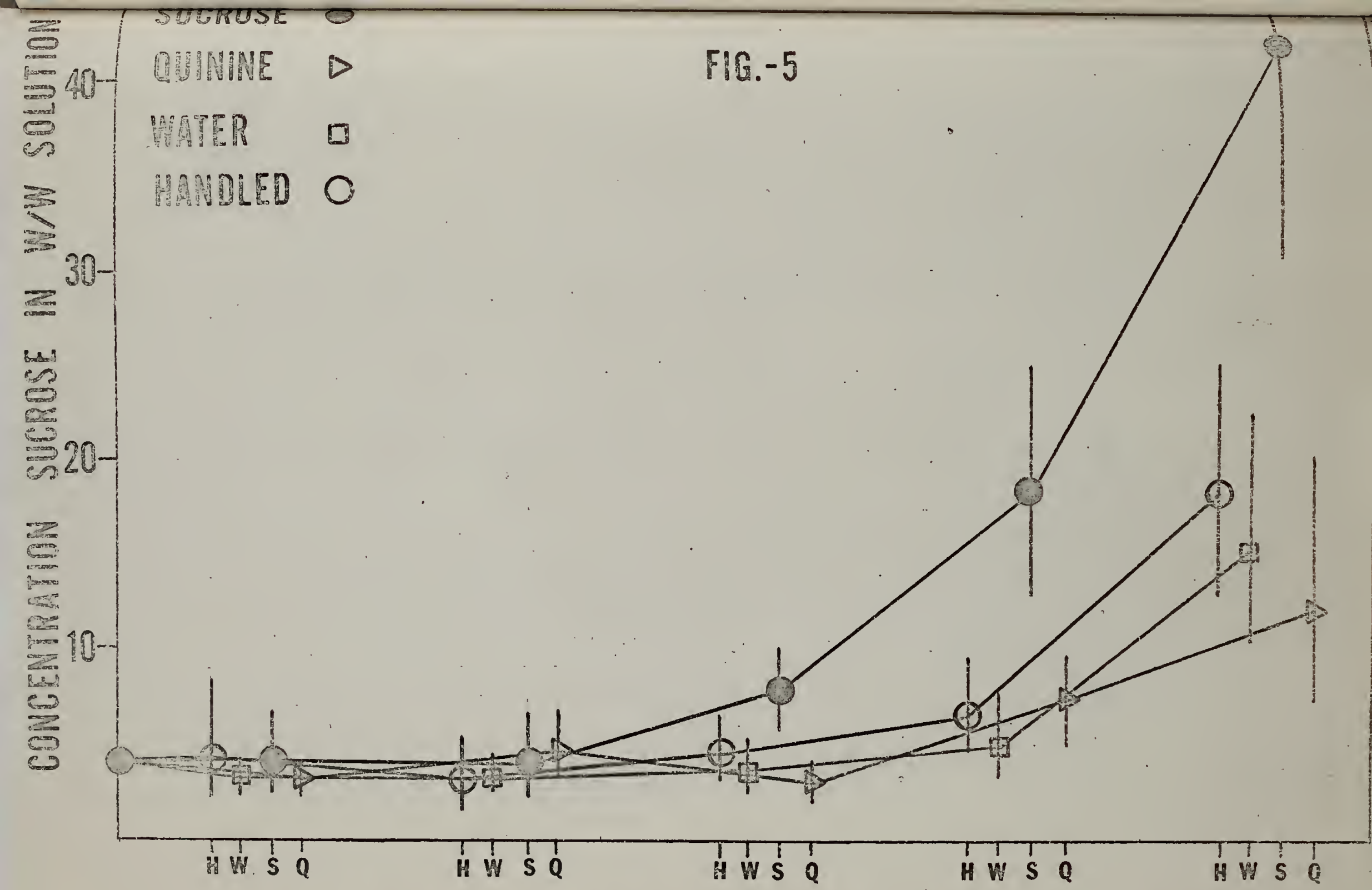
Figure-5 Isohedonic contours with .05 confidence intervals for isohedonic points. At the 4% standard, first exposure in the sucrose-quinine hydrosulfate stimulus area.

Figure-6 Isohedonic contours with .05 confidence intervals for isohedonic points. At the 4% standard, replicated in the sucrose-quinine hydrosulfate stimulus area.

Figure-7 Isohedonic contours with .05 confidence intervals for isohedonic points. At the 16% standard, first exposure in the sucrose-quinine hydrosulfate stimulus area.

Figure-8 Isohedonic contours with .05 confidence intervals for isohedonic points. At the 16% standard, replicated in the sucrose-quinine hydrosulfate stimulus area.

