THE UNIVERSITY OF MANITOBA

ZINC CONCENTRATION IN STIMULATED PAROTID SALIVA AND ITS RELATIONSHIP TO SWEET AND BITTER TASTES BEFORE AND AFTER JEJUNOILEAL BYPASS SURGERY

by

SANDRA MARGARET MacDONALD

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF FOODS AND NUTRITION

WINNIPEG, MANITOBA

SEPTEMBER, 1983.

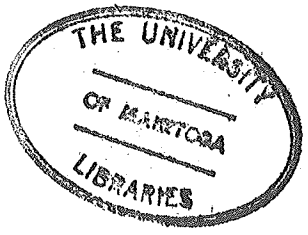
ZINC CONCENTRATION IN STIMULATED PAROTID SALIVA AND ITS RELATIONSHIP TO SWEET AND BITTER TASTES BEFORE AND AFTER JEJUNOILEAL BYPASS SURGERY

by

Sandra Margaret MacDonald

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE



ABSTRACT

The purpose of the study was to document parotid salivary zinc concentrations of morbidly obese and jejunoileal bypass subjects and to examine the relationship to sweet and bitter taste function. Stimulated parotid salivary (SPS) zinc concentration was quantitated by flame atomic absorption spectroscopy in 12 morbidly obese subjects, 17 bypass patients and 24 normal weight controls. Total SPS protein was quantitated for all saliva samples and flow rate was recorded. Samples were collected at the same time of day. The slope of magnitude estimation data, from a concurrent study on taste function in the same subjects, was the parameter used for taste perception. The molar concentration of the tastant after which pleasantness decreased was the parameter used for taste preference. No relationship was found in any subject group between SPS zinc and taste perception or preference for sweet and bitter tastants. Mean SPS zinc concentration of the morbidly obese subjects (35 $\pm$ 20 ng/mL) was significantly lower than that in matched normal weight controls (41 $\pm$ 20 ng/mL) but the mean SPS zinc concentration of the bypass patients (41 $\pm$ 24 ng/mL) did not differ from that of normal weight controls. Mean SPS zinc levels of 12 bypass patients were found to be correlated with postoperative time in months ($r = 0.74$). As postoperative time increased, the SPS zinc concentration increased. Flow rate and total SPS protein were not related to SPS zinc in this study. The reduced amounts of SPS zinc for morbidly obese subjects and the observed increase in SPS zinc concurrent with postoperative time suggests that the zinc nutritional status of morbidly obese subjects and bypass patients should be investigated.

ACKNOWLEDGEMENTS

I would like to express a special thank you to Dr. Vivian Bruce, my advisor, whose guidance and support were my cornerstone. To my other committee members, Dr. Colin Dawes and Marion Vaisey-Genser, thank you for being so generous with your time and so patient with my questions.

Thank you, Dr. Ken Thorlakson, for your interest and for allowing your patients to participate in the study. The subjects, who participated so cheerfully, are also thanked.

The funding provided by the Winnipeg Clinic Research Foundation and the University of Manitoba Research Board is gratefully acknowledged.

Andy Lutz, Freshwater Institute, Environment Canada is thanked for technical assistance.

I would like to thank my family for the emotional support given, albeit over the miles. To Michelle and Craig, thank you for accepting all that goes with a mother who decides to study for a Master's degree.

This work is dedicated to my father, Duncan J. MacDonald, who did not live to see its completion.

TABLE OF CONTENTS

	Page
ABSTRACT	i
ACKNOWLEDGEMENTS	ii
LIST OF TABLES	v
LIST OF FIGURES	vi
INTRODUCTION	1
REVIEW OF LITERATURE	3
Role of Saliva, Zinc and Gustin in Taste	3
Composition and Analysis of Saliva	7
Association of Taste Alterations and Biological Zinc	11
Jejunoileal Bypass, Zinc and Taste	20
MATERIALS AND METHODS	23
Subjects	23
Saliva Collection	25
Salivary Zinc Analysis	28
Salivary Protein Analysis	30
Taste	33
Statistical Analysis	34
RESULTS	36
SPS Zinc and Taste Perception	36
SPS Zinc and Taste Preference	36
Salivary Zinc	40
Flow Rate	43
Salivary Protein	44
DISCUSSION	51
Taste and Parotid Salivary Zinc	51
Parotid Salivary Zinc	53
Flow Rate and Parotid Salivary Zinc	55
Protein and Zinc in Parotid Saliva	57
Analysis of Parotid Salivary Zinc	58
CONCLUSION	62
REFERENCES CITED	63

TABLE OF CONTENTS (Cont'd)

	Page
APPENDICES	68
A - Consent Form	69
B - Molar Concentrations of Tastant Solutions and Distribution of Preferred Concentrations	70 71
C - Regression Equations for Salivary Flow Rate and Total SPS Protein	74
D - Comparison of Aqueous Standards and Standards Containing TCA	77

LIST OF TABLES

Table		Page
1.	Physical Characteristics of the Subjects	24
2.	Zinc Content of Human Control Serum	31
3.	Regression Equations by Group for Mean SPS Zinc on the Sweetness and Bitterness Slopes derived from the Magnitude Estimation Data.	37
4.	Group Means for SPS Zinc and Taste Perception and Preference.	38
5.	Regression Equations by Group for Mean SPS Zinc on the Highest Preferred Concentration of Sucrose and Caffeine Solutions.	39
6.	Paired Comparison t-Tests for Mean SPS Zinc	41
7.	Mean SPS Zinc for Untreated and Treated Subjects and Controls	45
8.	Mean Saliva Flow Rate for Untreated and Treated Subjects and Controls.	46
9.	Mean Total SPS Protein for Untreated and Treated Subjects and Controls	48
10.	Paired Comparison t-Tests for Mean SPS Protein	49

LIST OF FIGURES

Figure		Page
1.	Lashley cup with tubing and needle adaptor	27
2.	A comparison of aqueous standards and standards prepared with TCA(2):water(5)	32
3.	Linear regression of SPS zinc and postoperative months	42
4.	Linear Regression of SPS Zinc on flow rate by group	47
5.	Linear regression of SPS zinc concentration and total SPS protein	50

INTRODUCTION

There is evidence in both animals and humans that an association exists between zinc and gustatory function (Catalanotto, 1978b). Henkin and coworkers (1975b) have shown that oral administration of zinc to zinc-deficient human subjects was associated with improvements in taste function. A protein present in parotid saliva, gustin, has been shown to contain zinc (Henkin, et al 1978). Subjects with normal taste acuity have been reported to have higher parotid salivary zinc concentrations than subjects with idiopathic hypogeusia. Although zinc supplementation has improved taste function, researchers have not been able to correlate changes in biological zinc concentrations to changes in taste function (Catalanotto, 1978b). Altered taste response has been suggested as a causative factor in obesity. An increased preference for sweet solutions has been documented in obese subjects (Rodin, et al, 1976) but a decreased preference for sweet solutions was reported after jejunoileal bypass surgery (Bray, et al, 1976). Since weight loss following surgery is not totally explained by malabsorption, altered taste responsiveness could be a contributing factor leading to decreased food intake (Rodin, 1980). Plasma zinc concentrations were found to be low in morbidly obese and bypass subjects (Atkinson, et al, 1978)

but the zinc concentrations of saliva in these subjects has not been documented. Thus, individuals who have jejunoileal bypass surgery present an interesting group in which to study the relationship between taste function and salivary zinc.

REVIEW OF LITERATURE

Role of Saliva, Zinc and Gustin in Taste

Taste is defined as detection and recognition of a liquid phase stimulus and can be separated into neural and preneural events. The preneural events of taste involve 1) the taste bud support system 2) the characteristics of the taste bud receptors and 3) the mechanism of tastant receptor binding. It is within these three components of the taste process that saliva and zinc play a role, although relatively little is known about the specific biochemical mechanisms (Henkin, 1978).

Persons with decreased salivary flow (xerostomia) exhibit hypogeusia and demonstrate destruction or modification of the taste buds (Henkin, 1978). The return of salivary flow is followed by the reappearance of the taste buds, which supports the hypothesis that saliva in man and animals contains a stimulatory factor which may be necessary to maintain normal taste bud anatomy and function. On the other hand, persons with normal salivary flow, who have low zinc concentrations in parotid saliva, whole saliva, serum, plasma and hair also exhibit hypogeusia (Catalanotto, 1978b). The alteration in taste response was shown to be

corrected after treatment with zinc (Atkin-Thor, et al, 1978; Hambidge, et al, 1972; Henkin, et al, 1975c; Majahan, et al, 1980). Therefore, Henkin and co-workers (1975a) sought to identify a zinc containing protein in human parotid saliva. A low molecular weight protein (3.7×10^{-4}) was identified which contained 2 moles of zinc per mole of protein. The protein was named gustin. This protein differed from previously described zinc proteins on the basis of chemical and molecular analysis.

A taste function was suggested for gustin from the observation that patients with hypogeusia exhibited decreased levels of zinc in the fraction of the saliva which contained the zinc protein, gustin (Henkin, 1978). Langmyhr and coworkers (1979) studied the distribution of zinc in human parotid saliva and concluded that the fractions with the highest zinc concentration could contain the same zinc protein as that identified by Henkin and coworkers (1975a) as gustin.

Since taste function appears to be associated with zinc nutritional status, the potential of salivary zinc as an indicator of zinc nutritional status has been studied. Zinc has been quantitated for whole saliva, the supernatant of whole saliva, the sediment of whole saliva and whole

parotid saliva. The zinc content of whole saliva and the supernatant of whole saliva were not correlated with the zinc concentration in hair or serum for adolescent females (Gregor and Sickles, 1979). However, significantly lower zinc concentrations were found in the supernatant when the subjects were consuming a diet which contained 11.5 mg Zn daily rather than 14.7 mg Zn daily. Freeland-Graves and coworkers (1981), who studied young females, also found that whole saliva did not reflect a low zinc intake, although the zinc concentration of the salivary sediment had decreased significantly. Plasma zinc had decreased after 22 days when subjects consumed a low zinc (3.2 mg/day) diet, but the change was not significant. Earlier, Freeland-Graves and coworkers (1980) had investigated the zinc status of vegetarians. Serum zinc levels did not correlate with zinc concentrations in the diet or saliva (whole saliva and salivary sediment). However, vegetarians showed decreased zinc concentrations in hair and salivary sediment, which consists mainly of epithelial cells. Mathur and coworkers (1977) investigated the zinc content of whole blood, plasma, unstimulated whole saliva and stimulated parotid saliva for healthy human adults. The zinc concentration found in parotid saliva was only one tenth the concentration in unstimulated whole saliva; whereas, plasma and whole blood had higher zinc concentrations. Prior to this, Henkin and

coworkers (1975b) had reported lower serum and parotid zinc levels in patients with hypogeusia as compared with subjects with normal taste acuity. When one subject was administered an intravenous injection of ^{65}Zn, the ^{65}Zn appeared in the parotid saliva in 20 minutes and continued to rise for 6 - 8 hours, while the serum zinc level was falling. Therefore, one would expect absorbed dietary zinc to appear in parotid saliva relatively quickly. Yet the circadian rhythm observed for zinc concentration in whole saliva (Snowden and Freeland, 1978) does not appear to be a response to the usual mealtimes.

