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RATE OF FLOW AND PH OF PAROTID FLUID  
STIMULATED BY VARIOUS COMBINATIONS OF  
MECHANICAL AND CHEMICAL ELEMENTS

by

Raymond J. Bielinski, D.D.S.

A Thesis Submitted to the Faculty of the Graduate  
School of Loyola University of Chicago in Partial  
Fulfillment of the Requirements for the Degree of  
Master of Science

MAY

1984

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DEDICATION

To my parents.

## VITA

Raymond John Bielinski was born in Evanston, Illinois on December 13, 1953, to Dr. Raymond R. and Margaret M. Bielinski. He attended National College of Education, Clara Bell Baker Demonstration grade school in Wilmette, Illinois and attended high school at Loyola Academy in Wilmette, Illinois from which he received his diploma in 1972. He attended John Carroll University in Cleveland, Ohio, Lake Forest College, in Lake Forest, Illinois and Illinois Benedictine College, in Lisle, Illinois, where he received a Bachelor of Science degree in 1977.

He then enrolled at Loyola University of Chicago, School of Dentistry, where he began graduate study toward the degree of Master of Science in Oral Biology. Recently, Mr. Bielinski has received his doctorate in dental surgery from the Loyola University School of Dentistry.

## TABLE OF CONTENTS

|   | Page |
|---|------|
| ACKNOWLEDGMENTS . . . . .                                   | ii   |
| DEDICATION . . . . .  | iii  |
| VITA . . . . .  | iv   |
| LIST OF TABLES . . . . .                                    | vi   |
| LIST OF FIGURES. . . . .                                    | vii  |
| CONTENTS OF APPENDICES . . . . .                            | viii |
| <br>Chapter   |      |
| I. INTRODUCTION . . . . .                                   | 1    |
| II. REVIEW OF RELATED LITERATURE . . . . .                  | 3    |
| Secretion of Saliva . . . . .                               | 3    |
| Hydrogen Ion in the Parotid Gland Fluid . . . . .           | 5    |
| Bicarbonate in the Parotid Gland . . . . .                  | 5    |
| Mechanism for Salivary Secretion . . . . .                  | 9    |
| Collecting of Parotid Secretions . . . . .                  | 10   |
| Saliva Flow Relationship to Caries . . . . .                | 17   |
| Flow Rate Experiments . . . . .                             | 17   |
| Salivary Constituents Involved in Limiting Caries . . . . . | 19   |
| III. METHODS AND MATERIALS . . . . .                        | 23   |
| Subjects . . . . .  | 23   |
| Materials . . . . .   | 23   |
| Procedure . . . . .   | 24   |
| IV. RESULTS . . . . .                                       | 27   |
| V. DISCUSSION . . . . .                                     | 38   |
| VI. SUMMARY AND CONCLUSION . . . . .                        | 51   |
| BIBLIOGRAPHY . . . . .                                      | 52   |
| APPENDIX A . . . . .  | 56   |

LIST OF TABLES

| Table   | Page |
|---|------|
| 1. Relationship between Salivary Characteristics and Caries . .   | 21   |
| 2. Mean pH Values from Stimulated Parotid Secretion as a<br>Function of Citric Acid Concentration in Chewing Gum . . . .        | 29   |
| 3. Mean Flow Rate Measurements of Stimulated Parotid Saliva as<br>a Function of Citric Acid Concentration in Chewing Gum . . .  | 30   |
| 4. Mean Carbon Dioxide Values from Stimulated Parotid Secretion<br>as a Function of Citric Acid Concentration in Chewing Gum .  | 31   |
| 5. Mean Bicarbonate Values from Stimulated Parotid Secretion as<br>a Function of Citric Acid Concentration in Chewing Gum . . . | 32   |
| 6. One-Way Analysis of Variance and Scheffe Multiple<br>Comparison Procedure . . . . .  | 33   |
| 7. Parotid Secretion Data . . . . .   | 57   |

LIST OF FIGURES

| Figure  | Page |
|---|------|
| 1. Relationship of Various Ions in Parotid Fluid . . . . .  | 6    |
| 2. Cannula for Collecting Parotid Saliva in Man . . . . .   | 11   |
| 3. Self-Positioning Cannula #1 . . . . .                    | 13   |
| 4. Self-Positioning Cannula #2 . . . . .                    | 15   |
| 5. Distribution of pH Values . . . . .                      | 40   |
| 6. Rates of Flow for Stimulated Parotid Secretion . . . . . | 42   |
| 7. Distribution of Carbon Dioxide Pressures . . . . .       | 44   |
| 8. Distribution of Bicarbonate Concentrations . . . . .     | 47   |



CONTENTS OF APPENDICES

|                                  | Page |
|----------------------------------|------|
| APPENDIX A . . . . .             | 56   |
| Parotid Secretion Data . . . . . | 57   |

## CHAPTER I

### INTRODUCTION

This research was undertaken to assess secretions of the salivary glands, principally the parotid organ, with regard to composition and the role played as an integral part of host resistance against the challenge of dental caries. The pure cannulated fluid from stimulated human parotid glands was the material investigated.

One of the fundamental functions of saliva, in man, is the protection of tissues, hard and soft, involved in mastication. If the flow of saliva ceases for extended periods of time, immediate degenerative changes (severe tooth decay, for example) occur in the oral tissues (Allington, 1950, Faber, 1943 and Prinz, 1932). Protection provided by saliva results from the washing and demulcent actions of the fluid, from certain chemical effects of the dissolved salts and proteinaceous substances (Allington, 1950).

Of particular interest to this researcher is the fact that increases of salivary flow rate result in an elevation of pH and buffer capacity (Allington, 1950). Studies of buffer capacity by Spellman (Spellman, 1936) and Leung, (Leung, 1951) displayed the bicarbonate buffer system in saliva as uniquely important. Leung estimated that about 85% of the total buffer capacity of saliva is provided by the bicarbonate system (Leung, 1951). Thus the salivary bicarbonate content

(buffering capacity) should be directly proportional to the rate of flow of gland fluid.

In order to obtain parotid secretory material, the parotid gland was stimulated by chewing gum containing varying concentrations of citric acid. The hypothesis tested is whether there is a relationship between chewing gums, containing varying amounts of citric acid and a change in the parotid fluid constituents.

Specifically, the hypothesis to be tested is that there is no significant difference between the various gums identified by citric acid concentrations, for each of the following parameters of parotid saliva; pH, rate of flow,  $p\text{CO}_2$ , and  $[\text{HCO}_3^-]$ .

The parotid elements observed directly in order to reach specific goals were the salivary pH, the volume secreted and the partial pressure of carbon dioxide ( $p\text{CO}_2$ ) observed in the fluid. Calculations of the buffer capacity were made from observed pH and  $p\text{CO}_2$  values using the Henderson-Hasselbalch equation. The ability of the parotid gland to respond to the weak acid stimulants and produce bicarbonate buffer was considered a function of  $p\text{CO}_2$  and pH, the two values being proportional to the carbonic acid: bicarbonate buffer pair, respectively. The significance of the buffering capacity of the parotid is that it can neutralize certain acidic oral conditions which predispose the host to infection, i.e., caries and periodontal disease.

## CHAPTER II

### REVIEW OF RELATED LITERATURE

Salivary secretions are involved in many physiological functions; particularly, oral fluids facilitate swallowing and digesting of food along with bathing of the teeth. Saliva is a fluid secreted by three pairs of salivary glands, the parotid, submaxillary and sublingual assisted by a network of minor glands in the oral cavity (Allington, 1950).

Research involving the salivary glands and their secretions has revealed in recent years complex variability. A major importance of these glands and their substituents is maintenance of oral health. Loss of function of the major salivary glands leads to complex oral and dental disease.

This review deals with some of the research on the parotid gland, its components, its relationship to mastication and dental caries development.

### SECRETION OF SALIVA

The principal glands of salivation are the parotid, submaxillary and sublingual glands. In addition, there are many small buccal-mucosal glands. The daily volume of saliva secreted is about 1000 - 1500 ml (Schneyer, 1974).

Mixed whole saliva contains two different types of fluid:

1. Serous secretion contains ptyalin, for starch digestion.

This secretion comes from the parotid glands in many individuals. In others, amylase activity is associated with the submandibular gland secretions.

2. Mucous secretions are produced by the submaxillary glands, the sublingual glands and mucosal-buccal glands. The mucous is utilized in lubrication of intraoral surfaces (Brawley, 1935). Additionally, the glycoproteins of mucous secretions form a thin film on teeth (pellicle) that appears to play a role in adherence of plaque.

The most abundant constituent of saliva is water. A variety of components, including proteins are present. The concentrations of inorganic and organic components of saliva are generally lower compared to plasma (Baxter, 1929).

The total mixed saliva is a potpourri of secretions from the three major gland pairs, the many mucosal glands and epithelial and bacterial debris. On the other hand, pure parotid gland secretion, when collected without frothing or clotting, is clear. Some of its major cations are potassium, sodium, calcium, magnesium and hydrogen. Important anions are chloride, phosphate, iodide, fluoride and bicarbonate (Hildes et al., 1955). Since pH and bicarbonate ions are of concern in this research, hydrogen ion and bicarbonate will be discussed.

### HYDROGEN ION IN THE PAROTID GLAND FLUID

Saliva collected under conditions of minimal stimulation, generally has a higher concentration of hydrogen ions than blood. The pH of resting parotid saliva is relatively low, about 6.0 (Hildes et al., 1955 and Schmidt-Nielson, 1946). When parotid salivary flow is elevated by reflex stimulation, the pH increases to as much as 7.8 (Burgen et al., 1959, Shannon et al., 1960 and Yoshimura et al., 1954). This change in hydrogen concentration relate mainly to the ionization status of carbonic acid in the fluid. In the later case the  $[\text{HCO}_3^-]$  predominates.

### BICARBONATE IN THE PAROTID GLAND

Bicarbonate is in high enough concentration in saliva to contribute significantly to the total osmolality and buffer capacity of the secretions.

At very low flow rates bicarbonate concentration is characteristically near 5 mEq/liter in parotid secretion. As flow rate increases  $[\text{HCO}_3^-]$  of parotid saliva increases rapidly to a plateau which is reached at a flow rate of about one third the maximal value (Hildes et al., 1955). The  $[\text{HCO}_3^-]$  at this plateau is between 40 and 60 mEq/liter. In venous plasma the  $[\text{HCO}_3^-]$  is 27 mEq/liter (Thaysen et al., 1954). Thus the bicarbonate concentrations of saliva may exceed the plasma level. See figure 1.

