Parotid Gland Secretion Rate as Method for Measuring Response to Gustatory Stimuli in Humans.*† (25557)

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Four distinct submodalities, acid, salt, sweet and bitter, are generally acknowledged as comprising the gustatory sensation. These primary responses along with certain somatosensory components such as pain, temperature and touch contribute to the response aroused when receptor areas of oral cavity are activated by gustatory stimuli. Electrical recording of action potentials from single taste fibers (1) and response from total receptor field(2) provided a means of measuring nerve response to chemical stimulation of the tongue in animals. "Drop or sip" methods are generally employed to measure taste thresholds and sensitivity differentials in humans. Beebe-Center and Waddell(3) described a heteroqualitative taste scale using the subjective strength of 1.0% solution of sucrose, units being termed "gusts." Gust concentration curves were given for tartaric acid, sodium chloride, sucrose and quinine. Unfortunately, these measurements of human response to gustatory stimuli are based on subjective evaluations and are thus necessarily limited. Clearly, some other means of exploring taste in humans is needed before physiological mechanisms governing this sensation can be discovered. The present paper offers a new approach to quantitative measurement of the effect of gustatory stimuli.

Methods. One hundred thirty-eight male subjects between ages of 17 and 30 years, of sufficiently sound physical condition to qualify for military service, were utilized. *Stimuli*: The effect of each of 4 gustatory submodalities was determined using solutions of citric acid, sodium chloride, sucrose and quinine. Using wetted cotton applicator sticks, the solutions were applied by running wetted cotton around lateral edges of the tongue, followed by swabbing the entire dorsum of tongue from area delineated by the circumvallate papillae to the tip. A wall clock with sweep second hand was visible to all participants and supervisory personnel were available to insure that the subjects applied the stimuli in a proper manner and at specified intervals. *Secretory Rate*: Samples of parotid saliva, collected in tubes graduated to 0.1 ml were obtained by means of vacuum cups(4). Secretion rate (ml/min/gland) for each participant was determined by measuring time required to secrete a standard volume (5 ml) which permitted greatest accuracy in evaluating response to test stimuli. Response to different stimuli was quite variable and some individuals were unable to secrete the desired volume within a reasonable period. Since prolonged application of the stimuli caused undue discomfort or trauma to the subjects, (sodium chloride—drying of oral mucosa and lips; sucrose—nausea; quinine—retching; and citric acid—bleeding of the tongue and lips,) it was not feasible to continue testing for more than 4 hours. Therefore, maximum stimulation time, 20 minutes for sodium chloride and sucrose solutions and 15 minutes for citric acid and quinine solutions, was employed. If 5 ml was obtained before maximum time limit was reached the subject was permitted to terminate that sample and proceed to the next sample, to prevent glandular fatigue in persons who were rapid secretors. Secretion rate (volume collected/time of stimulation) for each individual and each variable was calculated. The results obtained, for each variable, were summed and mean rate for the group determined. Each experimental group received 4 to 8 test solutions. However, subjects were sampled only once for each variable tested. This procedure was employed after

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ascertaining in a pilot group (10 subjects) that both individual and group response to randomly chosen stimuli did not significantly vary when a given stimulus was applied several times within 4 hours. *Sampling*: A different group of individuals was employed for each series of experimental variables and the following standard procedure was established. After initial placement of vacuum cup a 10 minute acquaintance interval was given each subject. The secretion thus obtained was considered a *clearance sample* and was not used. Upon completion of the acquaintance session an *experimental sample* was collected. Since several successive samples were obtained from each individual, using both constant and variable stimuli, a 5 minute clearance sample was collected immediately prior to all other experimental samples. Participants were given a drink of water (room temp., 25°C) after collection of each experimental sample to make certain that the response measured was not affected by previously employed stimulus. Sampling was done both morning and afternoon without regard for possible effect of postprandial or diurnal variation. Three test conditions were utilized in determining parotid gland secretory response to the 4 gustatory submodalities. The effect of solution concentration, application rate (frequency of stimulation) and accommodation were investigated. Twelve experimental groups were tested. A control or sham group using only water was employed to determine response resulting from tactile sensation. Control values were thus subtracted from all experimental values, to give only the response arising from gustation.

