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Effect of Acid Solutions on Human Gustatory Chemoreceptors as Determined by Parotid Gland Secretion Rate.* (28210)

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In the past measurement of human responses to gustatory stimuli has been based on subjective evaluation. Recent studies(1) employing parotid gland secretion rate as a method for measuring response to gustatory stimuli in humans have offered an objective approach to quantitative measurement of the effect of these stimuli. Utilization of these techniques permits accurate assay of physiological responses to innumerable experimental conditions.

In this study the secretory response elicited by 17 organic acids was used to determine which chemical and physical properties of acids exerted an influence on their stimulatory action on human gustatory chemoreceptors, giving rise to the "sour" sensation.

Materials and methods. Solutions were applied using wetted cotton applicator sticks. One complete application consisted of running the wetted swab around the lateral edges of the tongue and a subsequent swabbing of the dorsum of the tongue from the circumvallate papillae to the tip. Applications were at the rate of 4 times per minute.

Acids were presented in groups of three. The 9 subjects (third year, male, dental students) utilized for this study were divided into 3 groups of 3. The first subject in each group received the acids in the order ABC, the second in the order BCA, and the third in the order CAB. Nose clamps were worn throughout the experiment to prevent olfactory stimulation.

Parotid saliva samples were obtained using a vacuum cap(2). The fluid was collected in 15 ml centrifuge tubes, graduated to 0.1 ml, and the volume read to the nearest 0.05 ml. Rate of secretion was determined by measuring volume secreted in a standard time period. A control series using only water was conducted to evaluate the response elicited by tactile stimulation. These control values were subtracted from all other experimental data so as to measure only the response elicited by gustation.

After initial placement of the vacuum cap, a 5-minute acquaintance interval of stimulation was allowed each subject. This collection was considered a clearance sample and was discarded. Upon completion of the acquaintance period, a 10-minute experimental sample was collected. This was followed by a 5-minute rest interval, during which each subject was given a drink of water. Using this procedure a total of 3 experimental samples were obtained during a single sitting. Total collection period, including the 2 rest intervals, was 55 minutes. All samples were collected preprandially.

Subjective evaluation of the degree of sourness was also undertaken. Participants swabbed their tongues in the manner previously described, using groups of 3 acids. Subjects were allowed to sample the acids as often as necessary and in any sequence so as to determine the relative sourness of the 3 acids.

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TABLE I. Result of 3 Successive Applications of Citric Acid.*

Sample	A	B	C
Mean flow rate (ml/10 min/gland)	4.78	4.78	4.79

* Acid concentration = .067 M.

TABLE II. Duplication of Flow Rates from Parotid Saliva Samples Collected on Different Days.

Citric acid (M)	Mean flow rate (ml/10 min/gland) Week I	Week II	Malic acid (M)	Mean flow rate (ml/10 min/gland) Week I	Week II
.025	2.31	2.40	.0375	2.95	2.90
.050	3.89	4.44	.0750	4.97	5.04
.075	5.03	5.03	.1250	6.27	6.26
.100	6.27	5.65	.1500	6.80	6.60
.125	7.65	7.98	.1875	7.72	8.09

Results. Statistical analysis revealed that the subjective evaluation and the objective parotid flow measurement were significantly correlated for any group of 3 acids tested ($r = .688$; $P = <.01$); however, there was a greater degree of variation in the subjective evaluation. Additional studies revealed that the variation in parotid flow rate between samples collected during a single collection interval (Table I) and successive collection intervals (Table II), using the same acid, was not significant. The measurement of parotid secretion had a definite advantage over the subjective technique, since a large series of stimulating substances could thus be ranked in order of effectiveness.

A diurnal variation (Table III) was noted between samples collected in the morning and afternoon. The results show that the secretion rate in the afternoon increased for 7 of the 10 acids tested. Two acids remained essentially constant and one acid elicited a significantly lower rate. Therefore, successive phases were conducted entirely either in the morning or afternoon.

TABLE III. Diurnal Variations of Flow Rate.