Thus, the evidence supporting the use of saliva as an indicator of human zinc status is inconclusive. Also, a study with rats (Everett and Apgar, 1979) indicated that zinc concentration in whole saliva does not appear to be a reliable index of zinc status in that species. A correlation between salivary and dietary zinc or other biochemical parameters has not been found. However the improvement in taste function with zinc supplementation and the identification of gustin in saliva suggests further investigations on the relationship of salivary zinc and taste function are merited.

Composition and Analysis of Saliva

Although parotid saliva contains relatively low concentrations of zinc, it presents a more homogeneous sample than whole saliva which can be contaminated with food debris, bacteria and desquamated epithelial cells (Dawes, 1978). In addition the sampling procedure, which involves the placement of a collection device over Stenson's duct, is painless and noninvasive. If the sample of parotid saliva is collected without further stimulation it is termed unstimulated; whereas, a sample elicited with the use of a stimulus, such as citric acid or sour lemon drops, is described as stimulated. The duration of stimulation and the salivary flow rate are known to affect the composition of parotid saliva (Dawes, 1969). However, the effect of flow rate on zinc concentration in stimulated parotid saliva was only recently reported (Warren, et al, 1981), but the nature of the effect was not determined. Other investigators (Henkin, et al, 1975b; Langmyhr, et al, 1979; Mathur, et al, 1977) have not attempted to control flow rate when collecting stimulated parotid saliva. Since parotid saliva is a more homogeneous sample and since it is possible to maintain a constant salivary flow rate from the parotid gland with well-trained subjects (Dawes, 1969), it is a preferred biological sample. Control of the flow rate

would minimize the changes in zinc concentration due to variation in flow rate and duration of stimulation.

The zinc content of whole saliva follows a circadian rhythm (Snowden and Freeland, 1978). An initial decrease in zinc concentration upon rising is followed by a peak before noon. By late afternoon and evening, zinc had returned to morning levels. A similar pattern was shown in parotid saliva by Warren and coworkers (1981). The zinc concentration of samples collected in the late afternoon were significantly lower than that in fasting morning samples. No correlation was found between dietary zinc and stimulated parotid salivary zinc concentrations. Zinc concentration did not change significantly over consecutive days or months but wide subject variability was observed. Henkin and coworkers (1975b) did not find any significant changes in parotid salivary zinc concentrations due to age or sex, but the time of sample collection was not a controlled variable. As well, the other reports on zinc concentration in parotid saliva (Langmyhr, et al, 1979; Mathur, et al, 1977) did not specify the time of sample collection. On the basis of published data, the diurnal variation in zinc concentration should be taken into account in any study of zinc levels in parotid saliva.

Other secretable proteins and glycoproteins are found in whole parotid saliva in addition to the zinc-containing proteins discussed by Langmyhr and coworkers (1979). The concentration of total protein in parotid saliva is influenced by flow rate, duration of stimulation, the nature of the stimulus, the time of day at which samples are collected, plasma composition and the serial dependancy of saliva sampling (Dawes, 1978). The protein concentration rises faster and to a higher level at higher flow rates; whereas, the protein concentration decreases initially and then rises again when flow rate is maintained constant (Dawes, 1967). Gregor and Sickles (1979) investigated zinc, protein and the zinc:protein ratio in whole saliva, but samples were obtained at different times of day and whole saliva can contain non-salivary proteins. As for parotid saliva, zinc and protein have not been reported for the same samples. Wide individual differences in total protein concentration of stimulated parotid saliva have been observed and range from 0.025 to 1.0 gm/100 mL (Dawes, 1978). Since zinc in saliva may be bound to salivary protein, analysis of protein would be a useful parameter to include in the study proposed here.

Zinc in parotid saliva of healthy adults has recently been analyzed using flame atomic absorption

spectrophotometry (AAS) by two laboratories (Mathur, et al, 1977; Warren, et al, 1981). The results are not comparable since sample preparation was different. Mathur and co-workers analyzed the samples directly after dilution with deionized water and found that mean zinc concentration was 0.046 ppm. Warren and coworkers treated the parotid saliva with 20% trichloroacetic acid (TCA). Different volumes had been collected from the subjects, so the supernatant was diluted to a 25 mL volume after centrifugation. The results, which were expressed as the zinc concentration in 25 mL diluted supernatant, cannot be compared with zinc concentrations reported for a known volume of saliva.

Prior to this, Henkin and coworkers (1975b) determined the zinc content of stimulated parotid saliva using both flame and flameless AAS. Samples were analyzed directly and the salivary zinc concentrations were calculated from the calibration curve of aqueous zinc standards. With flameless AAS, the mean zinc concentration for the subjects with normal taste acuity was 51 ppb which was similar to the results found by Mathur and coworkers (1977) when flame AAS was used. When twenty randomly selected samples were analyzed again using flame AAS, the mean signed difference between the two methods was -15%. This indicated a higher mean estimate of salivary zinc con-

centration when the flame technique was used. Henkin and coworkers (1975b) concluded that the flameless technique was more accurate with samples containing less than 20 ppb.

A parotid salivary zinc concentration of 49 ppb zinc for one female subject was reported (Langmyhr, et al, 1979) using the flameless technique and direct analysis of the sample. This compares well with the mean zinc concentrations (51 ppb) reported by Henkin and coworkers (1975b). However, the standard addition technique was used rather than the standard calibration curve. But the method of standard additions is cumbersome and less accurate due to the non-linearity of the calibration curve, so the use of aqueous zinc standards is a simpler, more direct method of calibration.

The final choice of analytical method is dependent on the concentration range expected in the samples. Saliva samples can be analyzed directly with a graphite furnace, but treatment with TCA to release the zinc from salivary proteins is preferable when using the flameless technique.

Association of Taste Alterations and Biological Zinc

Taste disorders, which have been reported in a

broad spectrum of clinical states, have been associated with decreased zinc concentrations in biological tissues and fluids. Laboratory studies with rats have supported the hypothesis that zinc-deficiency may be associated with decreased taste acuity. Food intake and feeding patterns of rats fed a zinc-deficient diet (less than 3 ppm Zn) were studied by Williams and Mills (1970). The voluntary food intake of zinc-deficient rats decreased and a cyclical feeding pattern was observed. When the diet was supplemented with 6, 9 or 12 ppm Zn, food intake increased and the cyclical feeding pattern was diminished. Similar observations were reported in a second study with zinc-deficient rats (Chesters and Quarterman, 1970).

A specific alteration in sodium chloride (NaCl) preference and anorexia was exhibited by zinc-deficient weanling rats (McConnell and Henkin, 1974). Zinc replete rats, which were fed ad libitum or pair-fed with the zinc-deficient rats, did not demonstrate anorexia or an altered preference for NaCl. Since altered preference for NaCl may be reflective of a pathophysiological process, Catalanotto and Lacy (1977) investigated the response of zinc-deficient weanling rats to other taste solutions. Using the two bottle preference test, zinc-deficient rats showed an increased preference for NaCl, sucrose, quinine

sulphate and hydrochloric acid when compared with zinc-replete pair-fed controls. This was considered to be supportive of the hypothesis that zinc-deficient animals have altered taste acuity.

Zinc-deficient rats develop acanthosis and mild parakeratosis of the tongue epithelium which may prevent the tastant from reacting with the taste bud. In order to study this possibility, Catalanotto and Nanda (1977) repeated the investigation of the taste response of zinc-deficient weanling rats and included histological examination of the tongue. Again the zinc-deficient rats exhibited significantly increased preferences for NaCl, quinine sulphate and hydrochloric acid. The preference for sucrose did not change; however a lower concentration (2.0×10^{-2} vs 3.0×10^{-2} M) was used in this experiment and this may account for the differences in the results from the initial study. The histology of the tongue epithelium of the zinc-deficient rats showed the characteristic changes but the pore region of the taste bud did not appear to be covered with parakeratotic epithelium. This suggests that blockage of the taste bud pores is not the explanation for impaired taste in zinc-deficient rats.

Since zinc-deficient weanling rats may not be capable of performing the two bottle preference test, Catalanotto (1978a) repeated the two bottle preference test with adult rats. The adult rats would be less debilitated by induced zinc deficiency. Again, the zinc-deficient rats exhibited increased preference for NaCl, quinine and hydrochloric acid. The two bottle preference test is accepted as a valid method for testing taste preference of rats. Analysis of water and fluid intake supported the conclusions of the previous experiments. Thus a relationship has been shown in rats between zinc deficiency and taste preference which is assumed to be indicative of taste acuity in these animals.

Idiopathic hypogeusia was described by Henkin and coworkers (1971) as a syndrome in humans with associated dysgeusia, hyposmia and dysosmia. No cause was apparent for the complexity of symptoms observed in 35 patients, which included decreased taste and smell acuity, with or without abnormal taste and smell. In a subsequent study by Schecter and coworkers which was reviewed by Catalanotto (1978b), 100 patients with a similar syndrome were treated with zinc sulphate supplements in a single blind study.

After treatment, significant improvements were found in all subjects whose taste thresholds had been affected. However, the same investigators (Henkin, et al, 1976) failed to confirm the previous results in a randomized double blind crossover study, for a second group of subjects with taste and smell dysfunction. The results indicated that zinc sulphate was equivalent to placebo in the effect on taste function. But not all cases of hypogeusia are related to zinc deficiency and the subjects in the study had more than 25 different clinical conditions. As well, the parameters used to indicate zinc status (serum zinc and alkaline phosphatase activity) may not be the appropriate biological zinc measures to investigate the relationship of zinc to taste function.

Other researchers have reported decreased taste function in humans in conjunction with low zinc concentrations in biological tissues and fluids. Solomons and coworkers (1976, 1977) found that patients with celiac disease and Crohn's disease, respectively, had plasma zinc and taste detection scores that were significantly lower than those in control subjects. No correlation was found between plasma zinc and the taste detection scores. School children with hypogeusia were reported to have significantly lower hair zinc concentrations than those in children with normal taste

acuity (Buzina, et al, 1980). Hypogeusia was defined as the failure to detect and recognize any of the four taste qualities at three levels of concentration. Plasma zinc was not correlated with mild, moderate or severe hypogeusia, although low values were more frequently observed in the children with hypogeusia. However, neither hair zinc (<70 ppm) nor plasma zinc (<70 μg/dL) for the children with hypogeusia were below values commonly considered to be borderline zinc deficiency. Casper and coworkers (1980) reported similar results for plasma zinc and taste acuity with anorexia nervosa subjects, but hair zinc levels were normal in these subjects. Bitter and sour taste were most severely affected; whereas, sweet and salt taste were more easily recognized. Plasma zinc levels of the anorexia nervosa patients were significantly lower than those in the control group but plasma zinc did not correlate with taste recognition scores. The mean detection threshold for cirrhotic patients was significantly lower than that in controls for salt and bitter taste, but not for sweet and sour taste (Burch, et al, 1978). Serum zinc concentrations of the cirrhotic patients were significantly lower than those in the controls, but no correlation was found between zinc and taste thresholds. Cirrhotic patients would not be an ideal model to study the relationship between zinc and taste since the taste alterations may be a

result of other metabolic abnormalities common to the disease.