FIG.-5



CONCENTRATION QUININE SULFATE IN W/W SOLUTION

FIRST EXPOSURE

FIG.-6

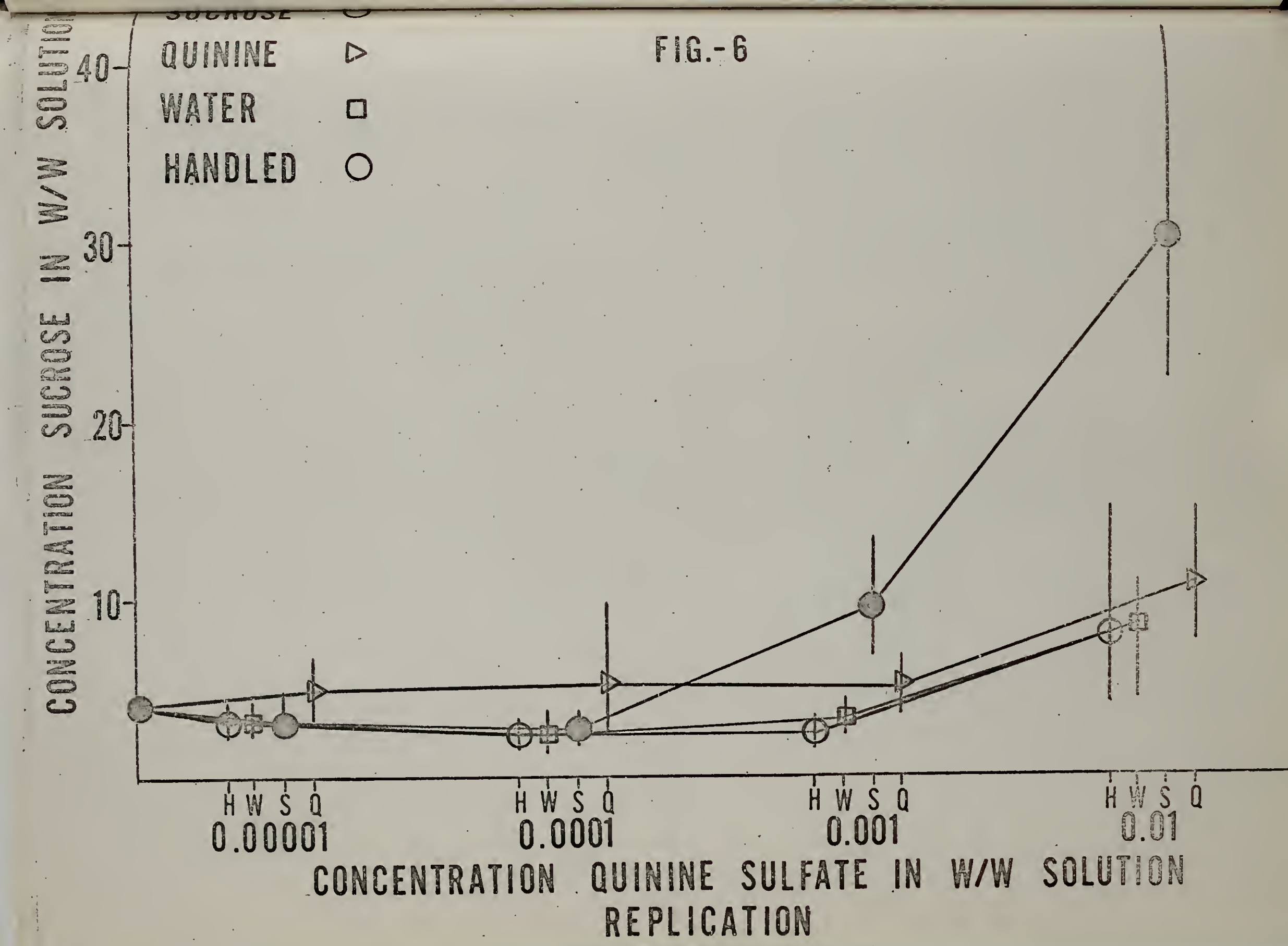
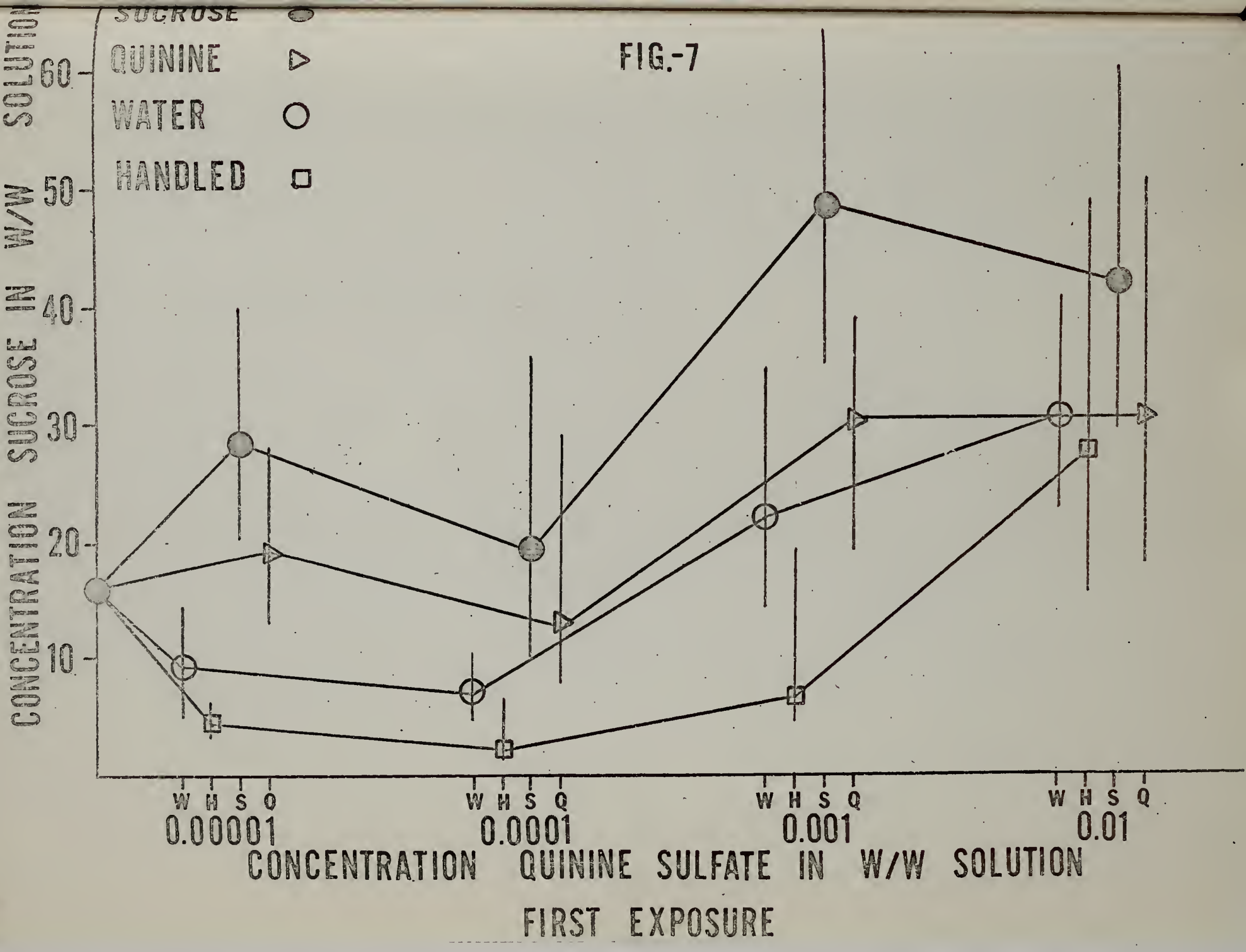
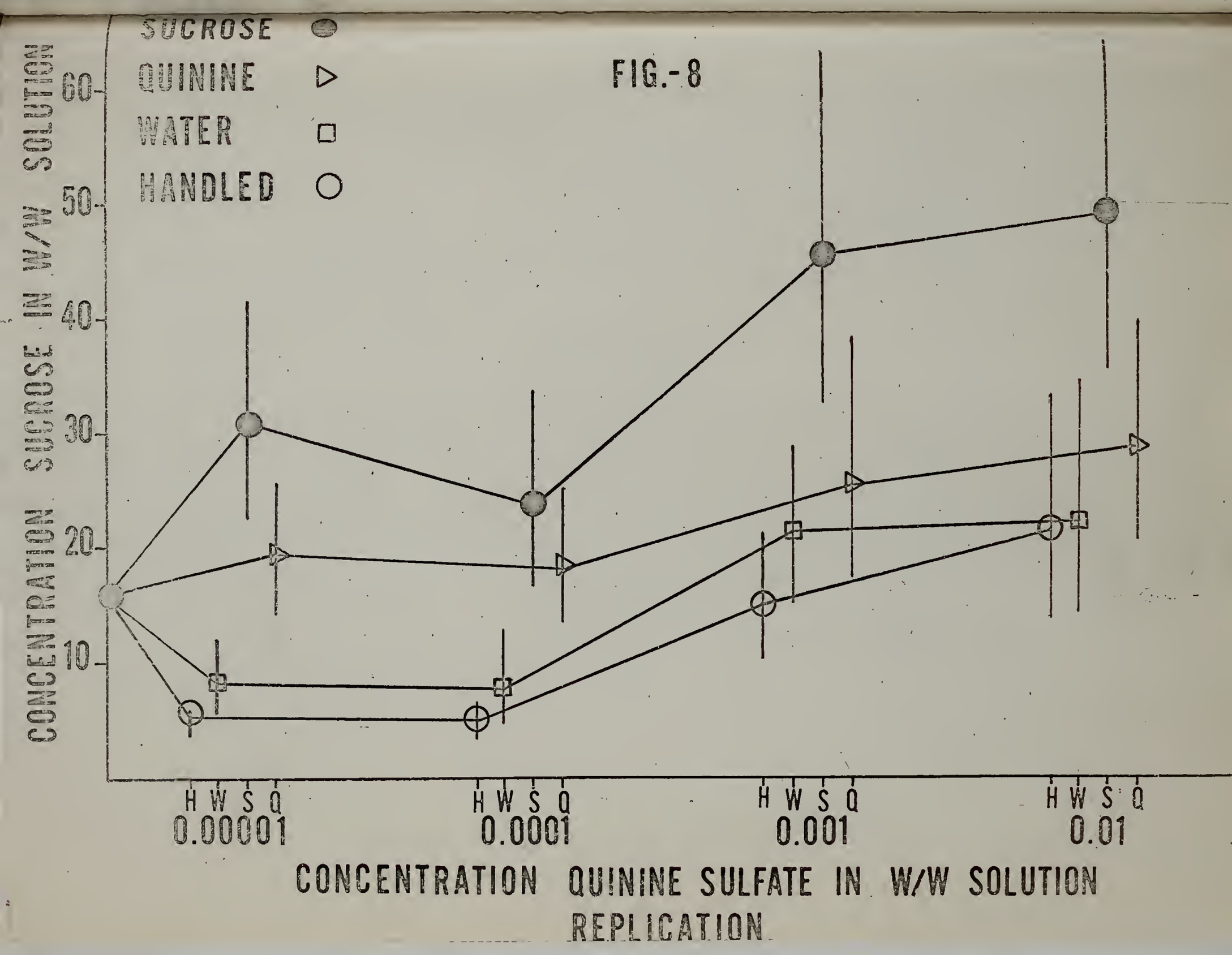