As the secretory rate of flow is increased both the pH and bicarbonate concentrations increase. The bicarbonate concentration may raise to 60 to 80 mEq/liter when the flow rate is beyond half maximum values (Yoshimura et al., 1959). Since  $\text{pCO}_2$  and pH are related, they are assayed for effect using the Hendersen-Hasselbalch equation. In this

FIGURE 1. Relationship of Various Ions in Parotid Fluid

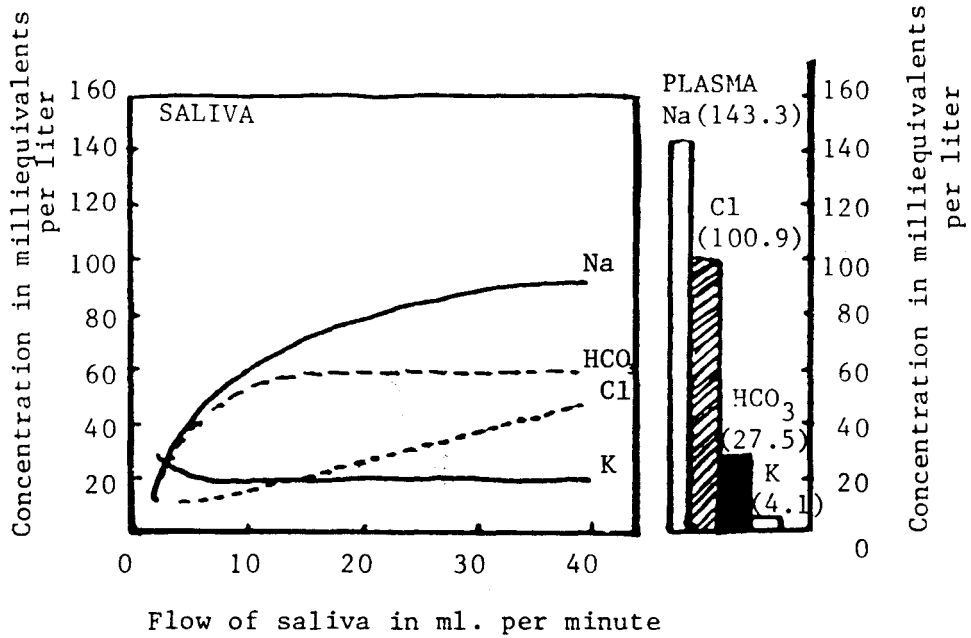


FIG. 12. Relationship between concentrations of sodium, potassium, chloride, and bicarbonate in human parotid saliva and rate of salivary flow. Plasma concentrations of the ions are also shown. (From Thaysen et al. 1954)



formula,

$$\text{pH} = \text{pKa} + \log \frac{[\text{HCO}_3^-]}{[\text{CO}_2]}$$

where pK is the negative log of the apparent dissociation constant for the acid, a value of 6.2 for saliva (Yoshimura et al., 1954). and CO<sub>2</sub> includes that in the form of H<sub>2</sub>CO<sub>3</sub>, which is proportional to the CO<sub>2</sub> tension. This value is usually 30 - 60 mmHg (Henderson et al., 1919, Yoshimura et al., 1959 and Henriques et al., 1958) and varies little with the salivary flow rate.

Thus, if salivary [H+] is primarily governed by the bicarbonate system then, changes in [H+] should be accompanied by reciprocal changes in [HCO<sub>3</sub><sup>-</sup>]. This relationship was established for total mixed and parotid saliva for man by Yoshimura and his colleagues (Yoshimura et al., 1959).

Studies of buffer capacity by Sellman (Sellman, 1936) also implicate the bicarbonate buffer system as the most important in saliva. Leung reported that about 85% of the total buffer capacity of saliva is provided by the bicarbonate system (Leung, 1951).

Salivary bicarbonate is derived from two sources, the metabolic activity in the gland and the plasma (Leung, 1951). The relationship of salivary bicarbonate to plasma is not clear, and there appears to be much debate in the literature as to the existence of a sole salivary gland source. However, a dual source (synthesis) does help to explain why salivary bicarbonate can exceed plasma bicarbonate levels.

### MECHANISM FOR SALIVARY SECRETION

Saliva is produced by the serous cells of the parotid glands, the mucous and serous cells of the submaxillary glands and sublingual gland, and numerous mucous cells in the labial, buccal, and palatal regions. Some of the saliva produced is the result of spontaneous secretion, that is, it is not the result of extrinsic stimuli and does not disappear on decentralization, but rather appears because of the intrinsic properties of a cell or gland.

The control of oral glandular secretions, both salivary and mucous glands, is regulated by the autonomic nervous system. Secretion is activated by reflex stimulation, which can be mechanical or of psychic nature. Both peripheral stimuli, transmitted via afferent nerve pathways from the oral cavity and psychic stimuli from other sensory centers (taste, smell, and sight) converge on the salivary nuclei in the medulla oblongata. The efferent pathway is via the parasympathetic and sympathetic parts of the autonomic nervous system. Main control of the salivary glands is exerted through the parasympathetic nerves which innervate them. In man, maximum secretion of saliva occurs when the parasympathetic fibers in the chorda tympani are stimulated. Increases or decreases in the rate of parasympathetic stimuli decrease the release of saliva. The saliva produced by parasympathetic stimulation is characteristically watery (serous) with a high concentration of amylase and a relatively low concentration of mucin. Associated with the secretion are increase of oxygen consumption and some nonpropagated changes in the transmembrane.

The final composition of saliva is a function of flow and, there-

fore, a function of both sympathetic and parasympathetic neural discharge to the gland. Therefore, salivary flow is initially determined by the individuals specific anatomy, which is determined by the genetics of that person.

#### COLLECTING OF PAROTID SECRETIONS

Early studies of human parotid gland secretions were made by Misterlich in 1832 who was studying a patient with a parotid fistula (Misterlich 1965). Some of the earliest cannulation work was performed in 1860 by Ordenstein. The early cannula had to be hand-held which severely limited masticatory movements. He reported that saliva tended to leak out of the cannula and that it was difficult to keep it in place.

In 1910, Carlson and Crittenden invented a collecting device that overcame these problems. It was held in place by vacuum and allowed parotid fluid to flow freely through the outlet tube under virually physiologic conditions (See Figure 2). Good stability was achieved by tooling the suction ring at a 60 degree angle to the surface of the oral mucosa.

Figures 3 and 4 show a collecting device developed which enables a subject to collect his own samples in an isolated state over prolonged periods of time. This device utilizes a bite block which when the subject fits his teeth into this block the central chamber of the suction cap automaticlly falls into position over the duct orifice, The cap is sealed to the buccal mucosa by squeezing and releasing the suction bulb, creating a negative pressure holding the collector to the mucosa (Shannon et al., 1965).

FIGURE 2. Cannula for Collecting Parotid Saliva in Man

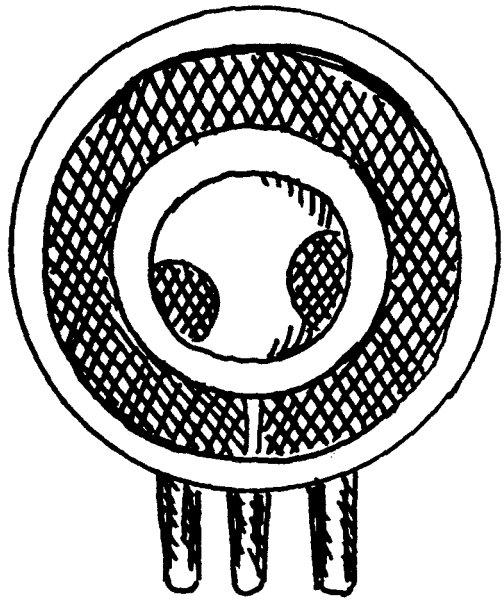
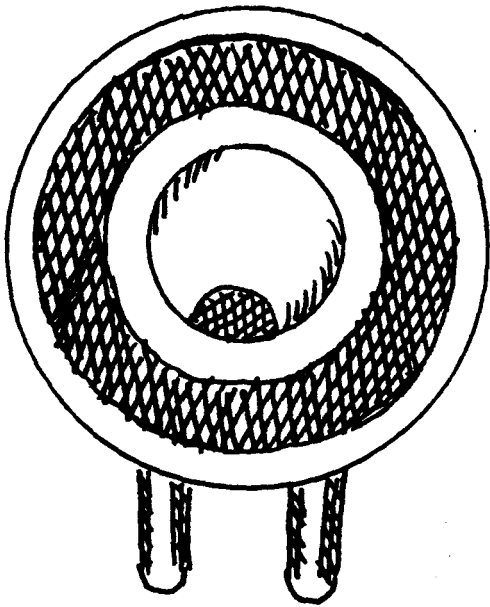


FIGURE 3. Self-Positioning Cannula #1

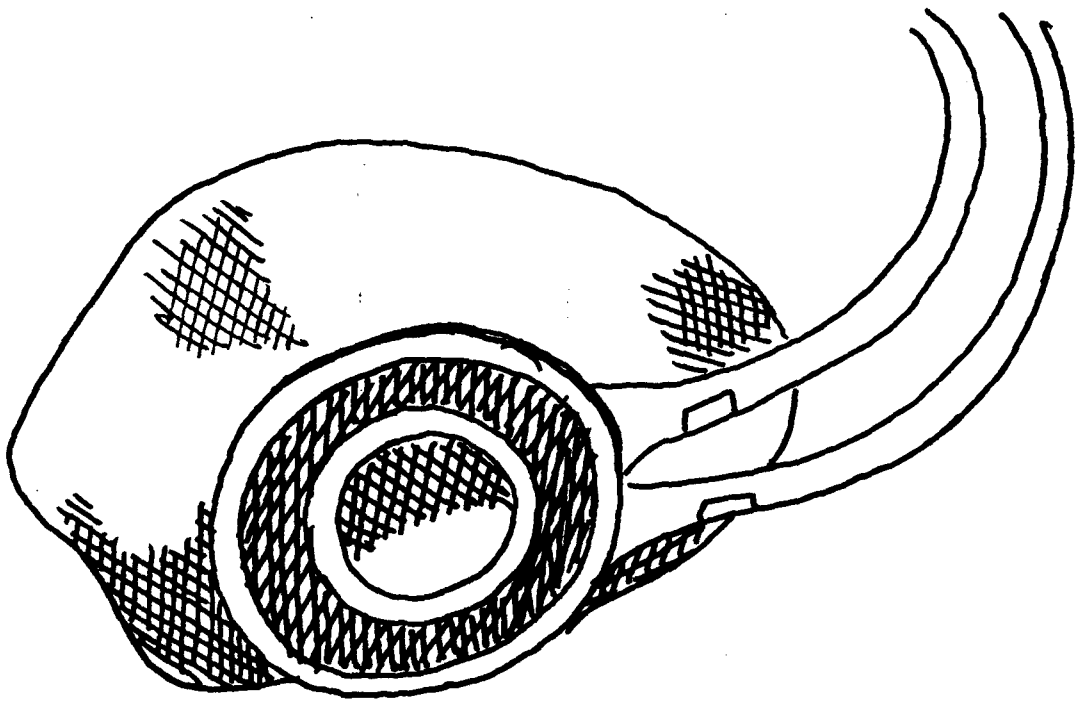
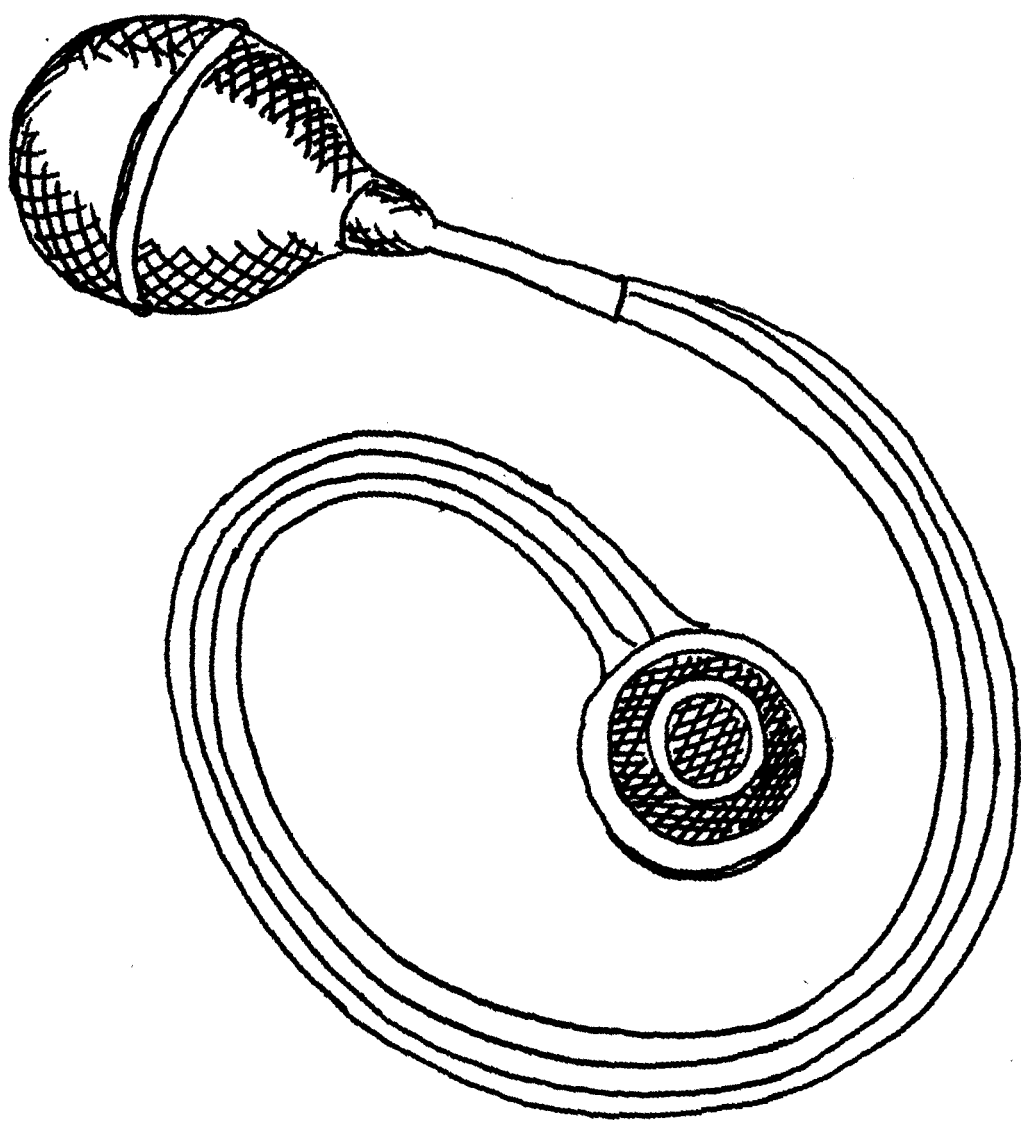