The taste changes reported for patients with chronic renal failure are not consistently related to zinc. Burge and coworkers (1979) observed an improvement in mean recognition thresholds for all four tastants when pre and post dialysis measures were compared. Mean serum zinc concentrations did not change with dialysis, so the improvement in taste acuity did not appear to be related to zinc. Vreman and coworkers (1980) measured plasma, red blood cell and hair zinc and mean detection thresholds for four tastants in patients with chronic renal failure. Mean detection threshold for sour was significantly lower than that in the controls for both male and female dialysis patients but sucrose and urea were only significantly lower for male dialysis patients. No significant change was found for the salt taste. The zinc concentrations in red blood cells and hair were significantly higher and plasma zinc levels were significantly lower for male dialysis patients as compared with controls. Females did not show significant differences when plasma, red blood cell and hair zinc concentrations were compared with controls. No correlation was found for any group between plasma, red blood cell and hair zinc concentrations and taste detection levels. On the other hand,

Majahan and coworkers (1980) demonstrated an improvement in uremic hypogeusia by zinc supplementation. In a double blind study, taste detection and recognition thresholds for salt, sweet and bitter tastants were improved 6 weeks after supplementation equivalent to 50 mg elemental zinc in the form of zinc acetate. Mean plasma zinc levels were significantly higher after the dialysis patients had been treated with zinc. No improvement in taste was shown in the patients taking the placebo. Similar results were found in a previous double blind crossover study with dialysis patients treated with 440 mg zinc sulphate three times per week (Atkin-Thor, et al, 1978). Patients who received the zinc supplement showed marked improvement in taste detection and recognition scores along with an increase in hair zinc concentration. Again, the metabolic abnormalities commonly present in dialysis patients make it difficult to isolate the effect of zinc on taste function. Yet the improvement of taste acuity following zinc supplementation is supportive of a relationship between taste and zinc.

Other investigators have shown an improvement in taste function in response to zinc supplementation. Earlier, Hambidge and coworkers (1972) had reported an improvement in taste function for school children after zinc supplements were given. In a survey of 132 school children, low hair

zinc concentrations were noted in 10 children who exhibited poor appetite and poor growth. Appetite, taste acuity and hair zinc concentrations all improved after 1 - 3 months of zinc supplementation with 1-2 mg $ZnSO_4$/Kg body weight/day. More recently, Friedman and coworkers (1980) investigated the effect of zinc supplementation on taste acuity in healthy young women. Zinc concentrations in plasma, hair and salivary sediment were measured and taste acuity was measured for sweet, sour, salty and bitter tastants. After 60 days, subjects who received 50 mg zinc showed a significant increase in taste acuity for sucrose. Plasma zinc showed a transient increase at 35 days and no significant change was observed in salivary sediment and hair zinc concentrations.

The methodology for these studies which investigated the association between biological zinc and taste function is variable with respect to 1) the tissues and fluids in which zinc was quantitated, 2) the methods used to assess taste function, 3) the use of supplemental zinc and 4) the ages and health of the subjects studied. Although this limits comparisons of the results, there is strong evidence, in both animals and humans, that a relationship between zinc and taste exists.

Jejunoileal Bypass, Zinc and Taste

Alterations in taste and decreased serum and plasma zinc have been reported in separate studies for morbidly obese subjects and subjects who have had jejunoileal bypass surgery. The altered taste response in morbid obesity was exhibited as an increased preference for sweet solutions (Rodin, et al, 1976). Following jejunoileal bypass, a dislike for sweet tastes developed in most patients (Bray, et al, 1976). The ratings of perceived intensity did not change, but rather the change was apparent in preference ratings. A review of approximately 15 diet records of morbidly obese subjects accepted for surgical treatment by Dr. Thorlakson (Department of Surgery, Winnipeg Clinic) showed that intake of bitter-tasting solutions (coffee, tonic, soda) was of high frequency. This observation suggested that bitter tastants should be studied.

Faber and coworkers (1978) evaluated the zinc status of 8 patients after bypass surgery, using serum zinc as the parameter. Six subjects had serum zinc concentrations below the normal range (80 - 110 g/dL). Dietary intake of zinc was calculated from 3-day food records or a 24 hour recall and was found to be adequate (8.8 - 29.7 mg/day). Statistical analysis was limited by the small subject

numbers but the two subjects who had the lowest serum zinc did not have the lowest estimated zinc intake. Although the number of observations was minimal, the authors concluded that malabsorption of zinc was the likely explanation for the low serum zinc. Similar results were reported by Atkinson and coworkers (1978), who measured plasma zinc concentrations of 15 morbidly obese patients before bypass surgery, 27 patients after bypass surgery and 52 lean controls. Mean plasma zinc concentration of the bypass patients was significantly lower than that of the lean controls and the morbidly obese subjects. Again, this result was assumed to be a consequence of malabsorption induced by jejunoileal bypass surgery, as deficiencies of magnesium, potassium and calcium had been reported previously. However, the unexpected result was the significantly lower mean plasma zinc concentrations in the morbidly obese group when compared with normal weight controls. No data was collected on dietary zinc intake, but the authors suggested that alterations in zinc metabolism may occur in obesity.

Although taste alterations and decreased zinc concentrations have been reported for morbidly obese and by-pass patients, an association between taste function and zinc has not been investigated in these subjects. In addition, parotid salivary zinc, which has been shown to be

associated with hypogeusia, has not been quantitated in morbidly obese or bypass patients. Therefore, this subject group was selected for study. The purpose of this study was to document the zinc concentration of parotid saliva and to examine its relationship to the taste response to sweet and bitter tastants.

EXPERIMENTAL METHODS

Subjects

The physical characteristics of the subjects, who were all white Caucasians, appear in Table 1. Twelve morbidly obese subjects formed the untreated group and 17 subjects, who had undergone surgery for jejunoileal bypass, comprised the treated group. Each subject was paired with a normal weight control of similar age, sex and smoking habit. The subgroups A and B were 5 subjects who were included in both the untreated and treated groups. These subjects served as matched pairs to compare treated and untreated groups directly. Morbid obesity was defined as 45 Kg in excess of ideal body weight[1] and normal weight was defined as within 20% of ideal body weight.

All subjects volunteered for the study and signed the consent form (Appendix A). All but two of the untreated and treated subjects were from the surgical practice of Dr. K. Thorlakson, Winnipeg Clinic, Winnipeg. The other subjects were obtained by personal contact. The study was approved by the Ethics Committee, Faculty of Medicine, University of Manitoba.

---

[1] Metropolitan Life Insurance Height and Weight Tables, 1975.

TABLE 1

PHYSICAL CHARACTERISTICS OF THE SUBJECTS

GROUP	NUMBER	SEX	AGE RANGE years	MEAN WEIGHT kg
Untreated	12	3M, 9F	23 - 47	122.4 ± 21.7*
Subgroup A	5	1M, 4F	26 - 47	136.0 ± 24.6
Treated	17	4M, 13F	20 - 49	97.0 ± 22.2
Subgroup B	5	1M, 4F	26 - 47	117.3 ± 15.3
Controls	24	6M, 18F	21 - 50	60.7 ± 11.4

* Group mean weight in kilograms ± S.D.

Saliva Collection

Parotid saliva was collected using a sterile teflon Lashley cup[2] placed over Stenson's duct. Proper placement of the Lashley cup was indicated when clear fluid appeared in the plastic tubing. Placement was facilitated when subjects were instructed to pull their cheek outward with the index finger and to bring the upper and lower tooth surfaces together before releasing the cheek. Gentle suction was applied to keep the Lashley cup in position and salivary flow was stimulated with sour lemon drops[3]. The stimulated parotid (SP) saliva was collected in a graduated 15 mL polystyrene vial with a conical bottom and a plastic screw cap.[4] The collection vials were checked for zinc contamination. By atomic absorption[5], 5 vials with distilled water or trichloroacetic acid (TCA) and distilled water (2 mL:5 mL) showed no detectable zinc and after 9 months vials with 5 mL distilled water and 2 mL TCA showed trace amounts of zinc. Therefore zinc contamination was minimal.

---

2 N.L. Harvey, 186 Auchinairn Road, Bishopbriggs, Glasgow, Scotland, U.K., G64 1NQ

3 Regal Crown Sour Lemon, Trebor Ltd., London, England.

4 2095 tube, Becton, Dickinson and Company, Parsippany, N.J. 07054

5 Varian Techtron Model AA5

During the initial collection, the subject was instructed to observe the flow rate with the aid of a mirror and stop watch and to keep it constant at 1 mL/min. by sucking more or less vigorously on the lemon drop. Three replicated 5 mL samples were collected on separate days from each subject and the duration of the collection (min) was recorded. All samples were collected between 1330 and 1700 hours and the samples from each individual subject were collected within the same hour whenever possible. Both the time of day and the time of rising for that day were recorded when a sample was collected. The samples were frozen for later analysis.

To assemble the collection unit, a 20 cm length of polyethylene tubing[6] was placed on the collection opening of the sterile Lashley cup (Figure 1) and a 40 cm length of the same tubing was placed on the suction opening. Care was taken to avoid zinc contamination during assembly and the assembled unit was placed in a plastic bag until used. Just prior to use, a rubber bulb was attached to the suction tubing using a sterile # 16 needle with a blunted end. After each use, the plastic tubing was discarded and the