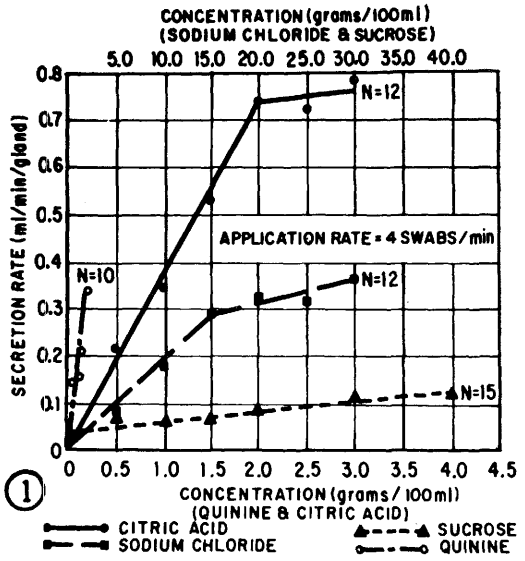
Results. Effect of concentration: Range of concentrations of the 4 solutions used was chosen so that minimal concentration would be above absolute threshold for each sensation. Maximum concentration was selected either on a basis of solubility or on concentration suitable for continuous use without damaging oral mucosa or inducing illness. Concentrations were always given in ascending order. Throughout entire series of concentrations all subjects were able to discern increase in gustatory intensity from one solution to the next. A separate group of individuals was used for each of the 4 stimuli. Application rate was maintained at 4 swabs/minute.

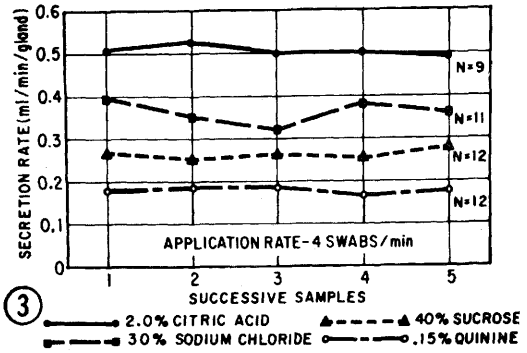
Relationship between secretory response and solution concentration is illustrated in Fig. 1. Citric acid evoked a response which had a linear relationship with concentration up to a 2% solution. Secretion rate then appeared to have reached a maximum and broke sharply. A similar, but not so severe, break occurred with sodium chloride where a marked decrease in rate was exhibited at concentrations above 15%. In contrast, rate of secretion appeared to have a linear relationship with amount of quinine and sucrose in solution throughout entire series of concentrations. This is in accord with observations of Pfaffman(1) that frequency of response in any one gustatory fiber was increased by increase of stimulus intensity (concentration) only up to some limiting value. Above this limit, more intense stimuli produced only the same maximal response.

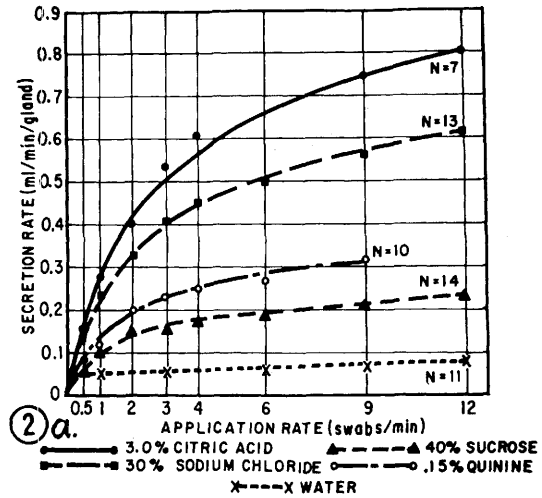
Effect of Application Rate: Intensity of response resulting from increasing rates of application is presented in Fig. 2. Solution concentrations were constant and rate of application (swabs/min) was increased throughout the series. Concentrations selected: citric acid—3%, sodium chloride—30%, sucrose—40% and quinine—0.15% induced maximal or near maximal stimulation. The results indicate that secretory response appears to be a curvilinear function of application rate (Fig. 2a). In the water series (control) secretion rate also increased as swab rate was raised, but here the relationship appeared to be a straight line function. When response (secretion rate) was plotted against logarithm of application rate, a linear relationship was established (Fig. 2b).