FIGURE 4. Self-Positioning Cannula #2





### SALIVA FLOW RELATIONSHIP TO CARIES

The relationship between salivary flow rate and dental caries activity has long been the subject of controversy. In animal experiments, gland removal and duct ligation have generally increased caries activity. In many human subjects with greatly impaired or completely absent salivary secretions, caries rampancy has usually been noted (Shannon et al., 1965).

### FLOW RATE EXPERIMENTS

Dr. Carl Rose, of Dresden, reported in 1908 that children drinking extremely hard water were more resistant to caries and were able to secrete more than three times as much saliva as those who were drinking soft water (Black, 1909). Early researchers studying paraffin-stimulated whole saliva reported contradictory results. Hewalt sampled 20 children and concluded a high rate of flow was associated with a high degree of immunity to caries (Hewalt, 1932). Hubbell and Bunting studied 75 children and found only a slight tendency towards greater flow rate in caries-free persons and found no satisfactory correlation between rate of flow and tooth preservation (Hubbell et al., 1932). McDonald, in 1950, studying 68 children, reported a statistically significant inverse correlation between DMF surfaces and saliva secretion rate (McDonald, 1950). Supporting these findings, Rovelstad and his colleagues in 1959, collected whole saliva samples from 1049 naval recruits and noted a significant inverse relationship between DMF values and flow rate (Rovelstad et al., 1958). H.C. Trimble concluded that the lower the secretion rate, the more frequent the incidence of new carious lesions (Trimble et

al., 1938). Cushman and his colleagues, in two studies, each with only 21 subjects, concluded that, while there was a trend that indicated a higher flow rate with reduced caries activity, there was no diagnostic importance, for the flow rates indicated other factors were at play other than simple flow rate (Cushman et al., 1940).

Contrary to previous findings, Becks, using nonparaffin stimulants reported that in 198 subjects, no statistically significant differences in flow rate could be detected that related to caries activity (Becks et al., 1941). To add to the confusion, Barany, in 1947, with 162 subjects, found that although there was evidence of an inverse relationship between caries and flow rate, it was not clearly established that individuals with little caries produced an increased quantity of saliva (Barney, 1947). Hathje, utilizing 100 subjects, found a higher rate of secretion in caries-resistant subjects (Hathje, 1951). However, the trend towards higher secretion rates in a caries resistant groups was insignificant according to Ericson (Ericson et al., 1953). Finally, Shannon found no significant difference in whole saliva flow for 537 subjects in three caries status groups (Shannon, 1958).

#### PAROTID FLUID RATE STUDIES

Parotid fluid secretion rate studies also show inconsistencies from one research team to the next.

Using chewing gum stimulation, Englander, et al, stimulated parotid gland secretions, and for 83 subjects concluded no significant differences in flow rate between caries-free and caries-rampant subjects (Englander et al., 1953).

Carter and his colleagues used chewing gum stimulation to collect

parotid fluid from 31 caries-free and 50 caries-rampant naval recruits. It was found that despite extreme dental differences, the mean parotid flow rates were the same (Carter et al., 1958). Weber, Weber and Mancinelli, with samples of chewing gum stimulated bilateral parotid gland fluid collections, found that caries immune subjects had a higher rate of parotid flow. However, these findings also indicated a considerable overlap existing between the immune group and the caries susceptible group (Weber, 1960 and Weber et al., 1961). Specht, in 1961, collecting parotid fluid with chewing gum stimulation from 116 children, found no correlation between flow rate and DMF teeth (Specht, 1961). Shannon and Terry, in 1965, using 3,786 subjects, found that there was a statistically significant correlation between DMFS values and flow rate. However, according to Shannon, "the high incidence of flow rate values among DMFS groups made it clear that the caries status of an individual could not be reliably predicted by this means" (Shannon, 1958).

#### SALIVA CONSTITUENTS INVOLVED IN LIMITING CARIES ACTIVITY

The preceding findings have led to the conclusion that saliva, in some ways, is necessary for maintaining the integrity of the teeth. Identification of some of the specific constituents in saliva, which might be responsible for limiting caries attack, have been difficult to determine. The findings have been, to date, contradictory with some investigators claiming a relationship between caries prevalence and salivary amylase, urea, ammonia, calcium, phosphate, pH, etc., and other finding no relationship (Leung, 1962). The main problem with saliva composition studies is that it will vary with flow rate, nature of stimulation, duration of stimulation, plasma composition, the time of day and even

previous stimulation.

As stated by Ernest Newbrun, Professor, Department of Oral Biology, University of California Red Center, "reviewing currently available information, there is no consistent relationship between dental caries prevalence and salivary amylase, ammonia, urea, calcium, phosphate, pH, or viscosity" (Newbrun, 1972). (See Table 1)

There is some evidence, however, for an inverse relationship between salivary flow rate and caries, as mentioned in some of the preceding reviews. The flow rate itself influences the salivary  $[Na^+]/[HCO_2^-]$  ratio and at higher flow rates there is an increased buffer capacity. The bicarbonate buffer is superior because it can buffer rapidly. Also, its pKa is close to that of plaque and with increased saliva flow rate the bicarbonate concentration increases.

Plaque microorganisms can convert urea, which is continuously secreted in saliva, to non-urea nitrogenous products and ammonia which can also serve as a buffer. On the other hand, salivary proteins can be disregarded as buffers because with the dialysis of saliva, which removes both phosphate and buffer but not protein, there is a total loss of buffer capacity.

Additional evidence of the importance of the buffering capacity of saliva has been found from studies of the pH of carious lesions and dental plaque. A pH gradient exists in the carious lesion, the deep advancing edges being more acidic and the shallower layers closer to the pH of saliva. In enlarged and exposed cavities, empty of their contents, the carious layer is shallower and more alkaline probably due to better access to saliva (Dikersen et al., 1963).

TABLE 1

## Relationship Between Salivary Characteristics and Caries

| Property        | Relationship | Property                      | Relationship |
|-----------------|--------------|-------------------------------|--------------|
| Flow rate       | +/-          | pH                            | -            |
|                 |              | Ca <sup>+</sup>               | -            |
| Buffer Capacity | +            | PO <sub>4</sub> <sup>-2</sup> | -            |
|                 |              | NH <sub>3</sub> <sup>-</sup>  | -            |
|                 |              | Amylase                       |              |
|                 |              | Viscosity                     | -            |
|                 |              | Urea                          | -            |

Positive = +

Some Relation = +/-

No Relation = -

The role of buffers in establishing the pH of saliva is further involved in providing a suitable microenvironment for the development of specific microflora (Chatterjee et al., 1978).

It has been shown that addition of whole saliva to the mixed oral bacteria results in glycolysis stimulation with a subsequent rise in pH (Kanapka et al., 1978). In attempting to isolate the specific component responsible for this effect, Kanapka and Kleinberg (1978) have demonstrated that arginine peptides, a naturally occurring salivary protein, could duplicate both the glycolysis enhancement and the subsequent rise in pH. Free arginine could only result in an increase in pH (Kanapka et al., 1978). It was found that lysine peptides were much less effective than arginine peptides in increasing pH but are much more readily available than is free lysine for the growth and metabolism of the oral bacteria (Kleinberg et al., 1978).

#### SUMMARY OF LITERATURE PROGRESS

Currently, while specific salivary proteins are being investigated a vast variety of other interrelated factors will probably have to come to the forefront before the mechanism of interaction is clearly resolved.

Thus, it can be seen that while investigators are showing continuous progress towards a saliva-carries inter-relationship, more research is needed.

One direction, not yet seen by this researcher, regards the genetic factor of salivary gland responses to stimuli and the possible use of responses in terms of buffering capacity as a correlate of host resistance to cariogenic challenge.

## CHAPTER III

### METHODS AND MATERIALS

#### SUBJECTS

Ten normal, healthy subjects, 7 males, 3 females were selected from a pool of young adults. All were in good oral health free of rampant caries or severe periodontal disease. The ages were from twenty-two to twenty-nine.

#### MATERIALS

The following is a list of all the materials utilized in this research.

1. One Carlson indirect cannulation cup, made of acrylic plexiglas, machined by Hoffman-LaRoche Laboratories, Basel, Switzerland.
2. Two, 12 cm. lengths of Tygon tubing, ID 1.5 mm.
3. One, 10 ml. insufflator bulb.
4. Mineral oil, reagent grade.
5. Stopwatch, Premier, 7 jewel, 1/10.
6. 30 pieces of Wrigley's gum base.
7. 120 pieces of chewing gum each weighing 3.2 grams and having



25% gum base by weight. There were four types of gum.

| # of pieces | gum type | mg. citric acid | mg. sorbitol |
|-------------|----------|-----------------|--------------|
| 30          | P        | 0               | 50           |
| 30          | N        | 32              | 50           |
| 30          | L        | 96              | 50           |
| 30          | M        | 224             | 50           |

8. One serological, B-D, Luer-Lok Tip, 3 cc. syringe.
9. One modified, blunt end, B-D plastic Luer-Lok Hub and needle.
10. One pH, blood gas analyzer Instrumentation Laboratory, Inc, Model 813.
11. 2 x 2 sterile swabs
12. 1000 ml. beaker
13. crushed ice, 600 ml.

#### PROCEDURE

Each subject was seated in a dental chair and placed into the supine position. The suction bulb of the indirect cannulating apparatus was given to the subject and he was instructed to squeeze its bulb firmly when directed by the investigator and then to release the bulb gently when instructed. This technique allowed the investigator to position the Carlson cup intraorally, as required. The orifice of the Stenson duct (parotid gland) was located and inspected for possible pathology. If

normal, the buccal mucosal surface was gently blotted dry to insure that the cup apparatus would adhere firmly.

The Carlson cup was placed on the left buccal mucosa so that the papilla was completely contained in the central chamber of the cup. The thumb reinforced the outside of the cheek. As the investigator placed the cup, the subject was asked to squeeze and then release the bulb, thus forming a suction in the outer ring of the cup causing it to adhere to the inside of the cheek.