---

6 Intramedic PE 190, Becton, Dickinson and Company, Parsippany, N.J. 07054

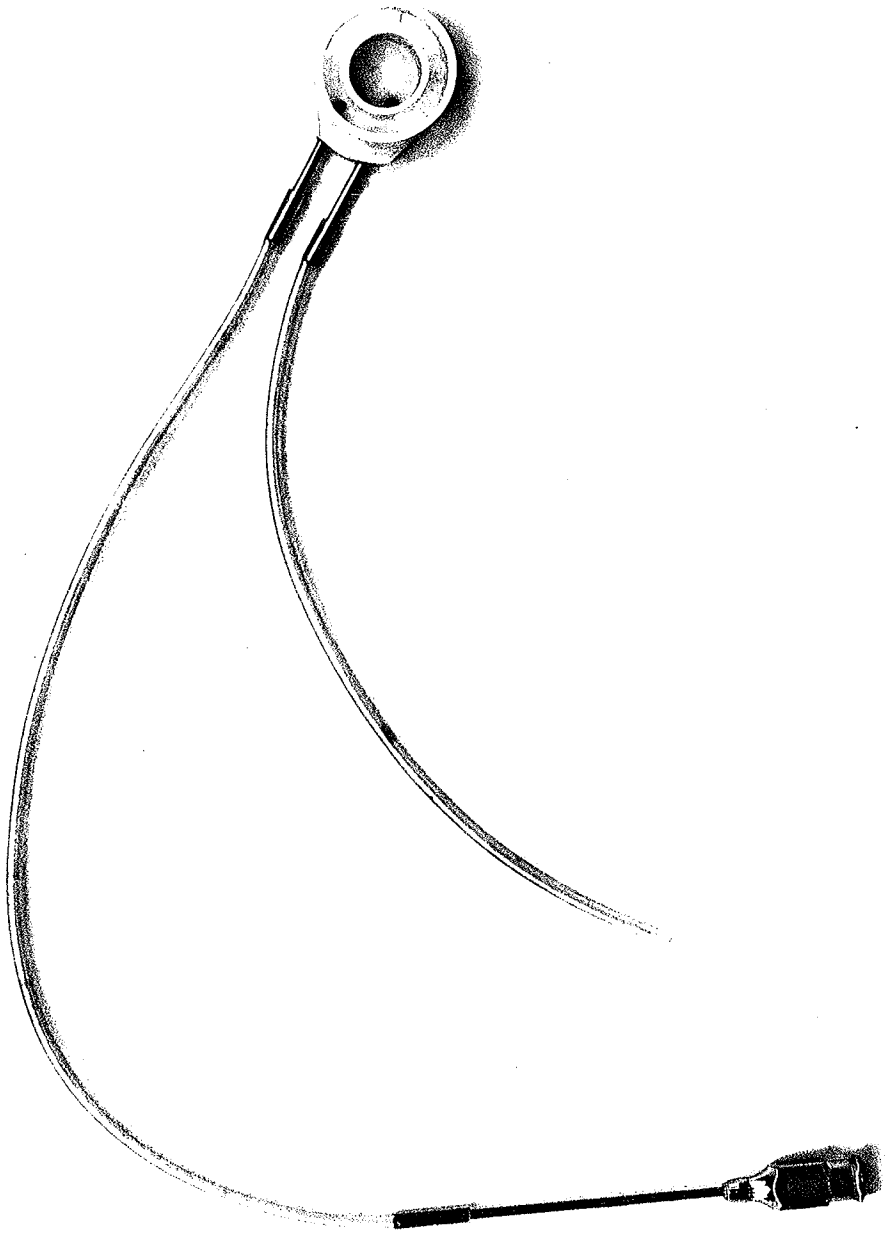


Figure 1 - Lashley cup with tubing and needle adaptor

Lashley cups were washed with soap and distilled water and rinsed with distilled water. The needle adaptors and the Lashley cups were placed in a glass Petri dish and autoclaved 20 min. at 84 kPa. The rubber bulbs were washed, rinsed and air dried.

Salivary Zinc Analysis

All equipment was acid washed before use. Samples were thawed at room temperature and each vial was vortexed before removing 10 μL of sample for protein analysis. The total weight of each vial and sample was recorded. The samples were prepared for analysis using a modification of the method described by Olson and Hamlin (1968) for serum zinc determinations. Instead of a 1:1 dilution of saliva and TCA, 5 mL of saliva was diluted with 2 mL TCA (20%).

Trichloroacetic acid (Fisher TCA, Heavy Metals 0.00003%) was prepared in a 20% solution and, when analyzed using atomic absorption spectrophotometry[6] (AAS), zinc contamination was found to be 40 ng/ml. To clean the TCA, 2 mL - 1% APDC (1-ammonium pyrrolidine dithiocarbamate) was added to 500 ml 20% TCA in a separatory funnel and shaken well. After 10 seconds carbon tetrachloride (10 mL) was added and the mixture shaken well. The bottom layer was

drawn off and this procedure was repeated three times. The TCA was heated gently to evaporate any remaining organic solvent and this TCA showed no detectable zinc when analyzed directly by AAS. The carbon tetrachloride solution was dried and the digested extract showed (13 ng/mL zinc by AAS. This method was repeated when additional 20% TCA was prepared, but for this solution, zinc contamination could not be corrected. To prepare the 20% TCA for the remainder of the analyses, Baker TCA (Heavy Metals 0.00006%) was analyzed using AAS and less than 10 ng/ml zinc was found to be present. This was diluted with distilled water in the same ratio as for the samples to be analyzed. In this dilution, zinc was not detectable using AAS.

Saliva samples were prepared for analysis in the original collection vial and zinc levels in the supernatant were determined directly by flame AAS, using a lean air/acetylene flame at a wavelength of 213.4 nm, a lamp current of 5ma and a slit width of 100 nm. After duplicate readings, all vials were washed, air dried and weighed. Blanks, prepared for each day of analysis with 5 mL distilled water and 2 mL TCA, did not have detectable amounts of zinc. An internal control was prepared for each analysis with 5 mL diluted human control serum (Fisher Diagnostics) and 2 mL TCA.

Differences between vials of human control serum were noted; however, replicates from the same vial were similar as shown in Table 2.

Aqueous zinc standards, containing 50, 75, 100, 150 and 200 ng/ml zinc were prepared from a stock solution. A few drops of nitric acid (HNO_3) were added to prevent the zinc from adhering to the glass container. Later, these aqueous standards were compared with standards containing TCA and distilled water in a 2:5 ratio (Figure 2). To further verify the results of the zinc analysis, 15 samples were taken to a second laboratory and analyzed by flame AAS (Perkin Elmer 4000) using zinc standards prepared with TCA and distilled water (2:5).

Salivary Protein Analysis

Total protein was determined by the Lowry Method (1951) with human control serum (Fisher Diagnostics, 6.0 $\pm$ 0.3g/dL total protein) as the standard. Standard solutions were prepared as follows - 100μL, 150 μL, 200 μL, 250μLand 500 μL of reconstituted serum were placed in separate 10 mL volumetric flasks, and distilled water was added to volume. These standards contained 60, 90, 120, 150 and 300 mg/dL, respectively. The Lowry method has been modified

TABLE 2

ZINC CONTENT OF HUMAN CONTROL SERUM[a]

Date Analysed	Zinc (μg/dl) Vial 1	Vial 2	Vial 3
Nov. 4	171 189		
Nov. 9	186 186		
Nov. 16		261 261	
Nov. 23		255 255	123 134
Nov. 25			105 115
Nov. 30			107 110
Dec. 2			114 122

[a] Fisher Diagnostics

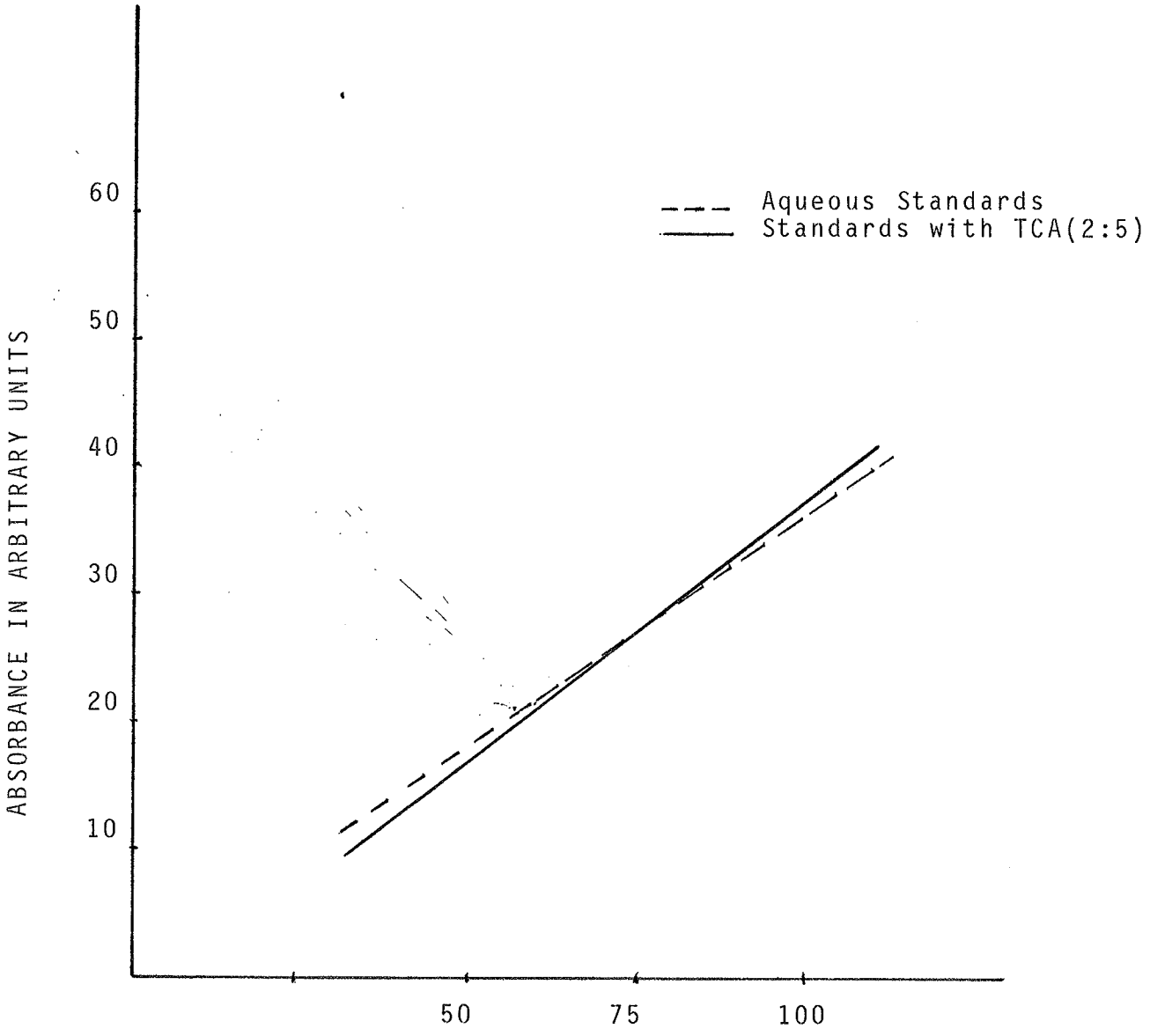


Figure 2 - A comparison of aqueous standards and standards prepared with TCA(2):water(5)

for analysis of parotid saliva (personal communication, Dr. C. Dawes, Faculty of Dentistry, University of Manitoba). A 10 μL sample of saliva was diluted with 200 μL distilled water. One mL of the alkaline copper solution was added to each sample and allowed to stand for 10 minutes. To each sample, 100 μL diluted Folin-Ciocalteu reagent was added rapidly and the mixture was vortexed 5 seconds to mix well. After 30 minutes, the optical density of the mixtures were read at 600 nm, using 10 mm light path cells. The reconstituted serum was stored in the refrigerator and new standards were prepared each day of protein analysis.

Taste

Magnitude estimation and hedonic response to two tastants, sweet and bitter, were evaluated (unpublished data, V. Murray, Dept. of Foods and Nutrition, University of Manitoba). Six serial dilutions of each tastant, which differed from one another by a factor of two, were presented to each subject on three occasions. The concentrations of the six sweet (sucrose) solutions were the same for all subjects; whereas, four series of concentrations were used for the six bitter solutions (Appendix B). Each subject received a bitter series which was within their sensitivity range.