Acid	Flow rate (ml/10 min/gland) 7 A.M.	1 P.M.	P
Glutaric	4.85	5.51	.05
Succinic	4.05	4.00	N.S.
Glycolic	3.92	5.70	.05
Isobutyric	1.93	1.83	N.S.
Propionic	1.83	2.72	.05
Butyric	1.28	1.97	N.S.
Crotonic	1.33	2.24	.05
Acetic	3.12	4.23	.05
Malic	4.18	6.34	.05
Tricarballylic	5.72	5.08	.05

Acid concentration = .1 M.

Six odoriferous acids were tested with and without nose clamps, so as to determine the effect of olfactory stimuli on the parotid gland secretion rate (Table IV). The results indicate that there was little or no odor effect with 4 of the 6 acids employed. However, when lactic acid and isobutyric acids were used without nose clamps there was a marked increase in secretion rate. Thus, it was deemed advisable to have the subjects wear nose clamps throughout the entire series of experiments.

TABLE IV. Effect of Olfactory Stimulation on Secretion Rate Utilizing Odiferous Acids.*

Acid	Mean secretion rate (ml/10 min/gland) Without nose clamps	With nose clamps
Acetic	3.61	3.74
Propionic	2.26	2.33
Butyric	2.34	2.30
Pyruvic	5.44	5.30
Lactic	4.44	3.57
Isobutyric	2.64	2.14

Acid concentration = .2 M.

Fig. 1 illustrates the results obtained with increasing solution concentrations for 3 organic acids. A plot of concentration *vs* secretion rate resulted in a curvilinear relationship which was distinct for each acid. However, when the (H^+) concentration was plotted against the parotid gland secretion rate (Fig. 2) a direct linear relationship was observed. Again, 3 distinct lines with different slopes were obtained, indicating that each acid evoked its own definitive response pattern.

Anion effect was determined in the subsequent series of experiments using 17 organic acids. Parotid gland response was measured using a constant pH of 2.60 ± 0.10 (Table V). Acids were ranked according to secretion rate. Thus, with a constant (H^+) concentration many of the acids elicited different

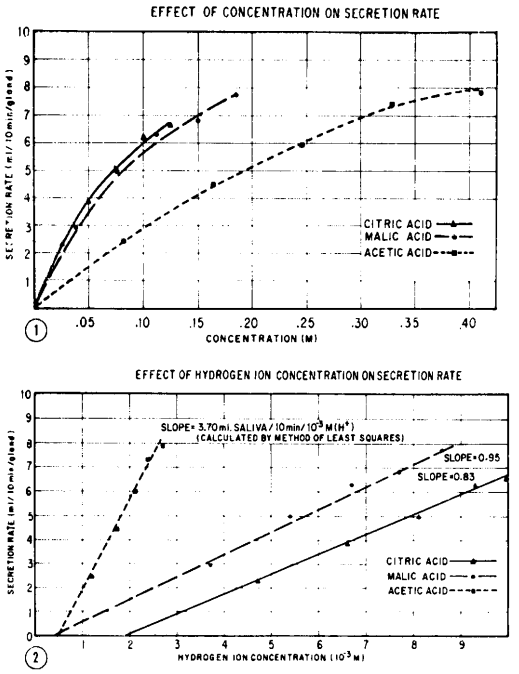

FIG. 1 and 2.

responses, indicating variation in degree of chemoreceptor stimulation.

To ascertain whether this variation was due to the structure of the anion or to the concentration of undissociated acid in solution, the same series of acids were tested

TABLE V. Effect of Constant Hydrogen Ion Concentration.*

Acid	Total concentration† (M)	Mean flow rate (ml/10 min/gland)
Acetic	.20	4.86
Glutaric	.10	4.85
Succinic	.10	4.05
Crotonic	.20	3.13
Tricarballylic	.113	3.07
Isobutyric	.20	3.04
Acrylic	.113	3.03
Propionic	.20	2.87
Butyric	.20	2.24
Glycolic	.0429	1.72
Malic	.0163	1.23
Citric	.00756	1.00
Malonic	.00426	.45
Lactic	.020	.39
Pyruvic	.00604	.37
Oxalic	.0027	.35
Tartaric	.0058	.21

* $[H^+] = 2.54 \times 10^{-3} \pm .25 \times 10^{-3}$ M.
† Undissociated acid concentrations = Total − $[H^+]$.