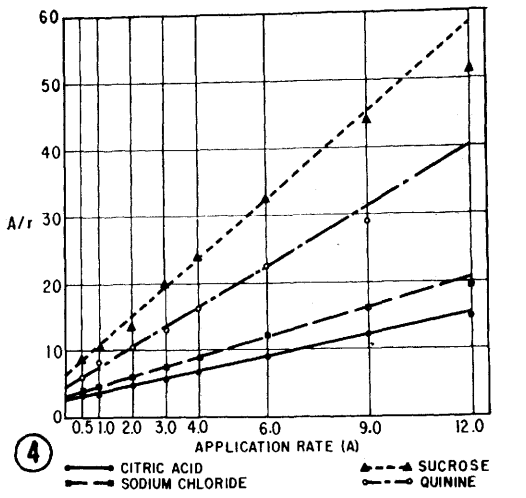
Accommodation. To ascertain if decreasing acceleration rate of response with increasing application rate was due to accommodation the following was undertaken. Five successive saliva samples were obtained, with exception of citric acid, using the same solution concentrations and collection procedures as for rate study. A lower concentration of citric acid (2%) was used because concentra-

tions greater than 2% had a keratinolytic action when used over prolonged periods. Application rate was maintained at 4 swabs/minute; this approximated a mean rate of 4.7 swabs/min calculated from the range (0.5-12/









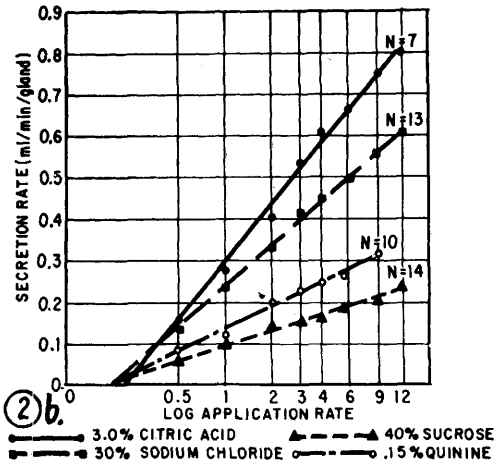


FIG. 1. Effect of solution concentration on secretion rate. One group of subjects used for each of 4 stimuli. N equals No. of individuals/group. Saliva samples were collected successively and stimuli given in ascending order. Solution temp. 25°C.

FIG. 2a and 2b. Effect of application rate on secretion rate. N equals No. of persons/group. A separate group was employed for each of 4 test solutions and the water control. Temp. of solutions 25°C. Saliva samples were collected successively and application rate modified in ascending order.

FIG. 3. Effect of continuous stimulation on secretion rate. N equals No. of subjects/group. A different group was used for each test solution. Solutions maintained at 25°C.

FIG. 4. Determination of equilibrium constant and maximal response for 4 gustatory stimuli. Plot of 8 application rates (swabs/min.) against application rate divided by secretory response (ml/min./gland).

min) of the rate series. Fig. 3 shows that the response remained quite consistent over entire stimulation period (citric acid—80 min; sodium chloride—100 min; sucrose—130 min; quinine—130 min). Only sodium chloride and sucrose solutions exhibited a significant change in secretion rate between first and subsequent samples, in that paired data analysis of results indicated that there was a slight, but significant, difference in rate of secretion between samples 1 *vs.* 2, and 1 *vs.* 3. The difference between 1 *vs.* 4, 1 *vs.* 5 or among samples 2 through 5 was not statistically significant. With citric acid and quinine solutions, there were no statistically significant differences among any of the 5 samples. Thus it can be assumed that there was no measurable accommodation under imposed experimental conditions. Furthermore, these findings serve to reemphasize the observation noted in the pilot study, that subjects respond in a fairly consistent manner to a given stimulus.

Discussion. Plotting secretory response against stimulation frequency resulted in a curve fitting the equation of Michaelis and Menten. The method of Lineweaver and Burk(5) which rearranges the equation to a straight line function makes the relationship between response and affecting variable more obvious. This relationship can be expressed mathematically as $\frac{A}{r} = \frac{1}{R}(A) + \frac{K}{R}$, where A is affecting variable, r is observed response, R is maximum response and K is equilibrium constant of the entire reflex. By plotting A against A/r (Fig. 4) the slopes of resultant lines are equal to $\frac{1}{R}$ and the ordinate intercept is equal to $\frac{K}{R}$. The method of least squares was used to calculate slopes and ordinate intercepts. The R max and the gustatory-salivary reflex K for the 4 gustatory modalities are presented in Table I. The K for sucrose and quinine appear to be of similar magnitude and markedly differ from values obtained for citric acid and sodium chloride.