SUCROSE
QUININE
WATER
HANDLED
40
30
20
10
CONCENTRATION SUCROSE IN W/W SOLUTION
H W S Q
0.00001
H W S Q
0.0001
H W S Q
0.001
H W S Q
0.01
CONCENTRATION QUININE SULFATE IN W/W SOLUTION
REPLICATION

FIG.-7



FIRST EXPOSURE


SUCROSE
QUININE
WATER
HANDLED
FIG.-8
CONCENTRATION SUCROSE IN W/W SOLUTION
60
50
40
30
20
10
H W S Q
0.00001
H W S Q
0.0001
H W S Q
0.001
H W S Q
0.01
CONCENTRATION QUININE SULFATE IN W/W SOLUTION
REPLICATION

to represent the % concentration of sucrose used as a standard. The levels of quinine are listed along the baseline. Each standard is represented by a separate graph and each replication of a standard is graphed separately. It was concluded that the reliability of an effect was best indicated by non-overlapping .05 confidence intervals. Therefore, .05 confidence intervals were drawn as vertical lines extending on both sides of each isohedonic point (mean); the isohedonic points were separated horizontally by a constant according to groups (set sequence). Appendix II (A-F) contains the G-factor, standard error of the mean, and .05 confidence intervals for all means.

It is apparent, at the 4% standard (Figures 5-6), that the Sucrose group differed from all the other groups across the last 2 levels of quinine (0.01 & 0.1) of the first exposure and the last 2 levels of quinine (0.001 & 0.01) of the replication, as indicated by non-overlapping .05 confidence intervals. The replication of the 4% standard indicates an increased sensitivity to quinine (0.001) and a resulting difference between the Sucrose group and the other groups. Experience with the test solutions and the increased practice in the previously described apparatus contributed to the observed effect. It is possible that the responding subjects were able to remember the comparison solution or their response to that solution (at that quinine level).

At the 16% standard (Figures 7-8), there is a clear separation (no overlap) of all groups. It is possible that the observed separation for three of the groups is due to chance but it is important to note that

there is no overlap or crossing of the groups (replicated). Therefore, there may be a trend in this data which will approach reliability with the testing of more subjects. In addition, all groups dip (isohedonic point drops) at 0.0001 quinine (replicated); consistent with Young & Schulte, 1963, and Kappauf, Burright, & DeMarco, 1963). A sudden drop of an isohedonic point at some low level of quinine, appears to be characteristic of all isohedons at the 16% standard (positive contribution of quinine to the quinine-sucrose mixture).