TABLE VI. Effect of Constant Total Acid Concentration.*

Acid	Hydrogen ion concentration	Mean flow rate (ml/10 min/gland)
Tartaric	1.12×10^{-2}	7.69
Oxalic	5.50×10^{-2}	7.55
Citric	1.78×10^{-2}	6.34
Malic	6.46×10^{-3}	6.34
Malonic	1.32×10^{-2}	5.94
Glycolic	5.62×10^{-3}	5.70
Glutaric	2.45×10^{-3}	5.51
Tricarballylic	5.25×10^{-3}	5.08
Pyruvic	2.24×10^{-2}	4.56
Acetic	1.51×10^{-3}	4.23
Succinic	3.02×10^{-3}	4.00
Lactic	5.62×10^{-3}	3.65
Propionic	1.38×10^{-3}	2.72
Acrylic	2.63×10^{-3}	2.53
Crotonic	2.82×10^{-3}	2.24
Butyric	1.55×10^{-3}	1.97
Isobutyric	1.99×10^{-3}	1.83

* Concentration = .100 ± .010 M.

TABLE VII. Effect of Constant Undissociated Acid Concentration.*

Acid	Hydrogen ion concentration	Mean flow rate (ml/10 min/gland)
Oxalic	5.45×10^{-2}	8.00
Malonic	1.58×10^{-2}	7.13
Citric	1.00×10^{-2}	6.70
Tricarballylic	6.03×10^{-3}	5.72
Succinic	3.16×10^{-3}	5.31
Tartaric	1.01×10^{-2}	4.88
Pyruvic	2.04×10^{-2}	4.23
Malic	6.61×10^{-3}	4.18
Glycolic	4.47×10^{-3}	3.92
Glutaric	2.40×10^{-3}	3.84
Acetic	1.48×10^{-3}	3.12
Lactic	5.25×10^{-3}	2.51
Acrylic	3.02×10^{-3}	2.31
Isobutyric	1.32×10^{-3}	1.93
Propionic	1.02×10^{-3}	1.83
Crotonic	1.66×10^{-3}	1.33
Butyric	1.41×10^{-3}	1.28

* Undissociated acid concentration = .100 ± .010 M.

maintaining a constant concentration of total acid (0.10 ± 0.01 M) and a constant concentration of undissociated acid (0.10 ± 0.01 M) (Tables VI, VII). Acids are again ranked according to secretion rate.

Discussion. Comparison of the rank order obtained in the constant total acid (0.10 M) phase with the acid threshold levels determined by other investigators shows that a high correlation (Table VIII) exists between our results and those obtained by prior investigators. This would tend to confirm that measurement of the parotid fluid secretion rate, induced by various acid solutions, is

TABLE VIII. Rank Order Comparison from Threshold Levels and Parotid Gland Response.

Rank order from threshold levels		Rank order from flow rate	
Taylor(3)	Pfaffman(4)	.1 M total acid	.1 M undissociated acid
Tartaric	Tartaric	Tartaric	Oxalic
Citric	Citric	Oxalic	Malonic
Oxalic	Malic	Citric	Citric
Tricarballylic	Lactic	Malic	Tricarballylic
Malonic	Oxalic	Malonic	Succinic
Malic	Succinic	Glycolic	Tartaric
Glycolic	Acetic	Glutaric	Pyruvic
Succinic	Butyric	Tricarballylic	Malic
Pyruvic		Pyruvic	Glycolic
Glutaric		Acetic	Glutaric
Lactic		Succinic	Acetic
Acetic		Lactic	Lactic
Propionic		Propionic	Acrylic
Butyric		Acrylic	Isobutyric
		Crotonic	Propionic
		Butyric	Crotonic
		Isobutyric	Butyric