Once the cup was secured, the subject was asked to close his mouth and move the mandible a few times to insure that the cup had not slipped out of place. The gum was then placed on the opposite side of the mouth and the subject was instructed to chew at a fixed stroke rate over a three minute period. After several pre-experiment collections, each subject became familiar with the device and procedure.

The saliva flowed down the collecting tube. The first two drops of saliva were discarded. The investigator recorded the volume of the collected fluid and the duration.

The saliva was collected under mineral oil in a chilled test tube. This procedure prevented the loss of  $\text{CO}_2$  gas from the fluid. After the three minute collection, the cup was removed carefully so as not to traumatize the papilla. This was accomplished by removing the suction bulb from the negative pressure tube first, then removal of the cup.

The pure parotid fluid was maintained in an ice bath to reduce enzymatic degradation of proteins. The volume was recorded and the rate (ml/min) was noted. The saliva, under oil, was withdrawn in a syringe from the bottom of the tube leaving the oil layer in the tube. The vol-

ume of saliva was measured in the syringe and then quickly transferred to the air-free chamber of the blood gas analyzer. The needle was removed from the syringe and the evacuator tip of the pH Blood Gas Analyzer 813 was placed into the syringe. The pH blood gas analyzer utilizes a 0.5 ml. sample to measure pH,  $pCO_2$ .

Descriptive statistics that were generated, i.e., mean and standard deviation, helped guide the further analysis of variance. It was noted that while collecting samples stimulating with the base gum that secretions for more than 3 minutes were required to obtain an adequate volume of saliva to introduce into the blood gas analyzer. This data could not be utilized in an analysis of variance however it still demonstrated a base line indicator that could be referred to as a point of reference.

## CHAPTER IV

### RESULTS

Samples of pure parotid secretions in an ice bath and under mineral oil were analyzed for pH and  $p\text{CO}_2$ . Then  $[\text{HCO}_3^-]$  values were calculated based on the two measured chemical properties. Table 6 of the appendix titled "Analysis of Parotid Secretion After Stimulation by Various Citric Acid Gums," presents the raw data which are: pH,  $p\text{CO}_2$ ,  $[\text{HCO}_3^-]$ , duration of cannulation, rate of flow and the date of collection for each type of gum for each of the three trials per gum type. Gum type is presented as per the increase in concentration of citric acid.

Tables 2 through 5 present the mean and standard deviation values for pH,  $p\text{CO}_2$ ,  $[\text{HCO}_3^-]$  and the rate of flow as a function of citric acid concentration in each of four sweetened experimental chewing gums and one unsweetened gum base as a control.

Table 2 demonstrates that the means of the pH measurements increase with the order of increasing citric acid concentration. Mean pH values increased when comparing sorbitol (0 mg. citric acid) to base gum.

Table 3 demonstrates that the means of the rates of flow increases with the order of increasing citric acid concentration. Mean rates of flow values increased when comparing sorbitol (0 mg. citric acid) to the base gum.

Table 4 demonstrates that the means of the  $p\text{CO}_2$  increases with the order of increasing citric acid concentration. Mean  $p\text{CO}_2$  values increased when comparing sorbitol (0 mg. citric acid) to base gum.

Table 5 demonstrates that the means of the  $[\text{HCO}_3^-]$  increases with increasing citric acid concentration. Mean  $[\text{HCO}_3^-]$  values increased when comparing sorbitol (0 mg. citric acid) to base gum.

Table 6 shows the mean and standard deviation as a function of citric acid concentration for all subjects, thus  $n = 30$ . The means of all parameters increased as citric acid concentration increased. Sorbitol also increased the compared to the Base.

In Table 6 the first column presents the mean value for each parameter for all the gum types. Columns 2 - 6 presents a list of  $f$  distribution scores that were derived from conducting the analysis of variance. The  $f$  distribution score serves as a statistic which enables one to accept or reject the null hypothesis. The null hypothesis was that the means for any given parameter for each gum type would not differ from one another. In other words, null hypothesis =  $X_{\text{base}} = X_{32\text{mg}} = X_{96\text{mg}} = X_{224\text{mg}}$ . All the  $f$  distribution scores that were presented were found statistically to be very highly significant at  $p < 0.001$ . Therefore the null hypothesis can be rejected as applies to each of the parameters.

The last column presents a list of  $r^2$  values x 100 to yield a percent amount of variance of the distribution of values. This was done for each parameter.

Table 2

Mean pH Values (+/- 1 SD) of Stimulated Parotid Saliva as a  
Function of Citric Acid Concentration in Chewing Gum

| Subject<br>(N=3) | GUM =>  | BASE* | P    | N     | L     | M      |      |      |      |      |
|------------------|---|-------|------|-------|-------|--------|------|------|------|------|
|                  | Citric Acid<br>Concentration<br>(mg/stick) ** | 0 mg  | 0 mg | 32 mg | 96 mg | 224 mg |      |      |      |      |
| 1                | 6.53  | 0.55  | 7.17 | 0.06  | 7.30  | 0.10   | 7.43 | 0.12 | 7.43 | 0.06 |
| 2                | 6.59  | 0.10  | 6.90 | 0.10  | 7.03  | 0.12   | 7.30 | 0.20 | 7.55 | 0.15 |
| 3                | 6.33  | 0.38  | 7.21 | 0.08  | 7.37  | 0.06   | 7.27 | 0.21 | 7.43 | 0.15 |
| 4                | 6.83  | 0.12  | 7.17 | 0.06  | 7.13  | 0.06   | 7.23 | 0.06 | 7.33 | 0.06 |
| 5                | 6.63  | 0.12  | 6.87 | 0.06  | 7.23  | 0.06   | 7.45 | 0.07 | 7.50 | 0.00 |
| 6                | 6.50  | 0.17  | 7.37 | 0.21  | 7.37  | 0.06   | 7.40 | 0.10 | 7.40 | 0.10 |
| 7                | 6.83  | 0.21  | 7.13 | 0.15  | 7.17  | 0.12   | 7.43 | 0.12 | 7.47 | 0.06 |
| 8                | 6.47  | 0.39  | 7.10 | 0.10  | 7.37  | 0.15   | 7.47 | 0.15 | 7.57 | 0.06 |
| 9                | 6.67  | 0.06  | 6.93 | 0.06  | 7.10  | ***    | 7.40 | 0.10 | 7.50 | 0.20 |
| 10               | 6.97  | 0.06  | 7.10 | 0.10  | 7.33  | 0.06   | 7.13 | 0.06 | 7.53 | 0.06 |

\* = unsweetened

\*\* = 3 gm stick of chewing gum

\*\*\* = for this subject N = 1

Table 3

Mean Flow Rate Measurements (+/- 1 SD) of Stimulated Parotid Saliva  
as a Function of Citric Acid Concentration in Chewing Gum

| Subject<br>(N=3) | GUM =>  | BASE* | P    |      | N     |       | L     |       | M      |        |
|------------------|---|-------|------|------|-------|-------|-------|-------|--------|--------|
|                  | Citric Acid<br>Concentration<br>(mg/stick) ** | 0 mg  | 0 mg | 0 mg | 32 mg | 32 mg | 96 mg | 96 mg | 224 mg | 224 mg |
| 1                | 0.10  | 0.05  | 0.57 | 0.21 | 1.01  | 0.22  | 1.18  | 0.28  | 1.23   | 0.43   |
| 2                | 0.09  | 0.04  | 0.40 | 0.06 | 0.51  | 0.02  | 0.55  | 0.06  | 1.14   | 0.10   |
| 3                | 0.09  | 0.06  | 0.44 | 0.02 | 0.74  | 0.07  | 0.74  | 0.25  | 1.33   | 0.06   |
| 4                | 0.07  | 0.02  | 0.32 | 0.07 | 0.56  | 0.05  | 0.64  | 0.04  | 0.04   | 0.20   |
| 5                | 0.05  | 0.05  | 0.76 | 0.98 | 0.74  | 0.13  | 1.28  | 0.78  | 1.50   | 0.39   |
| 6                | 0.10  | 0.01  | 0.88 | 0.41 | 0.94  | 0.25  | 1.54  | 0.16  | 2.40   | 0.52   |
| 7                | 0.08  | 0.01  | 0.81 | 0.15 | 1.60  | 0.52  | 1.89  | 0.97  | 2.66   | 0.32   |
| 8                | 0.07  | 0.02  | 0.38 | 0.14 | 0.74  | 0.23  | 1.22  | 0.55  | 1.90   | 0.09   |
| 9                | 0.12  | 0.04  | 0.36 | 0.06 | 0.33  | ***   | 1.39  | 0.41  | 1.93   | 0.84   |
| 10               | 0.10  | 0.02  | 0.81 | 0.15 | 1.18  | 0.20  | 1.28  | 0.14  | 1.64   | 0.08   |

\* = unsweetened

\*\* = 3 gm stick of chewing gum

\*\*\* = for this subject N = 1

Table 4

Mean Values (+/- 1 SD) for Stimulated Parotid Carbon Dioxide Pressure as a Function of Citric Acid Concentration in Chewing Gum

| Subject<br>(N=3) | GUM =>  | BASE* | P     |       | N     |       | L     |       | M      |        |
|------------------|---|-------|-------|-------|-------|-------|-------|-------|--------|--------|
|                  | Citric Acid<br>Concentration<br>(mg/stick) ** | 0 mg  | 0 mg  | 0 mg  | 32 mg | 32 mg | 96 mg | 96 mg | 224 mg | 224 mg |
| 1                | 28.50   | 6.42  | 38.87 | 6.33  | 44.80 | 3.82  | 36.90 | 10.74 | 44.43  | 5.28   |
| 2                | 20.93   | 2.14  | 41.77 | 3.70  | 44.87 | 7.41  | 44.03 | 12.32 | 43.13  | 3.56   |
| 3                | 20.93   | 2.67  | 39.80 | 2.95  | 40.93 | 6.62  | 38.67 | 6.65  | 46.97  | 7.98   |
| 4                | 28.43   | 1.25  | 35.63 | 11.30 | 41.13 | 2.61  | 41.53 | 2.82  | 40.00  | 2.03   |
| 5                | 16.50   | 2.76  | 26.20 | 8.62  | 37.37 | 4.65  | 41.95 | 2.33  | 41.03  | 0.23   |
| 6                | 26.00   | 1.93  | 40.83 | 6.00  | 44.50 | 7.08  | 43.87 | 13.53 | 48.03  | 10.91  |
| 7                | 30.27   | 3.40  | 44.73 | 9.63  | 31.33 | 4.23  | 37.73 | 10.22 | 40.97  | 0.59   |
| 8                | 21.87   | 3.45  | 36.27 | 4.93  | 32.00 | 14.18 | 45.73 | 5.95  | 44.30  | 4.45   |
| 9                | 25.43   | 3.84  | 32.27 | 7.35  | 37.80 | ***   | 38.33 | 0.98  | 42.77  | 2.01   |
| 10               | 26.60   | 2.36  | 33.47 | 3.56  | 41.33 | 3.45  | 46.97 | 3.78  | 48.70  | 4.35   |