The magnitude estimation data was analysed (unpublished data, V. Murray, Dept. of Foods and Nutrition, University of Manitoba). Sweetness exponents (slopes) and bitterness exponents (slopes) from this analysis were used to examine the relationship of perceived intensity and concentration to stimulated parotid saliva (SPS) zinc. The scores from hedonic scaling were examined to select the most concentrated preferred solution of sweet and bitter for each subject. The distribution of the preferred concentrations is shown in Appendix B. The highest molar concentration of sweet and bitter, which was scaled as most pleasant and after which a consistent decrease in pleasantness occurred, was taken as the point of change in preference. The actual molar concentrations of the most pleasant solution of sweet and bitter for each subject were plotted against SPS zinc to determine the relationship of SPS zinc to taste preference (pleasantness) for sweet and bitter.

Statistical Analysis

Paired comparison t-tests were employed to analyze the difference between matched groups for zinc and protein. The two sample t-test was used to compare group means for flow rate and protein. Simple linear regression was applied to determine the correlation between SPS zinc and protein,

flow rate, months after surgery, sweetness (sucrose) slope, bitterness (caffeine) slope, preferred sweet (sucrose) concentration and preferred bitter (caffeine) concentration.

RESULTS

SPS Zinc and Taste Perception

There was no relationship between SPS zinc and the perceived intensity and concentration of sucrose and caffeine solutions for any subject group, when tested by regression analysis. The regression equations of mean SPS zinc on the sweetness and bitterness slopes derived from the magnitude estimation data, for each subject group, are shown in Table 3. The small correlation coefficients (r less than 0.365), indicates that a relationship between SPS zinc and taste perception does not exist for the subjects in this study. The mean slopes for each group are shown in Table 4 for both sweet and bitter taste.

SPS Zinc and Taste Preference

No relationship was found between SPS zinc and the preferred concentrations of sweet and bitter solutions for any subject group, when tested by regression analysis. The regression equations for each subject group of mean SPS zinc on the highest molar concentration of sucrose and caffeine preferred by each subject are shown in Table 5. On the basis of the small correlation coefficients (r less

TABLE 3

REGRESSION EQUATIONS BY GROUP FOR MEAN SPS ZINC ON THE SWEETNESS AND BITTERNESS SLOPES DERIVED FROM THE MAGNITUDE ESTIMATION DATA[a]

Tastant	Group	Equation[b]	r
Sucrose	Untreated	Y = 43.63 - 3.41 (X)	0.365
	Treated	Y = 54.92 - 6.68 (X)	0.338
	Controls	Y = 43.84 - 1.16 (X)	0.084
	Overall	Y = 47.25 - 3.28 (X)	0.230
Caffeine	Untreated	Y = 36.79 - 1.35 (X)	0.152
	Treated	Y = 37.59 + 4.48 (X)	0.138
	Controls	Y = 39.69 + 1.70 (X)	0.071
	Overall	Y = 39.62 - 0.29 (X)	0.014

[a] Unpublished data, V. Murray, Dept. Food and Nutrition, U. of Manitoba.

[b] Y = a + b (X) where Y = zinc concentration (ng/mL) and X = slope derived from magnitude estimation data.

TABLE 4

GROUP MEANS FOR SPS ZINC AND TASTE PERCEPTION AND PREFERENCE

Subjects	Mean SPS Zinc ng/mL	Taste Perception[a]		Taste Preference[b]	
		Sucrose Slope	Caffeine Slope	Sucrose mol/L	Caffeine mmol/L
Untreated	35 (16)[c]	2.46 (1.33)	1.14 (1.41)	0.69 (0.82)	0.63 (0.64)
Treated	41 (24)	2.13 (1.06	0.59 (0.63)	0.52 (0.92)	1.13 (1.08)
Controls	41 (20)	2.48 (1.11)	0.74 (0.59)	0.32 (0.58)	1.10 (1.10)

[a] Slope of magnitude estimation derived from unpublished data, V. Murray, Dept. of Foods and Nutrition, University of Manitoba.

[b] Molar concentration of solution above which pleasantness decreased, V. Murray, Dept. of Foods and Nutrition, University of Manitoba.

[c] Standard deviation.

TABLE 5

REGRESSION EQUATIONS BY GROUP FOR MEAN SPS ZINC ON THE HIGHEST PREFERRED CONCENTRATION OF SUCROSE AND CAFFEINE SOLUTIONS

Tastant	Group	Equation[a]	r
Sucrose	Untreated	Y = 38.8 + 0.59 (X)	0.045
	Treated	Y = 44.0 - 7.03 (X)	0.319
	Controls	Y = 39.7 + 6.44 (X)	0.263
	Overall	Y = 43.2 - 1.39 (X)	0.122
Caffeine	Untreated	Y = 34.9 + 630.9 (X)	0.032
	Treated	Y = 45.3 - 4406.2 (X)	0.235
	Controls	Y = 47.7 - 5189.7 (X)	0.395
	Overall	Y = 40.8 - 2.0 (X)	0.095

[a] Y = a + b (X) where Y = zinc concentration (ng/mL) and X = molar concentration of the solution.

than 0.395), the relationship between salivary zinc and preference for sweet and bitter tastants is poor for these subjects. The group means of the preferred concentrations of sweet and bitter solutions are shown in Table 4.

Salivary Zinc

The results of the paired comparison t-tests are shown in Table 6. The SPS zinc for the untreated subjects is significantly lower than the SPS zinc for normal weight controls. The SPS zinc for treated subjects is not significantly different from that of the normal weight controls. The SPS zinc for the subgroup, was not significantly different before (A) and after (B) surgery. When the subgroup was removed from the treated group, the 12 subjects who had received surgery prior to the study did not have significantly different SPS zinc values as compared with the normal weight controls.

The zinc content of stimulated parotid saliva increases as the post operative months increase, for subjects who were surgically treated within the preceding 18 months. Figure 3 shows the relationship between mean SPS zinc and the number of months following surgery. The regression equation is $Y = 9.21 + 3.78 (X)$ with a correlation coefficient of 0.86. The 4 treated subjects who had received surgery

TABLE 6

PAIRED COMPARISON t-TESTS FOR MEAN SPS ZINC[a]

Paired Groups	Number	Mean of the Difference	t-value
Untreated : Controls	12	-9.9 (5.2)[b]	-1.89[c]
Treated : Controls	17	1.3 (3.9)	0.32[d]
Subgroup A : Subgroup B	5	8.1 (4.6)	1.75[d]
Treated : Controls	12	8.2 (7.6)	1.08[d]

[a] Mean of three replicates for each subject

[b] Standard error of the mean difference

[c] Using one tailed probability, $p<0.05$

[d] Using two tailed probability, $p>0.05$

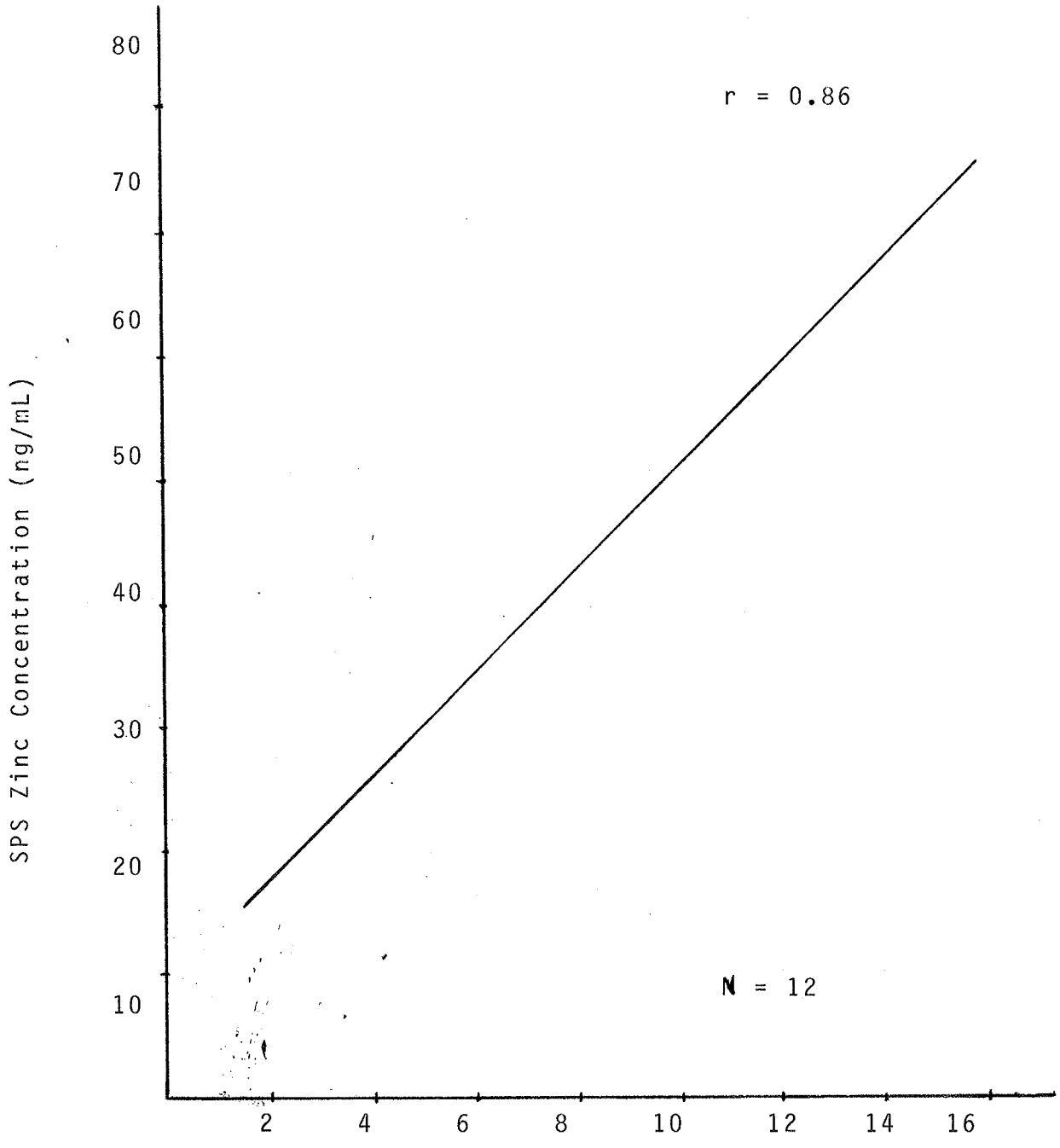


Figure 3 - Linear regression of SPS zinc and post operative months

23 or more months prior to their participation in the study did not show this relationship.