R = Spearman rank order coefficient.
R_1 (Taylor and .1 M total acid) = .889.
R_2 (Pfaffman and .1 M total acid) = .714.
R_3 (.1 M total acid and .1 M undissociated acid) = .828.

significantly related to acid threshold levels. When a comparison is made between total acid concentration data and undissociated acid concentration data, it can be seen that the rank order is similar (Spearman Rank Order Correlation Coefficient (R) = 0.828; P = <.01) for both phases. Taylor(3) and Biedler(5) have postulated that the concentration of the undissociated acid is either responsible for the effect produced or potentiates the action of the hydrogen ion. Evidence in contradiction of these theories can be seen in Table V where the hydrogen ion concentration (.00254 M) is constant. There are numerous examples where one acid which has a greater concentration of undissociated material produces a lower secretion rate than another acid which has less undissociated material, *e.g.*, crotonic *vs* glutaric; crotonic *vs* succinic; isobutyric *vs* tricarballylic; isobutyric *vs* succinic; isobutyric *vs* glutaric; lactic *vs* malonic; lactic *vs* malic, etc. Thus, it would appear that the undissociated portion of the acid does not facilitate stimulation by the hydrogen ion at receptor sites.

When the acids are categorized according to chemical configuration (Table IX), it becomes possible to evaluate the effect of carbon chain length, carbon to carbon double bonds, number of carboxyl groups, and polar groupings. In the monocarboxylic acids, increasing the carbon chain length of the anion decreases the stimulating action of the acid. The opposite is noted for dicarboxylic acids. The reason for this becomes apparent when the effect produced by mono-, di-, and tricarboxylic acids are compared. Acetic acid is more effective than all the dicarboxylic acids, except for glutaric acid. The presence of a second carboxyl group appears to reduce the stimulatory action of the acids and only when a sufficient number of intervening methyl groups are present is the stimulatory action returned to maximum activity. Thus, acetic and propionic acids are more effective than their dicarboxylic counterparts—oxalic and malonic acids. Only in the case of butyric and succinic acids is it noted that the dicarboxylic acid is more effective than the monocarboxylic acid. This is apparently due to the inhibitory action of C-C chain length in monocarboxylic acids while carbon chain length has an activator action in the dicarboxylic acids. The presence of a third carboxyl group also has an inhibitory effect. This is noted when glutaric and tricarballylic acids are compared.

The second column of Table IX illustrates

TABLE IX. Effect of Structure Relative to Constant Hydrogen Ion Concentration.*

CARBON LENGTH CHAIN

Acid	Structure	Conc (M)	Flow rate
Acetic	CH_3COOH	.20	4.86
Propionic	CH_3CH_2COOH	.20	2.87
Butyric	$CH_3CH_2CH_2COOH$	.20	2.24
Oxalic	$HOOCCOOH$	.0027	.35
Malonic	$HOOCCH_2COOH$	.0043	.45
Succinic	$HOOCCH_2CH_2COOH$	.10	4.05
Glutaric	$HOOCCH_2CH_2CH_2COOH$	.10	4.85

CARBOXYL GROUPS

Acid	Structure	Conc (M)	Flow rate
Acetic	CH_3COOH	.20	4.86
Oxalic	$HOOCCOOH$	.0027	.35
Propionic	CH_3CH_2COOH	.20	2.87
Malonic	$HOOCCH_2COOH$	.0043	.45
Butyric	$CH_3CH_2CH_2COOH$	.20	2.24
Succinic	$HOOCCH_2CH_2COOH$	.10	4.05
Glutaric	$HOOCCH_2CH_2CH_2COOH$	.10	4.85
Tricarballylic	$HOOCCH_2CH(COOH)CH_2COOH$	.113	3.07

CARBON - CARBON DOUBLE BOND

Acid	Structure	Conc (M)	Flow rate
Propionic	CH_3CH_2COOH	.20	2.87
Acrylic	$CH_2{=}CHCOOH$	.113	3.03
Butyric	$CH_3CH_2CH_2COOH$	.20	2.24
Crotonic	$CH_3CH{=}CHCOOH$	.20	3.13