\* = unsweetened

\*\* = 3 gm stick of chewing gum

\*\*\* = for this subject N = 1



Table 5

Mean Bicarbonate Values ( $\pm$  1 SD) from Stimulated Parotid  
Secretion as a Function of Citric Acid Concentration in Chewing Gum

| Subject<br>(N=3) | GUM =>  | BASE* | P     |      | N     |       | L     |       | M      |        |
|------------------|---|-------|-------|------|-------|-------|-------|-------|--------|--------|
|                  | Citric Acid<br>Concentration<br>(mg/stick) ** | 0 mg  | 0 mg  | 0 mg | 32 mg | 32 mg | 96 mg | 96 mg | 224 mg | 224 mg |
| 1                | 4.50  | 4.44  | 15.40 | 8.35 | 21.67 | 5.71  | 21.53 | 1.27  | 26.50  | 0.06   |
| 2                | 1.67  | 0.58  | 7.97  | 2.11 | 11.47 | 1.19  | 20.53 | 3.59  | 27.93  | 18.60  |
| 3                | 2.10  | 1.56  | 15.43 | 4.74 | 23.17 | 6.23  | 17.67 | 6.90  | 32.90  | 3.12   |
| 4                | 4.70  | 1.30  | 12.60 | 4.54 | 13.03 | 1.18  | 15.40 | 0.87  | 20.10  | 2.09   |
| 5                | 1.67  | 0.58  | 4.67  | 1.85 | 15.97 | 3.18  | 28.25 | 6.01  | 30.73  | 0.23   |
| 6                | 2.67  | 0.58  | 23.33 | 8.42 | 24.43 | 1.52  | 26.33 | 2.87  | 28.13  | 2.49   |
| 7                | 5.93  | 1.90  | 14.47 | 3.71 | 11.50 | 4.94  | 25.83 | 11.72 | 28.67  | 3.62   |
| 8                | 2.03  | 1.65  | 11.30 | 3.44 | 17.03 | 3.98  | 33.83 | 5.95  | 38.67  | 1.76   |
| 9                | 2.30  | 1.47  | 6.67  | 2.02 | 11.20 | ***   | 23.27 | 5.70  | 34.73  | 17.05  |
| 10               | 5.73  | 0.61  | 10.23 | 3.01 | 21.10 | 3.35  | 15.33 | 3.40  | 39.43  | 3.88   |

\* = unsweetened

\*\* = 3 gm stick of chewing gum

\*\*\* = for this subject N = 1

Table 6

## One-Way Analysis of Variance and Scheffe Multiple Comparison Procedure \*

| Parotid Secretion Parameters | Grand Mean | BASE**<br>0 mg | P     | N     | L     | M     | $f(3,113)$ | Explained Variance<br>( $\bar{r} \times 100$ ) |
|------------------------------|------------|----------------|-------|-------|-------|-------|------------|--|
| pH                           | 7.29       | 6.64           | 7.09  | 7.25  | 7.35  | 7.47  | 33.59      | 47.14%   |
| PCO                          | 40.58      | 24.55          | 36.98 | 39.74 | 41.56 | 44.03 | 5.24***    | 12.21%   |
| HCO                          | 20.81      | 3.33           | 12.21 | 17.48 | 22.61 | 44.03 | 33.39      | 46.99%   |
| Rate of Flow                 | 1.07       | 0.09           | 0.57  | 0.87  | 1.17  | 1.67  | 26.28      | 41.10%   |

\* = Scheffe multiple comparison tests were found to be significant at  $p < 0.05$ .

\*\* = Presented only for comparison--not included in one-way ANOVA design or Scheffe multiple comparison procedure.

\*\*\* = Barely significant at  $p < 0.05$  while other  $f$  distribution scores were found to be very highly significant at  $p < 0.001$ .

Example from Table 7:

|    | variance explained ( $\bar{r}^2 \times 100$ ) |
|----|---|
| pH | 71.4%   |

A great amount of variability (71.4%) in the distribution in pH values can be explained, in part, by the type of gum used.

The first step was to determine the existence of significant differences between the various gums for the measures of parotid secretion, i.e., pH,  $pCO_2$ ,  $[HCO_3^-]$ , and rate of flow. Very highly significant differences were found for all the measurements when an analysis of variance was conducted. The null hypothesis was able to be rejected at  $p < .001$ .

To define more clearly these differences a multiple comparison technique was employed. This technique, the Scheffe Test helps demonstrate which of the gums tested gave significantly different parotid secretion measurements. The Scheffe Test focuses on the overall significance level for the multiple comparisons and thus should be construed to be a conservative test. The Scheffe Test was conducted at the accepted alpha level of  $p < 0.05$ .

The Scheffe test (Table 6) conducted for the parameter pH demonstrated the existence of three gum type groupings. These are as follows:

- A) 224 mg. citric acid
- B) 96 mg. citric acid - 32 mg. citric acid
- C) Sweetner only

The Scheffe test (Table 6) conducted for the parameter  $pCO_2$  demonstrated the existence of two gum type groupings. These are as follows:

- A) 224 mg. citric acid - 96 mg. citric acid - 32 mg. citric acid
- B) 96 mg. citric acid - 32 mg. citric acid - sweetner only

The Scheffe test (Table 6) conducted for the parameter  $[HCO_3^-]$  demonstrated the existence of 3 gum type groupings. These are as follows:

- A) 224 mg. citric acid
- B) 96 mg. citric acid - 32 mg. citric acid
- C) 32 mg. citric acid - sweetener only

The Scheffe test (Table 6) conducted for the parameter Rate of Flow demonstrated the existence of 3 gum type groupings. These are as follows:

- A) 224 mg. citric acid
- B) 96 mg. citric acid - 32 mg. citric acid
- C) 32 mg. citric acid - sweetner only

Table 6 demonstrates which gum types can be grouped as similar and those that demonstrates significantly different characteristics. These groupings are indicated by the red bars under the mean value or a group of mean values. Thus this bar indicates that the gum groups can be considered similiar when referring to one of the parotid parameters tested (i.e., pH, Rate of Flow,  $pCO_2$ ,  $[HCO_3^-]$ .)

## EXPLANATION OF TABLE 7

Table 7 is located in the appendix. It presents all data collected. An explanation of the parameters investigated follows.

pH: The hydrogen ion concentration expressed as pH ranged from 6.0, with plain gum base, to 7.7 using gum "M" which contained the maximum concentration of citric acid (224 mg./stick). The pH values generally increased with a corresponding elevation in citric acid concentration.

pCO<sub>2</sub>: The pCO<sub>2</sub> values ranged from 18.8 to 56.4. PCO<sub>2</sub> values also increase generally as citric acid concentration of gum increases. Variation in pCO<sub>2</sub> values could reflect procedural experimental error due to loss of dissolved CO<sub>2</sub> to the air during the collection procedure.

[HCO<sub>3</sub><sup>-</sup>]: [HCO<sub>3</sub><sup>-</sup>] ranges from 0.3 to 5.35. The [HCO<sub>3</sub><sup>-</sup>] value are derived from the Henderson - Hasselbalch equation using the pKa of carbonic acid along with the observed pH and pCO<sub>2</sub> values. These values were automatically calculated by the pH blood gas analyzer, and thus have the specific values stated as mEq/l parotid fluid.

Duration: The times of collection ranged from 1 to 10 minutes. Duration is the time in minutes that the subject was cannulated. Duration was usually three minutes, but in some instances it was necessary to collect for a longer period of time in order to obtain a sample of sufficient volume to be utilized by the blood gas ana-

lyzer.

Volume: Volumes were recorded in ml to the tenth ml. Values ranged from 0.1 to 9.0 mls. This also reflects the same trend as pH,  $p\text{CO}_2$  and  $[\text{HCO}_3^-]$ .

Date: The date of all collections was recorded. All cannulations were done between the hours of 1 PM and 3:30 PM. This post-prandial collection time was utilized to avoid daily cyclic fluctuations in salivary flow, a common property of salivary secretion.

## CHAPTER V

### DISCUSSION

Table 2 presents the means and standard deviations of the pH values as a function of citric acid. Figure 5 illustrates the relationship of mean pH (hydrogen ion concentration) with the citric acid concentration (mg/3 gm stick of gum). The mean values increase when the base gum is compared to the sweetened (0 mg. citric acid) gum. This indicates that the parotid gland was stimulated. This psychic stimulus is probably a result of both taste and olfactory stimulations. As the citric acid values increase, the mean pH values increase, indicating that the gland was responding to the acid stimuli and trying to raise the pH of the oral cavity.

The measurements for base gum generally had a higher standard deviation than did the other means. This supports the idea that there was more variability in this parameter because more time was allotted to acquire the minimal amount of saliva necessary to be analyzed by the blood gas analyzer.

Table 3 presents the mean and standard deviation values for flow rates of stimulated parotid saliva as a function of citric acid concentration. Figure 6 illustrates this relationship of the mean rates of flow of parotid secretion (ml/min) with the the citric acid concentration (mg/3 gm stick of gum).

The mean values increase when comparing base gum vs. the sweetened (0 mg. citric acid gum). This indicates that the parotid was stimulated via psychic stimuli (taste and olfactory) with a resultant increased flow rate. As citric acid increased, the flow rate also increased indicating that the parotid gland was responding to the increase in citric acid delivered to the oral cavity. Increasing the flow rate acts to dilute the acid and subsequently deliver more buffering capacity to the area.

Table 4 demonstrates that the means of  $p\text{CO}_2$  increase with increasing citric acid concentration however, there was much variability in the results and no clear relationship can be established. Figure 7 illustrates the relationship of the mean  $\text{PCO}_2$  (mm/Hg) with the citric acid concentration (mg/3 gm stick of gum). Standard deviations were varied and had a great range indicating much variance.  $\text{PCO}_2$  measurements were difficult to achieve primarily because of loss of  $\text{CO}_2$  by diffusion.

Attempts were made to eliminate this diffusion by collecting the sample under oil. However, diffusion could have occurred anywhere in the collection apparatus between the Stensons duct and the collecting tube. Since air was present in the Carlson cup and the plastic tubing, salivary  $\text{CO}_2$  could have diffused into that air. More importantly, salivary  $\text{CO}_2$  could have been diffused through the oil itself.

Table 5 demonstrates that the mean  $\text{HCO}_3^-$  values increase with increasing citric acid concentration. Figure 8 illustrates this relationship of the mean  $\text{HCO}_3^-$  concentration (mEq/l) with the citric acid concentration (mg/3 gm stick of gum). A dramatic increase in mean  $\text{HCO}_3^-$  values between base gum and sweetened (0 mg. citric acid) gum indicates