The Sucrose group (first exposure, Figure-7 had .05 confidence intervals that did not overlap with the confidence intervals of any other group except for the last quinine level (0.01). At the 0.01 quinine concentration, .05 confidence intervals for the Sucrose group overlapped with both the quinine and control groups. However, there was a separation between the Sucrose group and all other groups at this last concentration of quinine. The separation was of smaller magnitude than expected because of chance variation attributable to extraneous factors. The replication (Figure-8) of the 16% standard supported this speculation. The Sucrose group was found to have .05 confidence intervals which did not overlap with the .05 confidence intervals of any group at any level of quinine. Consequently, early experience with sucrose seems to have an effect on adult taste preference. Re-examination of the 16% standard (Figure 7-8) results in the conclusion that the Quinine group did not differ from the control groups in terms of confidence intervals. However, there is no overlap of confidence

intervals between the Quinine group and control groups at the 0.0001 quinine level of the replication. In addition, the overlap of confidence intervals for the Quinine group with the control groups is frequently small. In fact there is no overlap of confidence intervals for the Quinine group and the Handled group except at the last level of quinine during the first exposure. You must recall that earlier it was noted that the Sucrose group also did not differ (confidence intervals) from the other groups at this level. The explanation for that observed result also has application here.

Future research in this area, probably should focus on the 16% standard since multiple group differences would occur at this level if they occur at all.

Sucrose effect. A sucrose effect is very consistent with a quinine effect; particularly, in light of the duplexity theory of taste. Preliminary experimentation demonstrated that the Sucrose group reacted more strongly (increased sensitivity) to quinine than did the Quinine group. The data collected here is consistent with that original finding. The Sucrose group does indeed take more sucrose at a given quinine level than does the Quinine group. It takes more sucrose to mask the unpleasant sensation of bitter. Also consistent with the theory is the observation that both the Quinine and Sucrose groups consume larger quantities of sucrose at a given quinine level than do control groups (the quinine effect being a trend and the sucrose effect a significant result).

Quinine effect. The effects of quinine (bitterness) are considerably longer lasting than the effects of sucrose (sweetness). It is possible

that the early exposure and lingering sensation of bitterness resulted in adaptation to quinine (increased threshold). Consequently, the ingestion of the mother's milk, which may be considered a mild sweet evoking substance, would be a stronger sensation (sweetness) since the overtones of bitterness would be minimized. This would account for the approach or closeness of the Sucrose and Quinine group isohedons at the 16% standard. Bekesy (1964) noted that the chemical stimulation of the tongue by four different sugar solutions (fructose, glycine, sucrose, and glucose) produced sensations of sweet that were more similar and purer to sensations evoked by electrical point stimulation when the tongue is adapted to the qualities of salt, bitter, and sour. In addition, Dallenbach and Dallenbach (1943) reported that bitter adaptation resulted in an increased sensitivity to sweet for most of their subjects (human).

Conclusions

The Sucrose group was the only experimental group whose .05 confidence intervals frequently failed to overlap with the .05 confidence intervals of the control groups. At the 4% standard, the Sucrose group differed (confidence intervals) from the controls over the last 2-3 levels of quinine. At the 16% standard, the Sucrose group differed (confidence intervals) from the controls over all the levels of quinine (one exception during the first exposure). Consequently, early experience with sucrose appears to have some effect on adult taste preference. The change in preference must be mediated via the Central Nervous System since the taste receptors (taste buds) are continuously being replaced (approximate 4 day life span; Bekesy, 1964).

The Sucrose effect may be likened to the child spoiled with candy. The child continues a physical development uncompromised by substitutes for sweet evoking substances. Early work by Richter et al., etc., is suggestive of the learning of inappropriate feeding patterns when a subject is given early exposure to sweet evoking substances. It is probably safe to say that desirable feeding patterns are dependant on early experiences. The lack of sweet evoking substances early in life may lead to the establishment of a proper food selection habit later in life. The Sucrose effect may be suggestive of a critical period during infancy. Whether or not the early exposure results in a permanent structural change (resulting in a preference change), which may be modified (partially or completely) through adult learning, cannot be answered at this time.

The present data also relates to soda mixtures bought commercially by a large number of people. It is interesting that such beverage mixtures are a combination of between 0.003 to 0.03% quinine and 4 to 10% sucrose. Kappauf, Burright, and DeMarco (1963) found that a maximal enhancement of palatability occurred at 0.00003 Q + 40.3 CHO. The data of Kappauf et al. is inconsistent with the concentrations of quinine and sucrose used in soda mixtures although one pertains to an animal (rat) experiment and the other to an observation of human behavior. It seems logical that a subject would consume more of the 10-8% sucrose than a higher preferred concentration (particularly in light of the data provided by Young & Green, 1953). Consequently, the industry makes more money and the consumer acquires (orally) more of a pleasing solution, thereby, quenching a thirst and satisfying an appetite. The stronger concentration of quinine in combination with sucrose would seem to indicate that bitter (for human) may be a pleasurable sensation (within limits) when in combination with the sensation of sweet.

The present study was an attempt to establish preliminary groundwork for future experimentation in a rather ambiguous area. The data is suggestive but by no means inclusive. There is an obvious need for a larger N and an analysis of variance with the appropriate correction formulas.

In addition, the use of all the subject's data may have influenced the conclusions drawn about the population. Meiselman and Dzendolet (1967), noted that the careful selection of subjects is

necessary in an experiment concerned with quality responses when the stimuli are relatively near absolute threshold.

The replication of the data in this experiment supported the original form of the data and the conclusions about that data. But there is the possibility of a warming up or learning habit associated with such a replication.

Bibliography

Bekesy, G. Von. Sweetness produced electrically on the tongue and its relation to taste theories. J. Appl. Physical., 1964, 19, 1105-1113.

Bekesy, George Von. Duplexity theory of taste. Science, 1964, 145, 834-835.

Benjamin, R. M. Cortical taste mechanisms studied by two different taste procedures. J. comp. physical. Psychol., 1955, 48, 119-122.

Bloomquist, D. W., and Candland, D. K. Taste preferences measured by tongue licks and bar presses as a function of age in the rat, Psychon. Sci., 1965, 3, 393-394.