POLAR GROUPS

Acid	Structure	Conc (M)	Flow rate
Propionic	CH_3CH_2COOH	.20	2.87
Lactic	$CH_3CHOHCOOH$	.020	.39
Pyruvic	$CH_3COCOOH$	.0060	.37
Acetic	CH_3COOH	.20	4.86
Glycolic	$HOCH_2COOH$	.0429	1.72
Succinic	$HOOCCH_2CH_2COOH$	.10	4.05
Malic	$HOOCCHOHCH_2COOH$	.0163	1.23
Tartaric	$HOOCCHOHCHOHCOOH$	.0058	.21
Tricarballylic	$HOOCCH_2CH(COOH)CH_2COOH$	.113	3.07
Citric	$HOOCCH_2HOC(COOH)CH_2COOH$	.0076	1.90

* Data based on Table 5.

TABLE X. Effect of Structure—Constant Undissociated Acid Concentration.*

Acid	[H⁺]	Flow rate
CARBON CHAIN LENGTH		
Acetic	1.48×10^{-2} M	3.12
Propionic	1.02×10^{-3}	1.83
Butyric	1.41×10^{-3}	1.28
Oxalic	5.45×10^{-2}	8.00
Malonic	1.58×10^{-2}	7.13
Succinic	3.16×10^{-3}	5.31
Glutaric	2.40×10^{-3}	3.84
CARBON - CARBON DOUBLE BOND		
Propionic	1.02×10^{-3}	1.83
Acrylic	3.02×10^{-3}	2.31
Butyric	1.41×10^{-3}	1.28
Crotonic	1.66×10^{-3}	1.33
CARBOXYL GROUPS		
Acetic	1.48×10^{-3}	3.12
Oxalic	5.45×10^{-3}	8.00
Propionic	1.02×10^{-3}	1.83
Malonic	1.58×10^{-2}	3.84
Butyric	1.41×10^{-3}	1.28
Succinic	3.16×10^{-3}	5.31
Glutaric	2.40×10^{-3}	3.84
Tricarballylic	6.03×10^{-3}	5.72
POLAR GROUPS		
Propionic	1.02×10^{-3}	1.83
Lactic	5.25×10^{-3}	2.51
Pyruvic	2.04×10^{-2}	4.23
Acetic	1.48×10^{-3}	3.12
Glycolic	4.47×10^{-3}	3.92
Succinic	3.16×10^{-3}	5.31
Malic	6.61×10^{-3}	4.18
Tartaric	1.01×10^{-2}	4.88
Tricarballylic	6.03×10^{-3}	5.72
Citric	1.00×10^{-2}	6.70

* Data based on Table VII.

that the presence of carbon to carbon double bonds slightly enhances the action of acids on the gustatory chemoreceptors. Conversely, the presence of polar groups such as hydroxyl and carbonyl groups has an inhibitory effect.

The hypothesis that these differences in effect are indeed due to structure is supported by Fig. 2 where it was seen that a characteristic slope exists for each of the 3 acids tested and that a 10-fold increase in (H^+) concentration of any one acid resulted in at least an 8-fold increase in secretion rate. When Table IX (data obtained with a constant H^+ concentration) is reexamined for concentration effect it can be seen that there are 4 acids—acetic, propionic, butyric, and crotonic—which have a concentration of 0.2 M, yet all 4 of these evoke significantly different gustatory responses. Similarly, there are 4 acids—succinic, glutaric, tricarballylic, and acrylic—which were used at a concentration of approximately 0.1 M, yet 3 of these evoke significantly different gustatory responses. On the other hand, there are 3 acid pairs—acetic-glutaric, crotonic-tricarballylic and isobutyric-acrylic—where secretion rates are almost identical but concentrations are different (Table V).