One subject in subgroup B was consuming a vitamin-mineral supplement, postoperatively, which contained the equivalent of 25 mg zinc per capsule. Preoperative mean SPS zinc was 33 ng/mL for this subject. Two months after surgery, mean SPS zinc was 66 ng/mL; whereas, the other 4 subjects in the subgroup had postoperative SPS zinc values below 25 ng/mL. Since the other treated subjects were not receiving a zinc supplement, this subject was not included when the relationship of SPS zinc to postoperative time was examined.

The group means for SPS zinc are shown in Table 7. The SPS zinc values are variable within each subject group. The mean SPS zinc for the twelve treated subjects, who had received surgery 6 - 36 months prior to the study is greater than the mean SPS zinc for subgroup B, who had received surgery during the study.

Flow Rate

The mean flow rates for the treated and untreated subjects are not significantly different, but the control

group had a mean flow rate that was significantly lower than that in the treated and untreated subjects. The group mean flow rates are shown in Table 8. There was no relationship between SPS zinc and flow rate, when tested by regression analysis. The regression lines for SPS zinc relative to flow rate are shown in Figure 4 and the regression equations are found in Appendix C1.

Salivary Protein

The group means for total protein in stimulated parotid saliva are not significantly different as shown in Table 9. When paired comparison t-tests were applied to matched groups (Table 10), no significant difference was found between treated and untreated subjects and matched controls. Nor was there a significant difference in total SPS protein between subgroup A and B. The regression of SPS zinc on total SPS protein is shown in Figure 5, and the regression equations for this data are shown in Appendix C2. The small correlation coefficients (r less than 0.386) suggests that this relationship may be non-existent.

TABLE 7

MEAN SPS ZINC FOR UNTREATED AND TREATED SUBJECTS AND CONTROLS

Group	Number	Mean SPS Zinc ng/mL
Untreated	12	35 (16)[a]
Subgroup A	5	28 (7)
Treated	17	41 (24)
Subgroup B	5	30 (19)
Treated	12	45 (18)
Controls	24	41 (20)

[a] Standard deviation of the group mean

TABLE 8

MEAN SALIVA FLOWRATE FOR UNTREATED AND TREATED SUBJECTS AND CONTROLS

Group	Number	Mean Flow rate[a] mL/min
Untreated	12	0.80* (0.32)[b]
Treated	17	0.87* (0.26)
Controls	24	0.62 (0.24)

[a] Using two tailed probability, means with the same superscript (*) are not significantly different ($p<0.01$).

[b] Standard deviation of the group mean

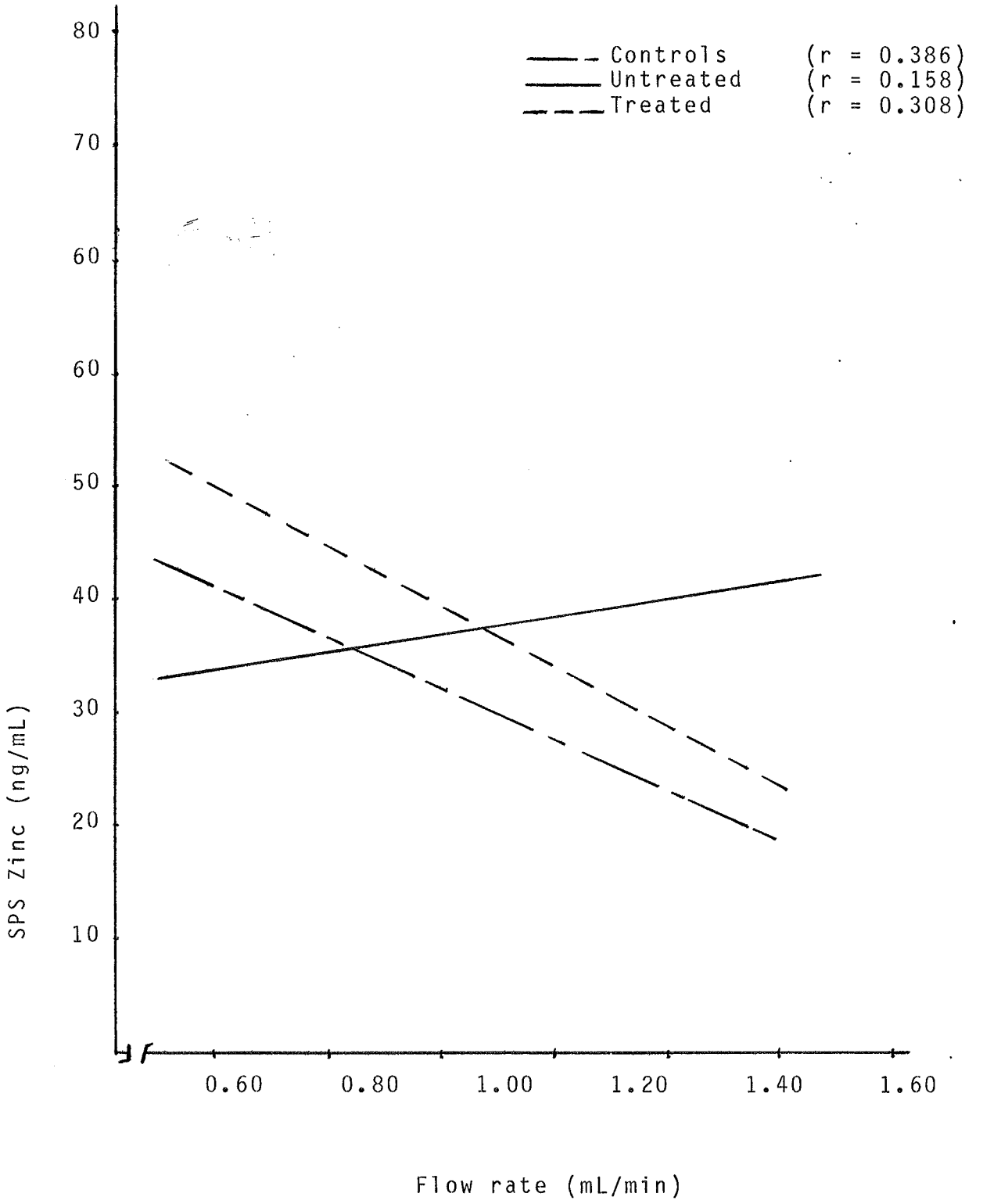


Figure 4 - Linear Regression of SPS Zinc on Flow rate by Group

TABLE 9

MEAN TOTAL SPS PROTEIN FOR UNTREATED AND TREATED SUBJECTS AND CONTROLS

Group	Number	Mean Total SPS Protein[a] mg/dL
Untreated	12	154* (57)[b]
Treated	17	144* (54)
Controls	24	151* (48)

[a] Using two tailed probability, means with the same superscript (*) are not significantly different ($p<0.01$).

[b] Standard deviation of group mean.

TABLE 10

PAIRED COMPARISON t-TESTS FOR MEAN SPS PROTEIN[a]

Groups Paired	Number	Mean of the Difference	t-value
Untreated : Controls	12	-5.0 (10.4)[b]	-0.48[c]
Treated : Controls	17	-0.5 (13.4)	-0.04[c]
Subgroup A : Subgroup B	4	-15.6 (30.9)	-0.51[c]

[a] Mean of three replicates for each subject

[b] Standard error of the mean difference

[c] Using two tailed probability, not significantly different ($p>0.05$).

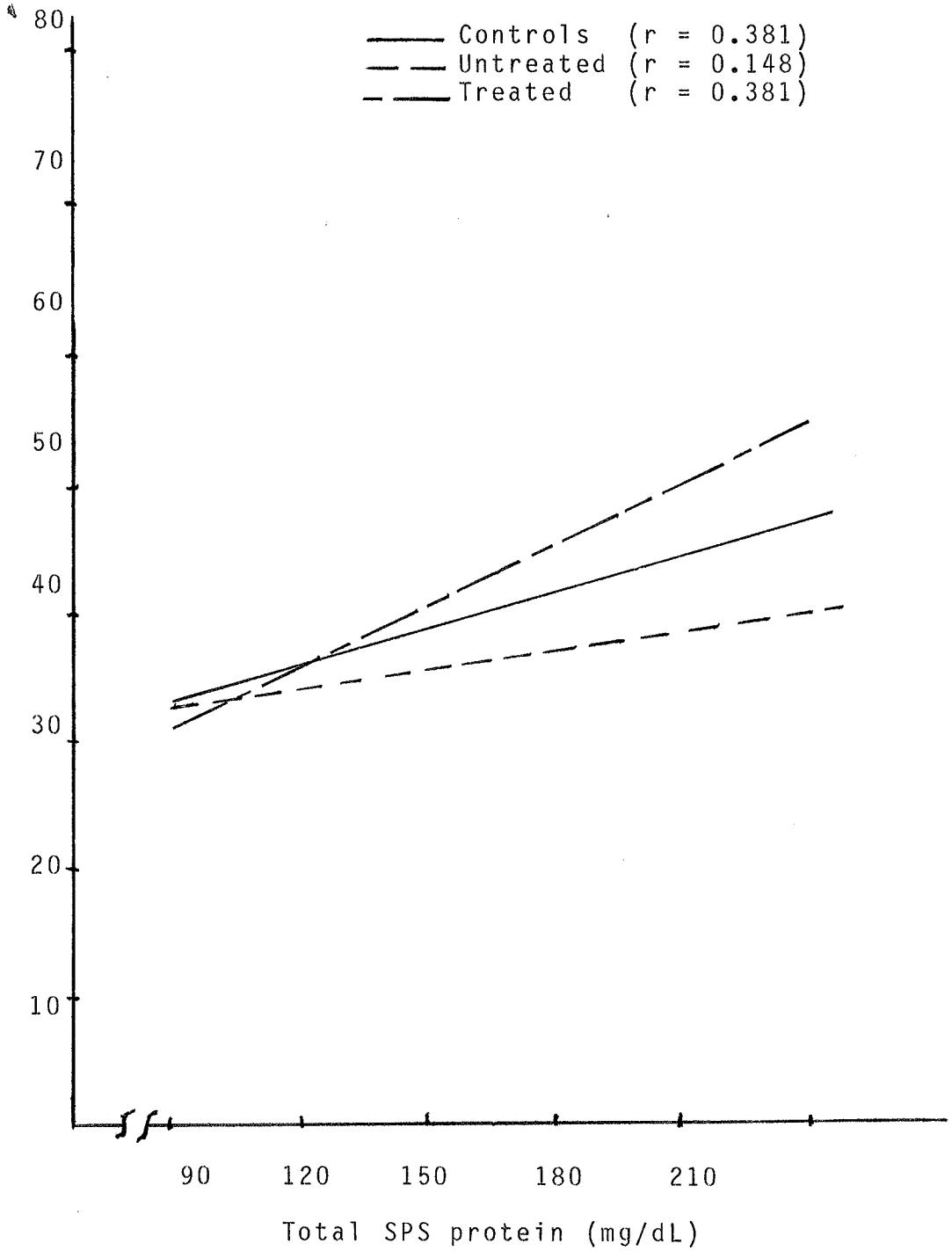


Figure 5 - Linear Regression of SPS zinc concentration and total SPS protein

DISCUSSION

Taste and Parotid Salivary Zinc

There are limited published reports (Henkin, et al, 1976; Shatzman and Henkin, 1980) which include a study of both parotid salivary zinc and taste, although an association between zinc nutritional status and taste function is well documented in the literature (Catalanotto, 1978b). One patient with hypogeusia, low parotid salivary zinc and low gustin levels responded to treatment with exogenous zinc (Shatzman and Henkin, 1980). After nine days, taste thresholds and magnitude estimation improved and salivary zinc and gustin levels increased. Henkin and coworkers (1975b) had previously reported significantly low concentrations of SPS zinc for patients with hypogeusia when compared with controls having normal taste function. The control subjects exhibited detection and recognition thresholds and forced scaling measurements which were within normal limits; whereas, patients with hypogeusia exhibited abnormalities for two or more threshold measurements and/or abnormalities in forced scaling for one or more taste qualities. In this study, no relationship was found between SPS zinc and perception of sweet and bitter taste for any subject group. The SPS zinc concentrations were higher than the mean SPS zinc concen-

tration (10 ppb) which Henkin and coworkers (1975b) reported for patients with depressed taste function. Zinc supplementation was not included in the experimental design. It is possible that the SPS zinc concentrations were not depressed to a level which would have an effect on perceived intensity and concentration.