Burright, R. G., and Kappauf, W. E. Preference threshold of the white rat for sucrose. J. comp. physical. Psychol., 1963, 56, 171-173.

Campbell, B. A. Absolute and relative sucrose preference thresholds for hungry and satiated rats. J. comp. physiol. Psychol., 1958, 51, 795-800.

Dallenbach, J. W., and Dallenbach, K. M. The effects of bitter adaptation on sensitivity to the other taste qualities. Amer. J. Psychol., 1943, 56, 21-31.

Dixon, W. J., and Massey, F. J. Introduction to statistical analysis. (2nd ed.) New York: McGraw-Hill, 1957.

Denenberg, V. H. An attempt to isolate critical periods of development in the rat. J. comp. physiol. Psychol., 1962, 55, 813-815.

Kappauf, W. E., Burright, R. G., and DeMarco, W. Sucrose-quinine mixtures which are isohedonic for the rat. J. comp. physiol. Psychol., 1963, 56, 138-143.

Pfaffmann, C., Young, P. T., Dethier, V. G., Richter, C. P., and Stellar, E. The preparation of solutions for research in chemoreception and food acceptance. J. comp. physiol. Psychol., 1954, 47, 93-96.

Richter, C. P. The self-selection of diets. In: Essays in Biology, in honor of Herbert M. Evans. Berkeley: University of California Press, 1943, Pl. 501-506.

Wagner, M. W. The effects of age, weight, and experience on relative sugar preference in the albino rat. Psychol. Sci., 1965, 2, 243-244.

Warren, R. P., and Pfaffmann, C. Early experience and taste aversion. J. comp. physiol., Psychol., 1958, 52, 263-266.

Young, P. T. Are there degrees of preference? Amer. J. Psychol., 1955, 68, 318-319.

Young, P. T. The role of effective processes in learning and motivation. Psychol. Rev., 1959, 66, 104-125.

Young, P. T. Appetite palatability and feeding habit: A critical review. Psychol. Bull., 1948, 45, 289-320.

Young, P. T., Burright, R. G., and Tromater, J. Preferences of the white rat for solutions of sucrose and quinine hydrochloride. Amer. J. Psychol., 1963, 76, 205-217.

Young, P. T., and Christensen, K. R. Algebraic summation of hedonic processes. J. comp. physiol. Psychol., 1962, 55, 332-336.

Young, P. T., and Green, J. T. Quantity of food ingested as a measure of relative acceptability. J. comp. physiol. Psychol., 1953, 46, 288-294.

Young, P. T., and Kappauf, W. E. Apparatus and procedures for studying taste preference in the white rat. Amer. J. Psychol. 1962, 75, 482-484.

Young, P. T., and Madsen, G. H., Jr. Individual isohedons in sucrose-sodium chloride and sucrose-saccharin gustatory areas. J. comp. physiol. Psychol., 1963, 56, 903-909.

Young, P. T., and Schulte, R. H. Isohedonic contours and tongue activity in three gustatory areas of the rat. J. comp. physiol. Psychol., 1963, 56, 465-475.

Young, P. T., and Trafton, C. L. Activity contour maps as related to preference in the four gustatory stimulus areas of the rat. J. comp. physiol. Psychol., 1964, 58, 68-75.

Footnotes

1. Fulfillment of a lab requirement for a course in Motivation by Marilyn K. Gorski (an undergraduate at the University of Massachusetts).

2. Symbols pertinent to this study are: CHO = sucrose (commercial cane sugar); Q = quinine sulfate; S = the standard solution of a pair presented for choice; C = the comparison solution of a pair presented for choice; X = the mean value of a variable component of the comparison solution; $\bar{X}$ = the mean value of a variable component which makes S and C isohedonic; and K = the constant concentration of a solution.

3. This table has been adapted from the table on pp. D-175 of the Handbook of Chemistry and Physics (47 th. edition; 1966-1967). The concentrations of Sucrose solutions are specified by different methods. In addition, the concentrations of sucrose-quinine mixtures are approximately equal to the values specified for a standard solution of sucrose.

The addition of quinine to sucrose in solution, results in a change in the Specific Gravity of that mixture; consequently, the molarity is altered. However, the levels of quinine used in this study are of such small magnitude that the density of the solution remains basically unchanged.

4. Early exposure to 0.01% quinine hydrochloride may be considered extremely strong or aversive by some experimenters because of the findings of past experimentation. However, the 0.01% quinine sulfate used in this study (during early exposure) is not equivalent to an 0.01% solution of quinine hydrochloride. It is contended that the 0.01% of quinine sulfate

evokes a sensation of bitter which is of lesser magnitude. In addition, it is noted that prior researchers frequently prepared solutions by methods other than the one used in this study (weight/weight).

If we assume that the magnitude of the sensation is related to the number of molecules in solution, then it is obvious that one formula-weight of quinine hydrochloride is equivalent to one formula-weight of quinine sulfate (Avogadro's number). However, a 10% solution (weight/weight) of quinine hydrochloride is not equivalent to a 10% solution of quinine sulfate. The formula-weight (mol. wt.) for quinine hydrochloride ($C_{20}H_{24}N_2O_2 \cdot HCl \cdot 2H_2O$) and quinine sulfate ($(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O$) is 396.91 grams and 782.97 grams respectively and the number of grams contained in a 10% (weight/weight) solution of quinine hydrochloride or quinine sulfate is 10 grams. Clearly, only 2.5% of the formula-weight of quinine hydrochloride is contained in a 10% solution (weight/weight) in contrast to the 1.28% of the formula-weight of quinine sulfate. In addition, the spatial arrangement of atoms composing quinine sulfate may be such that the quinine atom is surrounded and prevented from exerting a similar bitter evoking effect.

		Freq. x's	Freq. o's
2.0	x	1	0
1.7	x x x x x	10	0
1.4	x x x x o x x o x x x o o o x o x o x x o o x x x o x x	18	9
1.1	x o o o o o o o o o o o o o o o o x o	2	18
0.8	o o	0	2
		31	29

Fig.-9

Table-2

i	n_i	in_i	$i^2 n_i$
2	9	18	36
1	18	18	18
0	2	0	0
	29	36	54

Figure-9 is an example of a data sheet which might be used for the collection and analysis of sensitivity data. The letter x is used to designate (preference) the selection of the quinine-sucrose mixture and the letter o is used to designate the selection of the standard.