Table X indicates that in all structural categories both hydrogen ion concentration and anion structures are the governing factors. For the monocarboxylic acids, (H^+) concentrations are almost identical, but increasing carbon chain length decreases acid effectiveness. In the dicarboxylic acid series —oxalic through glutaric—(H^+) concentration is important, but is not the sole governing factor. Even though (H^+) concentration of the oxalic acid solution is more than 10-fold greater than the glutaric acid solution there is only slightly more than a 2-fold difference in secretion rate, instead of the marked increase which would be obtained if a single acid were employed. Similar results are noted when acetic and oxalic acids, propionic and pyruvic acids, and succinic and tartaric acids are compared. This again would seem to indicate that both structural configuration of the anion, and hydrogen ion concentration are the prime factors responsible for stimulation of chemoreceptors.

Summary. Seventeen organic acids were used to evaluate the effect of (H^+) concentration, total acid concentration, undissociated acid concentration, carbon chain length, polar groupings, and carbon to carbon double bonds on the stimulatory action of acids on human gustatory chemoreceptors. Parotid gland secretion rate was used to measure human response to the stimuli. Both hydrogen ion concentration and the chemical configuration of the anion were found to control the degree of response elicited.

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Bifidus Factor 2 for Growth of *Lactobacillus bifidus*. (28211)

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Lactobacillus bifidus, since its description by Tissier in 1899, has been studied in many laboratories. Raynaud(1) described a factor which is essential for the growth of *Lactobacillus bifidus in vitro* and which stimulates its appearance *in vivo*. This factor he calls bifidus factor 2 to distinguish it from the N-acetylglucosamine-containing substances required by *Lactobacillus bifidus* var. *pennsylvanicus*, György *et al.*(2), which he calls bifidus factor 1. Bifidus factor 2 appeared to be a peptide but differed from strepogenin or the "supplementary" factor of György and Rose(3). Through the kindness of Professor Raynaud, his strain, which has been maintained continuously since its isolation by Tissier in 1901, was made available to us so that we might compare its growth requirements with those of our strains under our laboratory conditions.

Materials and methods. The general techniques for carrying the cultures of *Lactobacillus bifidus* and for microbiological assay have been described(2,3). Our strains have been maintained for many years on a semisynthetic medium containing enzymatically hydrolyzed casein (NZCase, Sheffield). Initial trials indicated that this medium would not support growth of the "Tissier" strain of *L. bifidus* received from Professor Raynaud. In Raynaud's laboratory *L. bifidus* is maintained on Blaurock medium(1). With Blaurock medium we also obtained excellent growth of the strain. Morphologically it was typical *L. bifidus*. In carrying out microbiological assays for bifidus factor 2, two media were used: our regular NZCase medium, with modifications indicated by the requirements of the Tissier strain, and the "Garches" medium of Raynaud(1) which we will refer to as "Raynaud" medium. The latter is simpler in constitution than the NZCase medium. As a major source of nitrogen it contains a highly purified casein hydrolyzate, vitamin-free Casitone (Difco), since Raynaud found that almost all other casein preparations, including NZCase peptone, contained enough bifidus factor 2 to make them unsuitable for use in an assay medium. Several of the substances found to be active as bifidus factor 2 were analyzed for peptide and purine constitution by means of paper chromatography. Butanol-acetic acid was used as solvent system: peptides were detected by means of ninhydrin, purine bases with bromphenol blue-silver nitrate(4).

Results. Neither the NZCase medium nor the Raynaud medium alone supported growth of the Tissier strain of *L. bifidus*. With Raynaud medium a small amount of acid was produced after 48 to 72 hours incubation but it never exceeded about 2 ml of 0.1 N acid per 10 ml tube, even with a heavy inoculum. When this medium was supplemented with the crude amylase preparation from *Aspergillus oryzae* which Raynaud had found to be particularly active as bifidus factor 2 or with tryptose (Difco) there was growth in proportion to the amount of supplement added up to about 6 ml of 0.1 N acid after 48 to 72 hours incubation. NZCase peptone had much smaller activity as bifidus factor 2. NZCase medium would not support growth even when supplemented with amylase or tryptose. Additional supplementation with those components of the Raynaud medium which were not present in NZCase medium likewise failed to make it adequate for maintenance of the Tissier strain.

Among the components of the NZCase