In our study, no correlation was found in any subject group between SPS zinc and the molar concentration of sweet and bitter solutions above which pleasantness decreased. Rodin and coworkers (1976) found that obese subjects rated increasingly sweet solutions as more pleasant than did normal weight controls. After bypass surgery, the pleasantness ratings for sucrose solutions were more similar to those of normal weight controls. Perceived intensity did not differ between obese and bypass patients and normal weight controls. From the results of this study, it does not appear that the preference for sweet and bitter solutions shown by untreated, treated and normal weight subjects is dependant on salivary zinc.

Subject variability in salivary zinc concentrations, which has been discussed by Warren and coworkers (1981) may be the confounding factor in statistical significance. On the other hand, it is feasible, despite the identification of

gustin, that some other biological zinc component may be more functional in the taste process. The zinc content of whole saliva is ten times the concentration in parotid saliva (Mathur, et al, 1977) and taste bud membrane is rich in alkaline phosphatase, a zinc metalloenzyme (Henkin, 1978). Since saliva and alkaline phosphatase are present during the preneural events of taste, the zinc from these sources may be involved in the taste process. The relationship between zinc and taste may well remain unexplained until the biochemical mechanisms of taste, relative to zinc, are investigated more extensively.

Parotid Salivary Zinc

The increase in SPS zinc concurrent with an increase in postoperative time (Figure 3) is an interesting observation. Three possible sources of zinc could be dietary intake, improved absorption and/or a redistribution of endogenous zinc. The diets of both the treated and untreated subjects included 6 - 9 ounces of meat protein daily (personal communication, L. Sinabaldi, Dietitian, Winnipeg Clinic, Winnipeg) so it is unlikely that the diets were deficient in zinc. Malabsorption of nutrients, other than zinc, has been reported (DeWind and Payne, 1976) for bypass

patients, and low serum (Faber, et al, 1978) and plasma (Atkinson, et al, 1978) zinc have been reported following jejunoileal bypass surgery. However, the SPS zinc for the subject who was consuming a zinc supplement postoperatively was twice the concentration in saliva collected before the surgery. The SPS zinc for this subject was also greater than the mean SPS zinc of the control group. This suggests that this subject was absorbing dietary zinc. The third possibility, redistribution of endogenous zinc, could only be determined in humans using stable isotopes. A nutritional assessment study of zinc nutritional status after jejunoileal bypass would be of interest in order to confirm the observed postoperative increase for SPS zinc concentration.

Since taste alteration has been documented in obese subjects, and if low parotid zinc is associated with impaired taste, the low SPS zinc reported for the untreated subjects in this study would be expected. However these subjects were consuming diets which appeared to be adequate in zinc. Yet, Atkinson and coworkers (1978) found that plasma zinc levels of obese subjects were significantly lower than those of normal weight controls. In the study reported here, the subgroup of 5 untreated subjects had a lower mean SPS zinc concentration than that of the total untreated group. It was noted that the subjects in the sub-

group had higher percent ideal body weights than the other untreated subjects. These observations are suggestive of alterations in zinc metabolism in obesity and merit further study.

SPS zinc has limitations as an indicator of zinc nutritional status (Solomons, 1979) but low concentrations are suggestive of poor zinc nutritional status. Although the control group in the study had a mean SPS zinc concentration similar to values reported in the literature, many of the individual subjects had SPS zinc concentrations below the range reported in the literature for normal healthy subjects. An assessment of additional zinc-containing biological fluids of these individuals would be necessary to determine whether or not zinc deficiency was present.

Flow Rate and Parotid Salivary Zinc

It has been reported that flow rate affects the zinc concentration of parotid saliva (Warren, et al, 1981). However, the flow rate was examined in relation to SPS zinc concentrations in a 25 mL diluted volume which contained different amounts of saliva from each subject. This is not a valid way to express zinc concentration in saliva, therefore the reported results do not have any meaning. Protein,

chloride, sodium and bicarbonate concentrations of stimulated parotid saliva increase at higher flow rates; whereas, potassium and inorganic orthophosphate concentrations decrease at higher flow rates (Dawes, 1978). No relationship between SPS zinc concentrations and flow rate was found for the subjects reported here. Other investigators have analysed parotid saliva for zinc (Henkin, et al, 1975b; Langmyhr, et al, 1977) but did not document salivary flow rate. Mather and coworkers (1977) indicated that salivary flow rate was recorded, when collecting parotid saliva samples for zinc analysis, but the data was not reported. Salivary flow rate was not related to age in that study (Mather, et al, 1977).

A constant flow rate can be maintained in well trained subjects (Dawes, 1969). With the training time available, some subjects in our study were able to achieve the desired flow rate; whereas, others had more difficulty learning to control the flow rate. Warren and coworkers (1981) described a training effect for timed collections (10 min.) of stimulated parotid saliva. This was evidenced by an increase in salivary flow for day to day and month to month collections as the subjects adjusted to the presence of the Lashley cup over the parotid duct. Considering the effect of flow rate on salivary composition, it

would be useful to study the relationship of SPS zinc and salivary flow with a select group of well trained subject, and methodology similar to that reported by Dawes (1969) for other components of parotid saliva.

Protein and Zinc in Parotid Saliva

No relationship was found between SPS zinc and total protein in parotid saliva for the subjects in this study. Protein concentration is known to increase with duration of stimulation and at higher flow rates (Dawes, 1969). The sensitivity of protein to flow rate and stimulation may account for the poor correlation in these subjects. The quantitation of total protein and zinc in stimulated parotid saliva, obtained from a subject at specific flow rates, could be an indirect way to measure changes in the major zinc containing protein, gustin.

The protein concentrations for the subjects in this study were similar to values reported by Dawes (1969). The Lowry method was used to quantitate protein and spectrophotometric readings were obtained at a minimum time of 30 minutes following treatment of the standards and samples with the phenol reagent. The protein standards showed some variability which was thought to be due to readings ob-

tained later than 30 minutes, rather than exactly 30 minutes following treatment with the phenol reagent. In an analysis of milk protein conducted in the Nutrition laboratory, it was found that more consistent results were obtained with the Lowry method when the samples and standards were read at exactly 30 minutes following the phenol treatment. The published method indicates that samples should be read after 30 minutes but more precise timing is required.

Analysis of Parotid Salivary Zinc

The SPS zinc concentrations of the subject groups (Table 7) are similar to results reported in the literature, but comparison is limited by the fact that methodology differs in the studies reported. Mathur and coworkers (1977), who used flame AAS, reported a mean value of 46 ppb SPS zinc for 36 healthy subjects which is similar to that in the normal weight controls in this study. However, the saliva samples were analysed directly after dilution with deionized water. Warren and coworkers (1981) also used flame AAS and reported higher values for SPS zinc (73 and 111 ppb) for 10 healthy subjects. However, the methodology used is not valid. Yet, an earlier comparison of flame and flameless AAS (Henkin, et al, 1975b) indicated that flame AAS gave an increased mean estimate of salivary zinc con-

centration. The SPS zinc concentration of 51 ppb reported by Henkin and coworkers (1975b) for 34 subjects and 49 ppb reported by Langmyhr and coworkers (1979) for one subject were both obtained by flameless AAS. These results are similar to the group mean SPS zinc concentration for the healthy normal weight controls in this study.

The choice of flame AAS for this study was influenced by the fact that flameless AAS required more preparation of the sample and increased the possibility of zinc contamination. The optimum working range for the Varian Techtron with the graphite furnace was 1 - 20 ppb and the standard curve was nonlinear for concentrations greater than 30 ppb zinc (personal communication, A. Lutz, Freshwater Institute, Winnipeg). The controls and treated subjects were expected to be well above this range and it was not known how low the zinc concentrations would be in the untreated subjects. Therefore, all samples were analysed by flame AAS. The limit of detection was determined to be 5 ng/mL and sensitivity, which is the zinc concentration necessary for 0.0044 absorption units, was calculated as 16 pg Zn. It is preferable, in quantitative analysis, to specify a lower concentration limit of 10 times the detection limit, since the relative standard deviation at the limit of

detection can be 25 - 100% (Ingle, 1974). Yet many samples from the subjects in this study were in the 20 - 50 ng/mL range. Therefore, the experimental error would be expected to be greater for the subjects who had SPS zinc concentrations less than 50 ng/mL, although all replicates of the samples were in agreement.

During SPS zinc analysis aqueous standards were used but the saliva samples were treated with TCA. Warren and coworkers (1981), who analyzed parotid saliva, treated the samples with TCA, and Olson and Hamlin (1968), who analysed serum zinc after treatment with TCA, both used standards prepared in a w/v TCA solution. Other laboratories used aqueous standards (Henkin, et al, 1975b), method of additions (Langmyhr, et al, 1979) or did not report the treatment of the standards (Mather, et al, 1977). Standards and solutions analysed should be treated the same way. In retrospect, the choice of aqueous standards was prompted by a concern with zinc contamination from the TCA. A comparison of aqueous standards acidified with HNO_3 and standards in a TCA (2): water (5) solution is shown in figure 2, which indicates that the calibration curves are comparable. However, a comparison of results for samples analysed with both aqueous and TCA:water standards (Appendix D) indicates that higher zinc concentrations are obtained with the

standards containing TCA. Therefore, the SPS zinc concentrations reported here may be higher than the actual concentrations due to the treatment of the standards.