The estimate of $\bar{X}$ is given by the formula: $\bar{X} = y' + d(\frac{A}{n} \pm 1/2)$ where y' is the normalized height corresponding to the low-

EST CHO level on which the less frequent event occurs, $A = \sum_{i=0}^{k} i n_i$, $N = \sum n_i$, and d is the preliminary estimate of s (Dixon & Massey, 1957). The plus sign is used when the analysis is based on the zeros found in figure-9 and the minus sign is used when the analysis is based on the x's recorded in figure-9. A direct estimate of population variance is given by the following formula: $s = 1.62d\,(NB-A^2/N^2 + .029)$. $B = \sum_{i=0}^{k} I^2 n_i$ and $(NB-A^2)/N^2$ must be larger than 0.3 if the formula is to be accurate (Dixon & Massey, 1957). Figure-9 and Table-2 will illustrate the use of the above formulas. The y values given are 2,0, 1.7, 1.4, 1.1, and 0.8; the sucrose concentration used for the first test was 2.0 and d was 0.3. There are fewer o's (total) in Figure-9,hence the distribution of o's is used to estimate the parameters. The failures appear at 3 levels (0.8, 1.1, & 1.4) with frequencies: $n_o = 2$, $n_1 = 18$, and $n_2 = 9$. $N = 29$, $A = 36$, and $B = 54$. Therefore $\bar{X} = 0.8 + 0.3\,(36/29 \pm \frac{1}{2} = 1.32$ and $s = (1.620)\,(0.3)\,(270/841 \pm .029) = .17$. The standard deviation of a sample mean is given by $S_{\bar{X}} = Gs/\sqrt{N}$, $S_{\bar{X}} = (1.17)(1.12)/29 = .035)$ where G is a correction factor dependant on the ratio of d/s and on the position of the mean relative to the testing levels. .05 confidence interval for the above example is as follows: $1.32 \pm (1.96)(.035)$ or 1.25 to 1.39.

Appendix I-A

Analysis of Variance

1% Standard

Quinine Level	Group	$\bar{X} = y' / d$ 0.2 log units	$(A/N - 1/2)$ / Raw Data	$s = 1.62d(\sqrt{NB - A^2/N^2} / \sqrt{NB-A^2/N^2} \times .029)$	0.3	d/s
0.00001	H	0.43	02.73	.18	0.53	1.10
	W	0.13	01.38	.23	0.69	0.86
	S	-.37	00.87	.23	0.69	0.86
	Q	-.37	00.44	.19	0.56	1.06
0.0001	H	0.24	01.79	.17	0.49	1.19
	W	-.07	00.87	.23	0.69	0.86
	S	0.20	01.59	.20	0.58	1.01
	Q	-1.29	00.75	.21	0.61	0.96
0.001	H	0.21	01.66	.18	0.53	1.11
	W	0.10	01.30	.12	0.33	1.71
	S	0.07	01.21	.14	0.41	1.42
	Q	-.01	00.97	.14	0.41	1.42
0.01	H	0.64	04.51	.17	0.49	1.19
	W	0.44	02.84	.17	0.49	1.19
	S	0.44	02.84	.17	0.49	1.19
	Q	0.11	01.32	.23	0.67	0.89
0.1	H	1.03	11.06	.19	0.56	1.06
	W	1.13	12.90	.23	0.69	0.85
	S	1.07	12.18	.33	0.98	0.61
	Q	1.10	12.04	.23	0.67	0.89

1% Standard (Replication)

Quinine Level	Group	$\bar{X} = y' + d(A/N - 1/2)$ 0.2 log units	Raw Data	$s = 1.62(NB-A^2/A^2 + .029)$	$NB-A^2/N^2$ 0.3	d/s
0.00001	H	0.04	1.13	.17	0.49	1.19
	W	0.13	1.38	.33	0.98	0.61
	S	-0.10	0.82	.18	0.57	1.11
	Q	-0.19	0.66	.18	0.57	1.11
0.0001	H	0.21	1.66	.18	0.57	1.11
	W	0.04	1.13	.17	0.49	1.19
	S	-0.10	0.82	.27	0.82	0.73
	Q	-0.16	0.71	.17	0.49	1.19
0.001	H	0.07	1.21	.14	0.41	1.42
	W	0.07	1.19	.35	1.06	0.57
	S	0.10	1.30	.27	0.82	0.73
	Q	-0.24	0.58	.17	0.49	1.19
0.01	H	0.33	2.19	.23	0.69	0.86
	W	0.47	3.01	.17	0.49	1.19
	S	0.67	4.85	.14	0.41	1.42
	Q	0.50	3.26	.18	0.57	1.11

4% Standard

Quinine Level	Group	$\bar{X} = y' + d(A/N + 1/2)$ 0.2 log units	Raw Data	$s = 1.62d\left(\frac{NB-A^2}{N^2}\right) + .029$	$NB-A^2/N^2$ 0.3	d/s
0.00001	H	0.61	04.16	.37	1.10	0.55
	W	0.47	03.05	.14	0.41	1.42
	S	0.59	03.90	.27	0.82	0.73
	Q	0.47	03.00	.14	0.41	1.42
0.0001	H	0.47	03.02	.38	1.14	0.53
	W	0.47	03.05	.14	0.41	1.41
	S	0.64	04.51	.17	0.49	1.19
	Q	0.57	03.76	.30	0.90	0.67
0.001	H	0.64	04.51	.17	0.49	1.19
	W	0.56	03.68	.17	0.49	1.19
	S	0.87	07.69	.14	0.49	1.42
	Q	0.44	02.84	.17	0.49	1.19
0.01	H	0.81	06.61	.18	0.53	1.11
	W	0.67	04.83	.23	0.69	0.86
	S	1.24	18.02	.17	0.49	1.19
	Q	0.84	07.15	.17	0.49	1.19
0.1	H	1.24	18.02	.17	0.49	1.19
	W	1.19	15.59	.18	0.53	1.11
	S	1.61	41.98	.18	0.56	1.11
	Q	1.07	12.18	.27	0.81	0.74

Analysis of Variance

4% Standard (Replicated)