FIGURE 5. Distribution of pH Values

# Distribution of pH Values

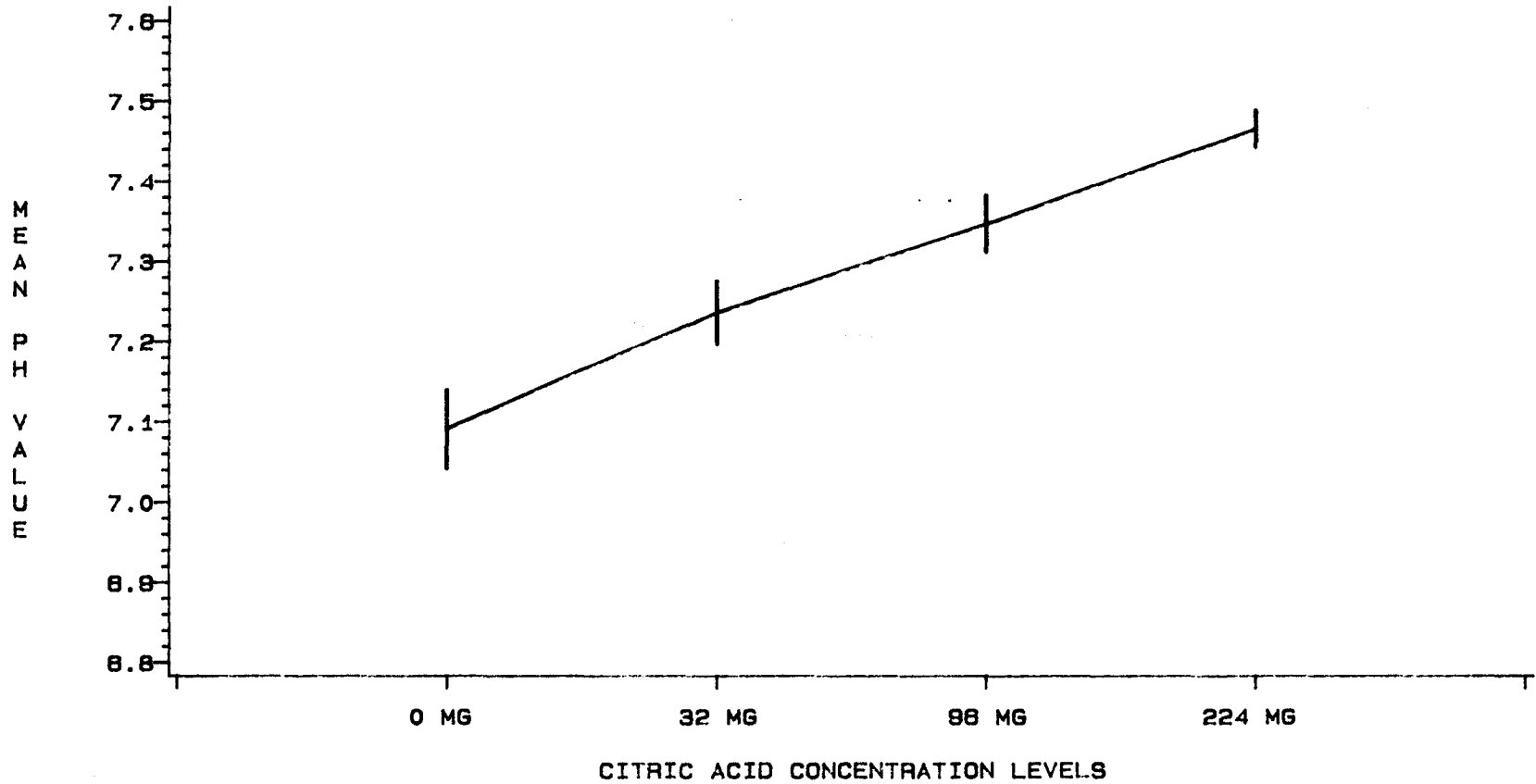


FIGURE 6. Rates of Flow for Stimulated Parotid Secretion

# Rates of Flow for Stimulated Parotid Secretion

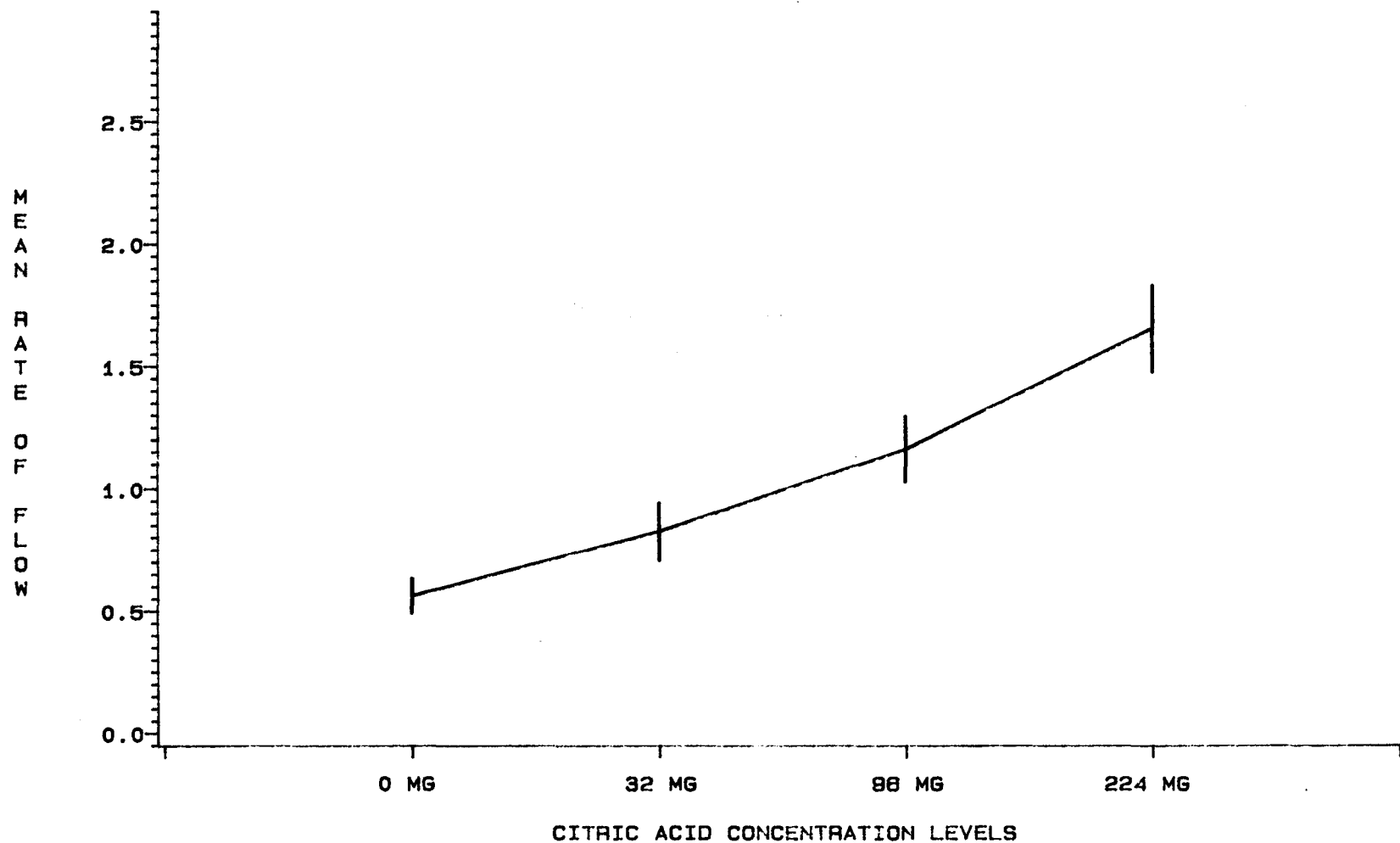
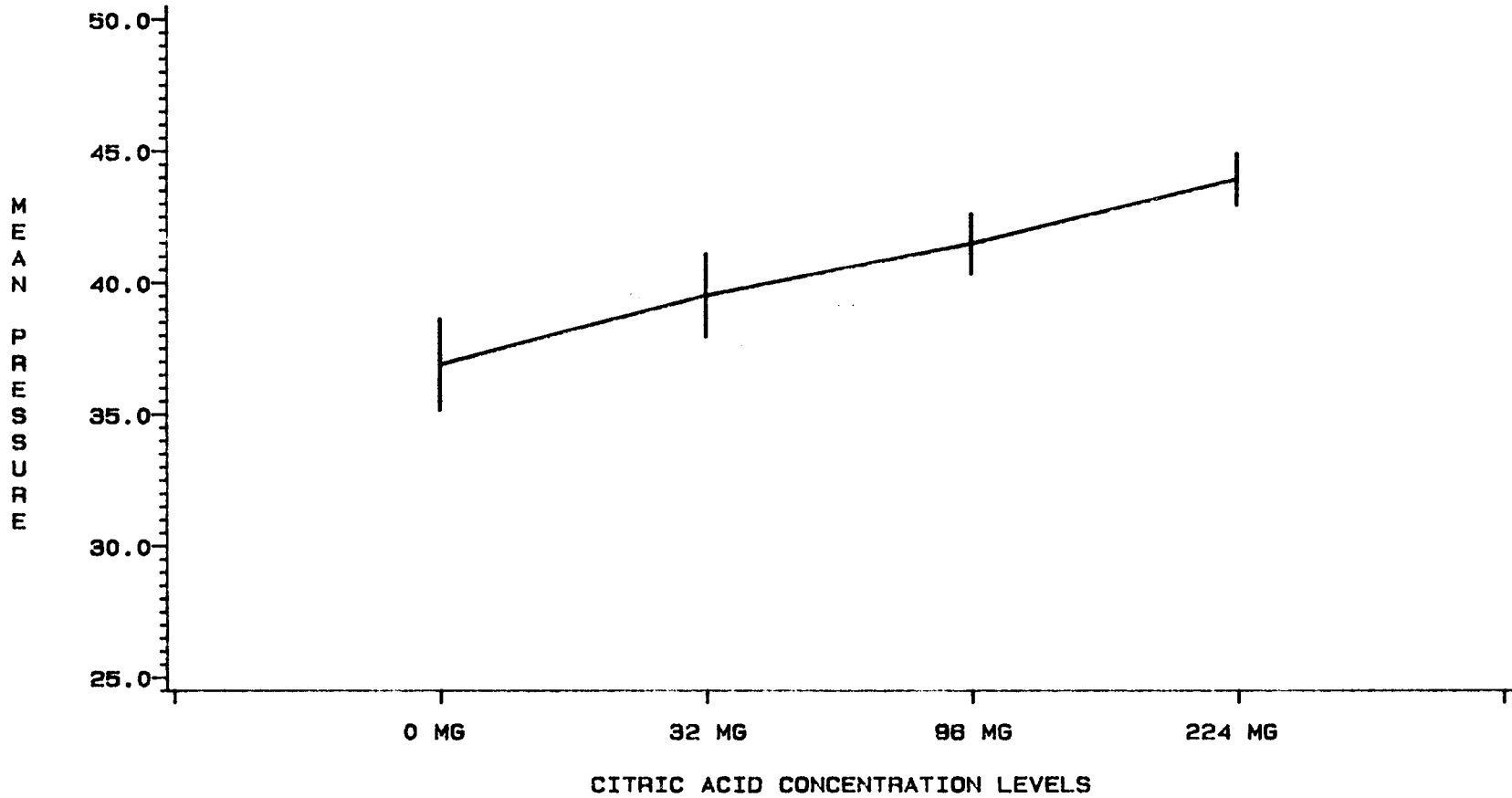


FIGURE 7. Distribution of Carbon Dioxide Pressures

# Distribution of CO<sub>2</sub> Pressures



that the gland was stimulated by the sweetener (psychic effect, taste and olfactory). As citric acid was added the parotid attempted to buffer this effect. This is most evident when reviewing the 224 mg. gum noting that the buffering effect between the sweetened gum and the 224 mg. citric acid gum almost doubled. Standard deviations varied but the variance was unremarkable.

Table 6 demonstrates the mean values and standard deviations of stimulated parotid saliva as a function of citric acid chewing gum for all subjects (N = 30). This table lists the distribution of the mean values of each parameter. These values are also presented in figures 5 through 8 and provide the reader easy review of Table 6. All parameters showed an increase when the sweetened gum (0 mg. citric acid) was utilized indicating psychic stimuli. Each parameter also increased when the citric acid concentration was raised indicating that the parotid gland was trying to neutralize and dilute the acid delivered to the oral cavity. Table 7 demonstrates the mean values of each parameter of all subjects as a function of citric acid chewing gum. It also lists the  $f$  distribution score and percent variance explained.