Due to the many problems which require further clarification, we can only speculate as to the meaning of these findings. Pfaffman (1) recording action potentials in cat chorda tympani and glossopharyngeal nerves was able to isolate 3 types of single fiber preparations. The first gave action potentials only when the tongue was stimulated with acid solution, the second responded only to acid and/or sodium chloride solution, while the third reacted to citric acid and/or quinine. Receptors for sugar were not usually found. Thus acid was a stimulus for all gustatory nerve endings while sodium chloride and quinine affected only certain types. In essence the 3 types were acid-salt, acid-quinine and pure acid fibers. In our investigation stimulation of the 3 primary modalities associated with fibers from the chordatympani which subserve, the sensation of taste in anterior two-thirds of tongue (acid, salt, and sweet) resulted in different responses as evidenced by equilibrium constants (K) for the gustato-salivary reflex. Although the bitter sensation is mediated, for the most part, through the glossopharyngeal nerve the equilibrium constant for quinine was similar in magnitude to that evidenced with sucrose. These findings appear to indicate that at least 3 different gustato-salivary reflex pathways are involved. Whether the difference lies merely in type of receptor ending stimulated, or depends also on different fiber types present in the gustatory nerve trunks cannot be discerned. However, it is believed that the procedure described herein offers a practical means of studying the physiology of human gustation and the mechanisms controlling salivary secretion.

TABLE I. Maximum Response and Equilibrium Constant of Gustatory Stimuli.

Stimulus	R max, ml/min./gland	Equilibrium constant
Citric acid	.97	2.63
Sodium chloride	.71	2.21
Sucrose	.23	1.45
Quinine	.34	1.53

Summary. Parotid gland secretion rate was employed for measuring human response to gustatory stimuli. Flow rate had a linear relationship to logarithm of frequency of stimu-

lation (swabs/min). Increasing concentration of gustatory stimuli resulted in increased rates of secretion. The response was linear throughout for sucrose and quinine, but with citric acid and sodium chloride it was linear only with lower concentrations. Mathematical expression of relationship between glandular secretion rate and frequency of stimulation permitted calculation of maximal response and reflex equilibrium constants for the various stimuli employed. Results indicated that at least 3 different gustato-salivary reflex pathways were involved.

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Urethan Induced Acceleration of Hexobarbital Metabolism.* (25558)

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Reduction and demethylation of certain azo dyes by rat liver microsomes have been increased by administration of such carcinogenic hydrocarbons as 3-methylcholanthrene and 3, 4 benzpyrene(1). The evidence is that induced enzyme synthesis occurs in these cases. Recently, increases in other hepatic microsomal enzyme activities have been obtained with several other classes of chemical compounds(2,3). The present article deals with data which suggest that urethan (ethyl carbamate) may similarly induce increases in activity of certain enzyme systems. Urethan is of particular interest owing to its cancer chemotherapeutic activity and carcinogenic activity.

Materials and methods. Sleeping times were measured in Swiss albino mice (20-35 g). Experimental groups were pretreated with urethan or 3-methylcholanthrene. After a definite time (in most cases 24 hours), these and control groups were given hexobarbital, sodium, and their sleeping times determined as duration of loss of righting reflex. Mice were considered asleep unless they could right themselves 3 times in one minute. Significance of the difference between sleeping times of control and experimental groups of mice was assessed by the "t" test. For determination of effect of urethan on hexobarbital body concentrations, the experimental mice were treated with urethan (1200 mg/kg, i.p.) and followed in 24 hours by hexobarbital sodium (150 mg/kg, i.p.). All drug solutions were of such concentration that .01 ml was given for each 1 gram body weight. Control mice received hexobarbital only. Determination of total body hexobarbital was done one hour after hexobarbital administration by homogenizing each mouse with 4 times its weight of water in a Waring Blender. Four ml of homogenate were extracted with chloroform by method similar to that of Axelrod *et al.*(4), except that the final NaOH extract of chloroform was immediately adjusted to pH 10.3 with $KHCO_3$ and read at 245 mμ in the Beckman DU spectrophotometer.

Results. In Table I pretreatment with urethan shortens hexobarbital sleeping time. Thus, if urethan is given 24 to 48 hours previous to hexobarbital, mean hexobarbital sleeping time is decreased from 41 to 31 minutes. This effect of urethan is largely gone by 72 hours. If the interval is only 12 hours between urethan and hexobarbital administration, sleeping time is prolonged beyond control levels owing possibly to persistence of CNS depressant effect of the urethan.

The most striking effects in shortening

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