Quinine Level	Group	$\bar{X} = y' + d(A/N \pm 1/2)$		$s = 1.62d\sqrt{NB-A^2/N^2}$	$NB-A^2/N^2$	d/s
		0.2 log units	Raw Data	+ .029	0.3	
0.00001	H	0.47	03.05	.17	0.49	1.19
	W	0.47	03.05	.17	0.49	1.19
	S	0.47	03.05	.23	0.69	0.86
	Q	0.67	04.80	.17	0.49	1.19
0.0001	H	0.33	02.21	.17	0.47	1.23
	W	0.33	02.21	.27	0.81	0.74
	S	0.41	02.62	.18	0.53	1.11
	Q	0.70	05.18	.37	1.10	0.55
0.001	H	0.33	02.19	.23	0.69	0.86
	W	0.47	03.02	.17	0.49	1.19
	S	0.96	09.33	.17	0.49	1.19
	Q	0.67	04.78	.17	0.49	1.19
0.01	H	0.87	07.58	.38	1.14	0.53
	W	0.90	08.21	.37	1.10	0.55
	S	1.47	30.32	.17	0.49	1.19
	Q	1.01	10.49	.18	0.53	1.11

Appendix I-E

Analysis of Variance

16% Standard

Quinine Level	Group	$\bar{X} = y' + d(A/N - 1/2)$ 0.2 log units	Raw Data	$s = 1.62d \sqrt{NB-A^2/N^2} \div .029$	$N/B-A^2/N^2$ 0.3	d/s
0.00001	H	0.63	04.44	.19	0.56	1.06
	W	0.96	09.26	.35	1.06	0.57
	S	1.44	28.53	.17	0.49	1.19
	Q	1.27	19.29	.23	0.69	0.86
0.0001	H	0.33	02.19	.33	0.98	0.61
	W	0.83	06.97	.19	0.56	1.06
	S	1.27	19.31	.33	0.98	0.61
	Q	1.07	12.15	.23	0.65	0.86
0.001	H	0.81	06.61	.18	0.53	1.10
	W	1.33	22.06	.23	0.69	0.85
	S	1.67	48.61	.14	0.41	1.41
	Q	1.47	30.23	.23	0.69	0.86
0.01	H	1.43	27.86	.30	0.90	0.67
	W	1.47	30.70	.14	0.41	1.41
	S	1.61	42.00	.18	0.53	1.11
	Q	1.47	30.47	.27	0.81	0.74

Analysis of Variance

16% Standard (Replicated)

Quinine Level	Group	$\bar{X}$ = y' + d(A/N - 1/2) 0.2 log units	Raw Data	s = 1.62d $NB-A^2/N^2$ + .029	$NB-A^2/N^2$ 0.3	d/s
0.00001	H	0.70	05.18	.19	0.57	1.03
	W	0.90	08.22	.19	0.57	1.03
	S	1.47	30.76	.14	0.41	1.42
	Q	1.27	19.38	.14	0.41	1.42
0.0001	H	0.67	04.78	.17	0.49	1.19
	W	0.87	07.58	.27	0.81	0.74
	S	1.36	23.52	.17	0.49	1.19
	Q	1.24	18.02	.17	0.49	1.19
0.001	H	1.16	14.80	.17	0.49	1.19
	W	1.31	21.26	.14	0.41	1.41
	S	1.64	45.42	.17	0.49	1.19
	Q	1.40	25.40	.20	0.58	1.01
0.01	H	1.31	21.26	.23	0.69	0.86
	W	1.33	22.02	.23	0.69	0.86
	S	1.67	48.73	.14	0.41	1.41
	Q	1.44	28.60	.17	0.49	1.19

Analysis of Variance

1% Standard

Quinine Level	Group	G-Factor	Standard Error of Mean	.05 Confidence Intervals 0.2 log units	Raw Data
0.00001	H	1.00	.080	.27 to .59	1.92 to 3.89
	W	0.95	.098	-.06 to .32	0.88 to 2.15
	S	0.95	0.98	-.26 to .12	0.56 to 1.36
	Q	1.00	.085	-.53 to -.20	0.30 to 0.63
0.0001	H	1.00	.076	.09 to .39	1.28 to 2.48
	W	0.95	.098	-.26 to .12	0.56 to 1.36
	S	1.00	.089	.03 to .37	1.08 to 2.40
	Q	1.00	.094	-.31 to .06	0.50 to 1.16
0.001	H	1.00	.080	.06 to .37	1.17 to 2.39
	W	1.13	.061	-.02 to .22	0.96 to 1.68
	S	1.08	.068	-.06 to .20	0.89 to 1.61
	Q	1.08	.068	-.15 to .12	0.73 to 1.59
0.01	H	1.00	.076	.59 to .79	3.96 to 6.26
	W	1.00	.076	.29 to .59	2.03 to 3.94
	S	1.00	.076	.29 to .59	2.03 to 3.94
	Q	0.98	.101	-.09 to .31	0.85 to 2.09
0.1	H	1.00	.085	.87 to 1.20	7.58 to 16.00
	W	0.95	.098	.90 to 1.30	8.25 to 20.61
	S	0.92	.136	.81 to 1.31	6.44 to 22.44
	Q	0.98	.101	.90 to 1.30	8.25 to 20.61

Analysis of Variance

1% Standard (Replicated)

Quinine Level	Group	G-Factor	Standard Error of Mean	.05 Confidence Intervals 0.2 log units	Raw Data
0.00001	H	1.00	.076	-.09 to 1.92	0.83 to 1.57
	W	0.92	.134	-.13 to 0.39	0.75 to 2.47
	S	1.00	.080	-.26 to 0.06	0.56 to 1.17
	Q	1.00	.080	-.34 to -.03	0.47 to 0.95
0.0001	H	1.00	.080	.06 to 0.37	1.17 to 2.39
	W	1.00	.076	-.11 to 0.19	0.80 to 1.57
	S	0.94	.113	-.32 to 0.12	0.54 to 1.36
	Q	1.00	.076	-.31 to -.01	0.51 to 0.99
0.001	H	1.08	.068	-.05 to 0.20	0.90 to 1.61
	W	0.88	.134	-.20 to 0.33	0.64 to 2.19
	S	0.94	.113	-.12 to 0.32	0.55 to 2.16
	Q	1.00	.076	-.39 to -.09	0.41 to 0.83
0.01	H	0.95	.098	.14 to 0.52	1.40 to 3.41
	W	1.00	.076	.33 to 0.60	2.20 to 4.00
	S	1.08	.068	.54 to 0.80	3.54 to 6.42
	Q	1.00	.080	.34 to 0.66	2.26 to 4.73