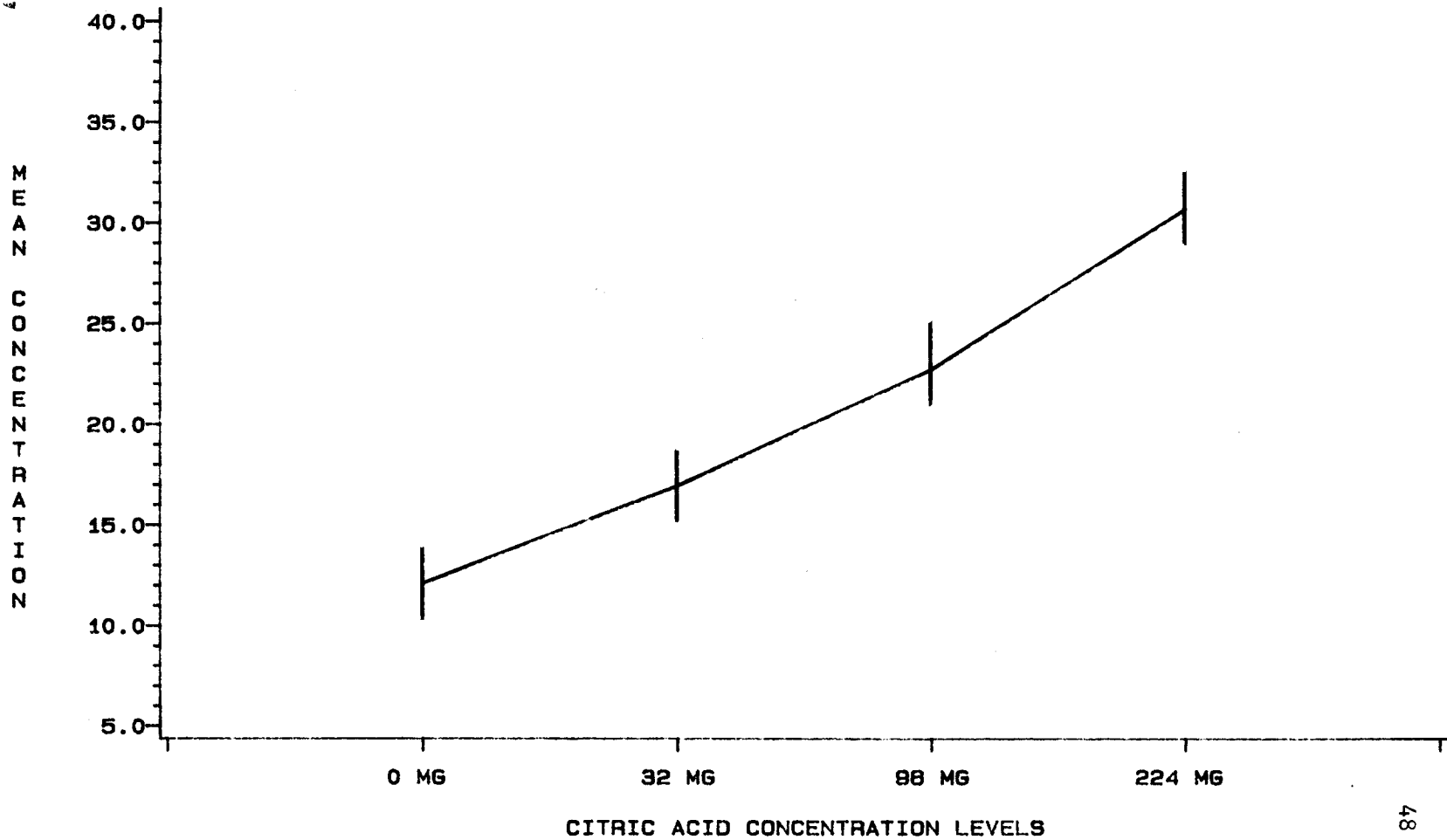
Since all the  $f$  distribution scores were found to be very highly significant at  $p < 0.001$  the null hypothesis can be rejected. Therefore the mean values obtained for each of the parameters are different. Part of variability in the distribution of the various parameters can be explained knowing the gum type. This is represented as a list of  $r^2$  values x 100 to yield a percent amount of explained variance of the distribution of values.

The amount of variability (47%) in the distribution of pH values

FIGURE 8. Distribution of Bicarbonate Concentrations



# Distribution of $\text{HCO}_3^-$ Concentrations



can be explained knowing the type of gum used. This is the highest percent variance listed. Calculating the pH of parotid saliva appears to be an appropriate parameter to measure distinctions in gum groups.

The amount of variability (41%) in the distribution of rate of flow values can be explained by knowing the gum type. This parameter also seems appropriate in the citric acid levels of the gums.

Experimental error could account for some of this decrease in percent variance explained. Loss of parotid fluid can occur when transferring the fluid from the test tubes to the calibrated syringes. Secondly, there is loss of parotid fluid in wetting the Carlson Apparatus. Thirdly, reading the syringe markings can also introduce error. Finally, error can be introduced in the timing of the trials.

The amount of variability (46.9%) in the distribution of bicarbonate values can be explained by knowing the gum types. This value has limited usefulness in determining the distinctions in citric acid levels of the gums. Bicarbonate is a calculated value utilizing  $p\text{CO}_2$  values. The  $p\text{CO}_2$  values showed the highest distribution of variance. Thus the Bicarbonate values should be considered only with care.

The amount of variability (12.2%) in the distribution of  $p\text{CO}_2$  values can be explained knowing the gum type. This is the least useful measurement and the  $p\text{CO}_2$  measurement should be considered a poor parameter to examine. Variance is due to loss of  $\text{CO}_2$  during the collecting procedure but mostly due to the loss through and into the mineral oil, which capped each sample. If the blood gas analyzer could have been immediately accessible,  $p\text{CO}_2$  values probably would show less variance. However, in this case, the blood gas analyzer was not

immediately accessible.

Utilizing the Scheffe Test, the gum groups were distinguished based on significant differences found in the means of the parameters. The 224 mg. citric acid gum distinguishes itself, from the other gums, in all parameters of parotid fluid. This gum showed the most elevated values. The 96 mg. and 32 mg. citric acid gums can be paired. There were few differences that were significant between these two gums. The 'sweetened only' gum stood alone in every parameter except in Rate of Flow where there was no significant difference between the sweetener and 32 mg. citric acid gum. This indicate that the psychic affects of the gum was very strong on the parotid and it overwhelmed the effects of the 32 mg. citric acid.

Correlation studies were also conducted. The 96 mg. citric acid gum was the only gum which showed a positive correlation between pH, Bicarbonate, and Rate of Flow ( $p < 0.001$ ) with Bicarbonate for all the gums ( $p < 0.0001$ ). This would indicate that all the parameters except  $pCO_2$  were systematically working to titrate the acid delivered by the 96 mg. gum.

## CHAPTER VI

### SUMMARY AND CONCLUSION

It has been clearly demonstrated that the parotid gland can actively respond and secrete in a protective manner in response to citric acid gum stimulation. This protective function is to produce a buffer which has the capacity to neutralize acid delivered to the oral cavity. When compared to the levels obtained while chewing base gum, the pH, rate of flow,  $pCO_2$ , and bicarbonate concentrations of pure parotid saliva all positively increase in a systematic manner with increasing citric acid gum levels.

The order of usefulness of the measured parameters of stimulated parotid salivary secretions seem to be pH, Rate of Flow, Bicarbonate and  $pCO_2$  respectively. Of the different gum formulation tested it would appear that the 96 mg. citric acid gum would be the best in elicit the most complete response of the parotid gland secretions.

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APPENDIX A

PAROTID SECRETION DATA

-- Subject #1 --

Gum: Base Gum

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 6.0 | 21.1             | 1.0              | 10.0     | 0.68   | 0.068 |
| 2     | 6.5 | 32.6             | 3.0              | 5.0      | 0.80   | 0.160 |
| 3     | 7.1 | 31.8             | 9.5              | 7.0      | 0.60   | 0.086 |

Gum: Sweetener only

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 6.9 | 33.2             | 6.2              | 3.0      | 1.00   | 0.333 |
| 2     | 7.2 | 45.7             | 17.5             | 3.0      | 1.90   | 0.633 |
| 3     | 7.4 | 37.7             | 22.5             | 3.0      | 2.20   | 0.733 |

Gum: 32 mg Citric Acid

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.4 | 47.2             | 28.2             | 3.0      | 3.20   | 1.067 |
| 2     | 7.3 | 40.4             | 19.2             | 3.0      | 3.60   | 1.200 |
| 3     | 7.2 | 46.8             | 17.6             | 3.0      | 2.30   | 0.767 |

Gum: 96 mg Citric Acid

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.5 | 27.7             | 20.8             | 2.0      | 2.90   | 1.450 |
| 2     | 7.3 | 48.7             | 23.0             | 3.0      | 2.70   | 0.900 |
| 3     | 7.5 | 34.3             | 20.8             | 3.0      | 3.60   | 1.200 |

Gum: 224 mg Citric Acid

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.4 | 46.7             | 27.6             | 3.0      | 2.30   | 0.767 |
| 2     | 7.4 | 48.2             | 28.2             | 3.0      | 4.00   | 1.333 |
| 3     | 7.5 | 38.4             | 23.7             | 3.0      | 4.80   | 1.600 |

## -- Subject #2 --

Gum: Base Gum

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 6.5 | 19.6             | 2.0              | 10.0     | 0.50   | 0.050 |
| 2     | 6.6 | 19.8             | 2.0              | 5.0      | 0.55   | 0.110 |
| 3     | 6.7 | 23.4             | 1.0              | 5.0      | 0.61   | 0.122 |

Gum: Sweetener only

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 6.9 | 37.5             | 6.9              | 3.0      | 1.00   | 0.333 |
| 2     | 6.8 | 43.8             | 6.6              | 3.0      | 1.30   | 0.433 |
| 3     | 7.0 | 44.0             | 10.4             | 3.0      | 1.30   | 0.433 |

Gum: 32 mg Citric Acid

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.1 | 41.1             | 12.3             | 3.0      | 1.50   | 0.500 |
| 2     | 6.9 | 53.4             | 10.1             | 3.0      | 1.60   | 0.533 |
| 3     | 7.1 | 40.1             | 12.0             | 3.0      | 1.50   | 0.500 |

Gum: 96 mg Citric Acid

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.3 | 47.3             | 22.4             | 3.0      | 1.50   | 0.500 |
| 2     | 7.1 | 54.6             | 16.4             | 3.0      | 1.60   | 0.533 |
| 3     | 7.5 | 30.5             | 22.8             | 3.0      | 1.86   | 0.620 |

Gum: 224 mg Citric Acid

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.5 | 41.6             | 48.5             | 3.0      | 3.50   | 1.167 |
| 2     | 7.7 | 40.6             | 23.0             | 3.0      | 3.70   | 1.233 |
| 3     | 7.4 | 47.2             | 12.3             | 3.0      | 3.10   | 1.033 |

## -- Subject #3 --

Gum: Base Gum

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 6.5 | 19.7             | 3.0              | 10.0     | 0.60   | 0.060 |
| 2     | 6.6 | 24.0             | 3.0              | 5.0      | 0.80   | 0.160 |
| 3     | 5.9 | 19.1             | 0.3              | 10.0     | 0.50   | 0.050 |

Gum: Sweetener only

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.2 | 39.2             | 14.7             | 3.0      | 1.30   | 0.433 |
| 2     | 7.1 | 37.2             | 11.1             | 3.0      | 1.30   | 0.433 |
| 3     | 7.3 | 43.0             | 20.5             | 3.0      | 1.40   | 0.467 |

Gum: 32 mg Citric Acid

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.3 | 33.4             | 16.0             | 3.0      | 2.40   | 0.800 |
| 2     | 7.4 | 45.8             | 27.3             | 3.0      | 2.00   | 0.667 |
| 3     | 7.4 | 43.6             | 26.2             | 3.0      | 2.30   | 0.767 |

Gum: 96 mg Citric Acid

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.5 | 33.3             | 24.7             | 3.0      | 2.90   | 0.967 |
| 2     | 7.1 | 36.6             | 10.9             | 3.0      | 1.40   | 0.467 |
| 3     | 7.2 | 46.1             | 17.4             | 3.0      | 2.40   | 0.800 |

Gum: 224 mg Citric Acid

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.6 | 38.4             | 36.2             | 3.0      | 4.20   | 1.400 |
| 2     | 7.4 | 54.2             | 32.5             | 3.0      | 3.90   | 1.300 |
| 3     | 7.3 | 48.3             | 30.0             | 3.0      | 3.90   | 1.300 |

## -- Subject #4 --

Gum: Base Gum

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 6.7 | 27.0             | 3.2              | 9.0      | 0.50   | 0.056 |
| 2     | 6.9 | 29.0             | 5.4              | 7.0      | 0.50   | 0.071 |
| 3     | 6.9 | 29.3             | 5.5              | 7.0      | 0.60   | 0.086 |

Gum: Sweetener only

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.1 | 35.3             | 10.6             | 3.0      | 1.20   | 0.400 |
| 2     | 7.2 | 24.5             | 9.4              | 3.0      | 0.80   | 0.267 |
| 3     | 7.2 | 47.1             | 17.8             | 3.0      | 0.90   | 0.300 |

Gum: 32 mg Citric Acid

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.2 | 38.2             | 14.4             | 3.0      | 1.50   | 0.500 |
| 2     | 7.1 | 42.0             | 12.3             | 3.0      | 1.80   | 0.600 |
| 3     | 7.1 | 43.2             | 12.4             | 3.0      | 1.70   | 0.567 |

Gum: 96 mg Citric Acid

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.2 | 42.8             | 16.0             | 3.0      | 2.00   | 0.667 |
| 2     | 7.3 | 43.5             | 15.8             | 3.0      | 2.00   | 0.667 |
| 3     | 7.2 | 38.3             | 14.4             | 3.0      | 1.80   | 0.600 |

Gum: 224 mg Citric Acid

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.4 | 37.8             | 22.5             | 3.0      | 2.50   | 0.833 |
| 2     | 7.3 | 41.8             | 18.7             | 3.0      | 2.30   | 0.767 |
| 3     | 7.3 | 40.4             | 19.1             | 1.5      | 1.70   | 1.133 |

## -- Subject #5 --

Gum: Base Gum

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 6.5 | 13.9             | 1.0              | 10.0     | 0.10   | 0.010 |
| 2     | 6.7 | 16.2             | 2.0              | 10.0     | 0.45   | 0.045 |
| 3     | 6.7 | 19.4             | 2.0              | 7.0      | 0.70   | 0.100 |

Gum: Sweetener only

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 6.9 | 18.3             | 3.5              | 3.0      | 5.70   | 1.900 |
| 2     | 6.8 | 24.9             | 3.7              | 3.0      | 0.50   | 0.167 |
| 3     | 6.9 | 35.4             | 6.8              | 3.0      | 0.90   | 0.225 |

Gum: 32 mg Citric Acid

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.2 | 42.1             | 17.9             | 3.0      | 1.80   | 0.600 |
| 2     | 7.2 | 32.8             | 12.3             | 2.0      | 1.60   | 0.800 |
| 3     | 7.3 | 37.2             | 17.7             | 3.0      | 2.50   | 0.833 |

Gum: 96 mg Citric Acid

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.4 | 40.3             | 24.0             | 3.0      | 2.20   | 0.733 |
| 2     | 7.5 | 43.5             | 32.5             | 3.0      | 5.50   | 1.833 |

Gum: 224 mg Citric Acid

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.5 | 40.9             | 30.6             | 3.0      | 5.50   | 1.833 |
| 2     | 7.5 | 40.9             | 30.6             | 3.0      | 3.20   | 1.067 |
| 3     | 7.5 | 41.3             | 31.0             | 3.0      | 4.80   | 1.600 |

## -- Subject #6 --

Gum: Base Gum

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 6.7 | 24.3             | 3.0              | 10.0     | 1.00   | 0.100 |
| 2     | 6.4 | 25.6             | 3.0              | 8.0      | 0.90   | 0.112 |
| 3     | 6.4 | 28.1             | 2.0              | 8.0      | 0.70   | 0.087 |

Gum: Sweetener only

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.3 | 40.9             | 19.4             | 3.0      | 2.30   | 0.767 |
| 2     | 7.2 | 46.8             | 17.6             | 3.0      | 1.60   | 0.533 |
| 3     | 7.6 | 34.8             | 33.0             | 3.0      | 4.00   | 1.333 |

Gum: 32 mg Citric Acid

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.4 | 38.1             | 22.8             | 3.0      | 3.50   | 1.167 |
| 2     | 7.3 | 52.1             | 24.7             | 3.0      | 3.00   | 1.000 |
| 3     | 7.4 | 43.3             | 25.8             | 3.0      | 2.00   | 0.667 |

Gum: 96 mg Citric Acid

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.5 | 28.5             | 23.9             | 3.0      | 5.20   | 1.733 |
| 2     | 7.3 | 54.0             | 25.6             | 3.0      | 4.40   | 1.467 |
| 3     | 7.4 | 49.1             | 29.5             | 3.0      | 4.30   | 1.433 |

Gum: 224 mg Citric Acid

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.5 | 35.7             | 26.9             | 3.0      | 3.50   | 1.167 |
| 2     | 7.3 | 56.4             | 26.5             | 3.0      | 3.00   | 1.000 |
| 3     | 7.4 | 52.0             | 31.0             | 3.0      | 2.00   | 0.667 |

## -- Subject #7 --

Gum: Base Gum

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 6.6 | 26.4             | 4.0              | 7.0      | 0.60   | 0.086 |
| 2     | 6.9 | 31.6             | 6.0              | 6.0      | 0.50   | 0.083 |
| 3     | 7.0 | 32.8             | 7.8              | 7.0      | 0.50   | 0.071 |

Gum: Sweetener only

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.3 | 35.6             | 16.9             | 3.0      | 2.70   | 0.900 |
| 2     | 7.1 | 54.8             | 16.3             | 3.0      | 2.70   | 0.900 |
| 3     | 7.0 | 43.8             | 10.2             | 3.0      | 1.90   | 0.633 |

Gum: 32 mg Citric Acid

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.3 | 36.2             | 17.2             | 3.0      | 3.40   | 1.133 |
| 2     | 7.1 | 28.5             | 8.5              | 3.0      | 6.50   | 2.167 |
| 3     | 7.1 | 29.3             | 8.8              | 3.0      | 4.50   | 1.500 |

Gum: 96 mg Citric Acid

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.3 | 26.3             | 12.5             | 2.0      | 1.60   | 0.800 |
| 2     | 7.5 | 46.0             | 34.5             | 3.0      | 8.00   | 2.667 |
| 3     | 7.5 | 40.9             | 30.5             | 3.0      | 6.60   | 2.200 |

Gum: 224 mg Citric Acid

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.5 | 41.4             | 31.0             | 3.0      | 9.00   | 3.000 |
| 2     | 7.5 | 40.3             | 30.5             | 3.0      | 7.80   | 2.600 |
| 3     | 7.4 | 41.2             | 24.5             | 3.0      | 7.10   | 2.367 |



-- Subject #8 --

Gum: Base Gum

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 6.9 | 21.2             | 3.7              | 6.5      | 0.50   | 0.077 |
| 2     | 6.1 | 18.8             | 0.4              | 10.0     | 0.45   | 0.045 |
| 3     | 6.4 | 25.6             | 2.0              | 8.0      | 0.60   | 0.075 |

Gum: Sweetener only

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.0 | 30.7             | 7.6              | 3.0      | 0.80   | 0.267 |
| 2     | 7.2 | 38.0             | 14.4             | 3.0      | 1.00   | 0.333 |
| 3     | 7.1 | 40.1             | 11.9             | 3.0      | 1.60   | 0.533 |

Gum: 32 mg Citric Acid

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.5 | 27.0             | 20.3             | 2.0      | 1.90   | 0.950 |
| 2     | 7.4 | 21.0             | 12.6             | 3.0      | 2.30   | 0.767 |
| 3     | 7.3 | 48.0             | 18.2             | 3.0      | 1.50   | 0.500 |

Gum: 96 mg Citric Acid

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.3 | 39.1             | 18.5             | 3.0      | 1.80   | 0.600 |
| 2     | 7.6 | 47.5             | 45.0             | 3.0      | 4.90   | 1.633 |
| 3     | 7.5 | 50.6             | 38.0             | 3.0      | 4.30   | 1.433 |

Gum: 224 mg Citric Acid

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.6 | 40.5             | 38.5             | 2.0      | 4.00   | 2.000 |
| 2     | 7.5 | 49.2             | 37.0             | 3.0      | 5.50   | 1.833 |
| 3     | 7.6 | 43.2             | 40.5             | 3.0      | 5.60   | 1.867 |

## -- Subject #9 --

Gum: Base Gum

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 6.7 | 27.7             | 3.9              | 8.0      | 0.70   | 0.087 |
| 2     | 6.6 | 21.0             | 2.0              | 5.0      | 0.80   | 0.160 |
| 3     | 6.7 | 27.6             | 1.0              | 7.0      | 0.70   | 0.100 |

Gum: Sweetener only

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.0 | 36.0             | 8.5              | 3.0      | 1.30   | 0.433 |
| 2     | 6.9 | 23.8             | 4.5              | 4.0      | 1.30   | 0.325 |
| 3     | 6.9 | 37.0             | 7.0              | 4.0      | 1.30   | 0.325 |

Gum: 32 mg Citric Acid

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.1 | 37.8             | 11.2             | 3.0      | 1.00   | 0.333 |

Gum: 96 mg Citric Acid

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.4 | 38.9             | 23.2             | 3.0      | 3.50   | 1.167 |
| 2     | 7.5 | 38.9             | 29.0             | 3.0      | 5.60   | 1.867 |
| 3     | 7.3 | 37.2             | 17.6             | 3.0      | 3.40   | 1.133 |

Gum: 224 mg Citric Acid

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.5 | 40.9             | 30.5             | 3.0      | 7.30   | 2.433 |
| 2     | 7.3 | 42.5             | 20.2             | 3.0      | 2.90   | 0.967 |
| 3     | 7.7 | 44.9             | 53.5             | 3.0      | 7.20   | 2.400 |

## -- Subject #10 --

Gum: Base Gum

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 6.9 | 27.6             | 5.2              | 7.0      | 0.80   | 0.114 |
| 2     | 7.0 | 28.3             | 6.4              | 8.0      | 0.60   | 0.075 |
| 3     | 7.0 | 23.9             | 5.6              | 7.0      | 0.70   | 0.100 |

Gum: Sweetener only

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.2 | 36.4             | 13.7             | 3.0      | 2.90   | 0.967 |
| 2     | 7.1 | 29.5             | 8.7              | 3.0      | 2.00   | 0.667 |
| 3     | 7.0 | 34.5             | 8.3              | 3.0      | 2.40   | 0.800 |

Gum: 32 mg Citric Acid

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.4 | 41.3             | 24.5             | 3.0      | 4.20   | 1.400 |
| 2     | 7.3 | 44.8             | 21.0             | 3.0      | 3.40   | 1.133 |
| 3     | 7.3 | 37.9             | 17.8             | 3.0      | 3.00   | 1.000 |

Gum: 96 mg Citric Acid

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.1 | 46.4             | 14.0             | 3.0      | 3.50   | 1.167 |
| 2     | 7.2 | 51.0             | 19.2             | 3.0      | 3.70   | 1.233 |
| 3     | 7.1 | 43.5             | 12.8             | 3.0      | 4.30   | 1.433 |

Gum: 224 mg Citric Acid

| Trial | pH  | PCO <sub>2</sub> | HCO <sub>3</sub> | Duration | Volume | Ratio |
|-------|-----|------------------|------------------|----------|--------|-------|
| 1     | 7.6 | 45.3             | 43.0             | 3.0      | 4.70   | 1.567 |
| 2     | 7.5 | 53.6             | 40.0             | 3.0      | 5.20   | 1.733 |
| 3     | 7.5 | 47.2             | 35.3             | 3.0      | 4.90   | 1.633 |

APPROVAL SHEET

The thesis submitted by Raymond J. Bielinski has been read and approved by the following committee:

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The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the Committee with reference to content and form.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science in Oral Biology.

4/18/84  
Date

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