Analysis of Variance

4% Standard

Quinine Level	Group	G-Factor	Standard Error of Mean	.05 Confidence Intervals 0.2 log units	Raw Data
0.00001	H	0.89	.147	0.33 to 0.90	02.18 to 08.25
	W	1.08	.068	0.34 to 0.60	02.23 to 04.05
	S	0.94	.113	0.37 to 0.81	02.36 to 06.48
	Q	1.08	.068	0.34 to 0.60	02.23 to 04.05
0.0001	H	0.89	.151	0.17 to 0.76	01.50 to 05.92
	W	1.08	.068	0.34 to 0.60	02.23 to 04.05
	S	1.00	.076	0.49 to 0.79	03.22 to 06.26
	Q	0.92	.123	0.33 to 0.81	02.18 to 06.50
0.001	H	1.00	.076	0.49 to 0.79	03.22 to 06.26
	W	1.00	.076	0.41 to 0.71	02.58 to 05.24
	S	1.05	.066	0.74 to 1.00	05.68 to 10.08
	Q	1.00	.076	0.29 to 0.59	02.03 to 03.93
0.01	H	1.00	.080	0.66 to 0.97	04.67 to 09.54
	W	0.95	.098	0.48 to 0.86	03.10 to 07.53
	S	1.00	.076	1.09 to 1.39	12.86 to 25.02
	Q	1.00	.076	0.69 to 0.99	05.10 to 09.93
0.1	H	1.00	.076	1.09 to 1.39	12.86 to 25.02
	W	1.00	.080	1.03 to 1.34	10.94 to 22.72
	S	1.00	.080	1.46 to 1.77	30.69 to [illegible]
	Q	0.94	.113	0.85 to 1.29	[illegible]

Appendix II-D

Analysis of Variance

4% Standard (Replicated)

Quinine Level	Group	G-Factor	Standard Error of Mean	.05 Confidence Intervals 0.2 log units	Raw Data
0.00001	H	1.00	.076	0.32 to 0.62	02.16 to 04.25
	W	1.00	.076	0.32 to 0.62	02.16 to 04.25
	S	0.95	.098	0.38 to 0.66	02.43 to 04.75
	Q	1.00	.076	0.52 to 0.82	03.39 to 06.65
0.0001	H	1.00	.076	0.19 to 0.48	01.58 to 03.07
	W	0.92	.111	0.11 to 0.56	01.34 to 03.67
	S	1.00	.080	0.26 to 0.57	01.86 to 03.79
	Q	0.89	.147	0.41 to 0.99	02.61 to 09.86
0.001	H	0.95	.098	0.34 to 0.52	01.40 to 03.37
	W	1.00	.076	0.32 to 0.62	02.14 to 04.19
	S	1.00	.076	0.81 to 1.11	06.56 to 13.30
	Q	1.00	.076	0.52 to 0.82	03.39 to 06.63
0.01	H	0.89	.151	0.57 to 1.16	03.78 to 14.86
	W	0.89	.147	0.61 to 1.19	04.14 to 10.93
	S	1.00	.076	1.32 to 1.62	21.50 to 42.10
	Q	1.00	.080	0.86 to 1.17	07.41 to 15.12

Analysis of Variance

16% Standard

Quinine Level	Group	G-Factor	Standard Error of Mean	.05 Confidence Intervals 0.02 log units	Raw Data
0.00001	H	1.00	.085	0.47 to 0.80	03.01 to 06.35
	W	0.88	.134	0.70 to 1.22	05.10 to 16.84
	S	1.00	.076	1.29 to 1.59	20.42 to 39.72
	Q	0.92	.094	1.09 to 1.46	12.70 to 27.94
0.0001	H	0.92	.251	0.16 to 0.82	01.48 to 06.74
	W	1.00	.085	0.67 to 1.00	04.77 to 10.08
	S	0.92	.136	1.00 to 1.54	10.08 to 35.68
	Q	0.95	.098	0.88 to 1.26	07.84 to 18.91
0.001	H	1.00	.080	0.66 to 0.97	04.67 to 09.54
	W	0.95	.098	1.14 to 1.52	14.12 to 34.41
	S	1.15	.073	1.53 to 1.81	34.86 to 64.00
	Q	0.95	.098	1.28 to 1.66	19.71 to 47.78
0.01	H	0.90	.122	1.20 to 1.67	15.82 to 48.85
	W	1.08	.068	1.34 to 1.60	22.69 to 40.79
	S	1.00	.080	1.46 to 1.77	29.65 to 60.58
	Q	0.92	.111	1.25 to 1.69	18.16 to 50.75

Analysis of Variance

16% Standard (Replicated)

Quinine Level	Group	G-Factor	Standard Error of Mean	.05 Confidence Intervals 0.2 log units	Raw Data
0.00001	H	1.00	.086	0.53 to 0.73	03.50 to 04.14
	W	1.00	.086	0.73 to 1.07	04.14 to 12.09
	S	1.08	.068	1.34 to 1.60	22.49 to 40.79
	Q	1.08	.068	1.14 to 1.40	14.15 to 25.70
0.0001	H	1.00	.076	0.52 to 0.82	03.39 to 06.63
	W	0.92	.111	0.65 to 1.09	04.53 to 12.65
	S	1.00	.076	1.21 to 1.51	16.52 to 33.52
	Q	1.00	.076	1.09 to 1.39	12.85 to 25.02
0.001	H	1.00	.076	1.01 to 1.31	10.40 to 21.12
	W	1.08	.068	1.18 to 1.44	15.36 to 28.75
	S	1.00	.076	1.49 to 1.79	32.40 to 63.07
	Q	1.00	.089	1.23 to 1.57	17.22 to 38.36
0.01	H	0.95	.098	1.12 to 1.50	13.62 to 33.15
	W	0.95	.098	1.14 to 1.52	14.12 to 34.41
	S	1.08	.068	1.54 to 1.80	35.68 to 64.00
	Q	1.00	.076	1.29 to 1.59	20.42 to 39.70

DATE DUE			