CONCLUSION

In this study, no relationship was found between zinc concentration in parotid saliva and taste for any subject group. Taste perception (the slope of magnitude estimation data) for sweet and bitter did not correlate with SPS zinc for the untreated, treated and normal weight subjects. A poor correlation was also found in these subject groups for SPS zinc and taste preference for sweet and bitter tastants. However, mean SPS zinc concentration for the untreated groups was significantly lower than that for normal weight controls. The mean SPS zinc concentration of the treated subjects did not differ from the normal weight controls. Flow rate was not related to SPS zinc concentration. However, it is necessary to confirm this result with a selected group of well trained subjects. No relationship was found between SPS zinc and total SPS protein, but the sensitivity of protein to flow rate may have been a confounding factor. The concentration of SPS zinc was found to increase concurrent with post operative times in months. In addition, the low SPS zinc in the untreated group is suggestive of altered zinc metabolism in obesity. A nutritional assessment study of zinc nutritional status before and after jejunoileal bypass would be of interest to confirm these observations.

REFERENCES CITED

Atkinson, R.L., W.T. Dahms, G. Bray, R. Jacob and H. Sandstead. 1978. Plasma zinc and copper in obesity and after intestinal bypass. Ann. Int. Med. 89:491.

Atkin-Thor, E., B.W. Goddard, J. O'Nion, R.L. Stephen and W.J. Kolff. 1978. Hypogeusia and zinc depletion in chronic dialysis patients. Am. J. Clin. Nutr. 31:1948.

Bray, G.A., R. Barry, J.R. Benfield, P. Castelnuovo-Tedesco and J. Rodin. 1976. Intestinal bypass surgery for obesity decreases food intake and preferences. Am. J. Clin. Nutr. 29:779.

Burch, R.E., D.A. Sackin, J.A. Ursick, M.N. Jetton and J.F. Sullivan. 1978. Decreased taste and smell acuity in cirrhosis. Arch. Intern. Med. 138:743.

Burge, J.C., H.S. Park, C.P. Whitlock and R. Schemmel. 1979; Taste acuity in patients undergoing long term hemodialysis. Kid. Internat. 15:49.

Buzina, R., M. Jusic, J. Sapunar and N. Milanovic. 1980. Zinc nutrition and taste acuity in school children with impaired growth. Am. J. Clin. Nutr. 33:2262.

Casper, R.C., B. Kirschner, H. Sandstead, R.A. Jacob and J.M. Davis. 1980. An elevation of trace metals, vitamins and taste function in anorexia nervosa. Am. J. Clin. Nutr. 33:1801.

Catalanotto, F.A. 1978a. Gustatory preferences in zinc deficient adult rats. Chem. Senses Flavor 3:299.

Catalanotto, F.A. 1978b. The trace metal zinc and taste. Am. J. Clin. Nutr. 31:1098.

Catalanotto, F.A. and P. Lacy. 1977. Effect of a zinc deficient diet upon fluid intake in the rat. J. Nutr. 107:436.

Catalanotto, F.A. and R. Nanda. 1977. The effects of feeding a zinc deficient diet on taste acuity and tongue epithelium in rats. J. Oral Path. 6:211.

Chesters, J.K. and J. Quarterman. 1970. Effects of zinc deficiency on food intake and feeding patterns of rats. Br. J. Nutr . 24:1061.

Dawes, C. 1978. The chemistry and physiology of saliva. Pages 593-616 in J.H. Shaw, E.A. Sweeney, C.C. Cappuccino and S.M. Meller, eds. Textbook of Oral Biology. W.B. Saunders Co., Philadephia.

Dawes, C. 1969. The effects of flowrate and duration of stimulation on the concentrations of protein and the main electrolytes in human parotid saliva. Arch. Oral Biol. 14:277.

Dawes, C. 1967. The effect of flowrate and length of stimulation on the protein concentration in human parotid saliva. Arch. Oral Biol. 12:783.

DeWind, I.T. and H. Payne. 1976. Intestinal bypass surgery for morbid obesity. J. Am. Med. Assoc. 236:2298.

Everett, G.A. and J. Apgar. 1979. Effects of zinc status on salivary zinc concentrations in the rat. J. Nutri. 109:406.

Faber, J., J.G. Randolph, S. Robbins and J.C. Smith, 1978. Zinc and copper status in young patients following jejunoileal bypass. J. Surg. Res. 24:83.

Freeland - Graves, J.H., P.J. Hendrickson, M.L. Ebangit and J.Y. Snowden. 1981. Salivary zinc as an index of zinc status in women fed a low zinc diet. Am. J. Clin. Nutr. 34:312.

Freeland-Graves, J.H., P.W. Bodzy and M.A. Eppright. 1980. Zinc status of vegetarians. J. Am. Diet. Assoc. 77:655.

Friedman, B.J., J. Freeland-Graves, W-S. Han, R. Young, M. Schorlemer and J. Geders. 1980. Effect of zinc supplementation on taste acuity in young women. Fed. Proc. 39:651. (Abstr.)

Gregor, J.L. and V.S. Sickles. 1979. Saliva zinc levels: potential indicators of zinc status. Am. J. Clin. Nutr. 32:1859.

Hambidge, K.M., C. Hambidge, M. Jacobs and J.D. Baum. 1972. Low levels of zinc in hair, anorexia, poor growth and hypogeusia in children. Pediat. Res. 6:868.

Henkin, R.I. 1978. Zinc, saliva and taste : interrelationship of gustin, nerve growth factor, saliva and zinc. Pages 35-48 in K.M. Hambidge and B.L. Nichols, Jr., eds. Zinc and copper in clinical medicine. S.P. Medical and Scientific Books, New York, N.Y.

Henkin, R.I., P. Schecter., W.T. Friedwald, D.L. Demets and M. Raff. 1976. A double blind study of the effects of zinc sulphate on taste and smell dysfunction. Amer. J. Med. Sci. 272:285.

Henkin, R.I., R.E. Lippoldt, J. Bilstad and H. Edelhoch. 1975a. A zinc protein isolated from human parotid saliva. Proc. Nat. Acad. Sci. 72:488.

Henkin, R.I., C.W. Meuller and R.O. Wolf. 1975b. Estimation of zinc concentration of parotid saliva by flameless atomic absorption spectrophotometry in normal subjects and in patients with idiopathic hypogeusia. J. Lab. Clin. Med. 86:175.

Henkin, R.I., B.M. Patten, P.K. Re and D. Bronzert. 1975c. A syndrome of acute zinc loss. Arch. Neurol. 32:745.

Henkin, R.I., P.J. Schecter, R. Hoye and C.F.T. Mattern. 1971. Idiopathic hypogeusia with dysgeusia, hyposmia and dysosmia. J. Am. Med. Assoc. 217:434.

Ingle, J.D., Jr. 1974. Sensitivity and limit of detection in quantitative spectrometric methods. J. Chem. Educ. 51:100.

Langmyhr, F.J., B. Eyde and J. Jonsen. 1979. Determination of the total content and distribution of cadmium, copper and zinc in human parotid saliva. Anal. Chim. Acta 107:211.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265.

Majahan, S.K., A.S. Prasad, J. Lambujon, A.A. Abbasi, W.A. Briggs and F.D. McDonald. 1980. Improvement of uremic hypogeusia by zinc: a double blind study. Am. J. Clin. Nutr. 33:1517.

Mathur, A., K. Wallenius and M. Abdulla. 1977. Relationship between zinc content in saliva and blood in healthy human adults. Scand. J. Clin. Lab. Invest. 37:469.

McConnell, S.D. and R.I. Henkin. 1974. Altered preference for sodium chloride, anorexia and changes in plasma and urinary zinc in rats fed a zinc deficient diet. J. Nutr. 104:1108.

Olson, A.B. and W.B. Hamlin. 1968. Serum copper and zinc by atomic absorption spectrophotometry. Atomic Absorption Newsletter. 7:69.

Rodin, J. 1980. Changes in perceptual responsiveness following jejunoileostomy: their potential role in reducing food intake. Am. J. Clin. Nutr. 33:457.

Rodin, J., H.R. Moskowitz and G.A. Bray. 1976. Relationship between obesity, weight loss and taste responsiveness Phys. Behav. 17:591.

Shatzman, A.R. and R.I. Henkin. 1980. Saliva zinc - gustin-taste interrelationships. Fed. Proc. 39:897. (Abstr.)

Snowden, J. and J.H. Freeland. 1978. Circadian rhythm of zinc in saliva. Fed. Proc. 37:890. (Abstr.)

Solomons, N.W. 1979. On the assessment of zinc and copper nutriture in man. Am. J. Clin. Nutr. 32:856.

Solomons, N.W., I.H. Rosenberg and H.H. Sandstead. 1976. Zinc nutriture in celiac sprue. Am. J. Clin. Nutr. 29:371.

Solomons, N.W., I.H. Rosenberg, H.H. Sandstead and K.P. Vo-Khactu. 1977. Zinc deficiency in Crohn's disease. Digestion 16:87.

Vreman, H.J., C. Ventu, J. Leegwater, C. Oliver and M.W. Weiner. 1980. Taste, smell and zinc metabolism in patients with chronic renal failure. Nephron 26:163.

Warren, D.C., H.W. Lane and M. Mares. 1981. Variability of zinc concentrations in human stimulated parotid saliva. Biol. Tr. Elem. Res. 3:99.

Williams, R.B. and C.F. Mills. 1970. The experimental production of zinc deficiency in the rat. Br. J. Nutr. 24:989.

APPENDIX A

CONSENT FORM

CONSENT FORM

I agree to be a subject in a research study designed to relate sweetness and bitterness to the amount of zinc in saliva. I will be asked to taste six solutions of a bitter tastant (caffeine) and six solutions of a sweet tastant (sucrose) and rate them according to two scales. I will be asked to do this at three different times. This will require 1/2 hour each time.

I will be asked to supply the investigators with some saliva. A small suction cup will be fitted on one of the salivary glands in my cheek and the saliva suctioned into a test tube. I understand that the investigators will need three different samples of saliva. Each sample collection will require about 20 minutes.

If I decide to withdraw from the study, I will continue to receive the normal services of my physician.

Interviewer

_____

Subject: _____

Address: _____

Telephone: _____

APPENDIX B

MOLAR CONCENTRATIONS OF TASTANT SOLUTIONS AND DISTRIBUTION OF PREFERRED CONCENTRATIONS

MOLAR CONCENTRATION[a] OF CAFFEINE FOR EACH SOLUTION IN THE FOUR SERIES

Series	Solution					
	1	2	3	4	5	6
A	0.06	0.12	0.24	0.48	0.96	1.92
B	0.09	0.18	0.36	0.72	1.44	2.88
C	0.12	0.24	0.48	0.96	1.92	3.84
D	0.15	0.30	0.60	1.20	2.40	4.80

[a] Expressed as mmol/L

MOLAR CONCENTRATION[a] OF SUCROSE IN EACH SOLUTION OF THE SERIES

Solution	1	2	3	4	5	6
	0.093	0.185	0.370	0.740	1.480	2.960

[a] Expressed as mol/L

FREQUENCY DISTRIBUTION OF PREFERRED BITTER CONCENTRATION BY CASE[a], SERIES[b] AND NUMBER

Series	A			B			C			D		
Solution Number	C	T	U	C	T	U	C	T	U	C	T	U
1	2	-	1	-	1	-	-	1	1	-	-	-
2	3	1	1	-	-	-	-	-	-	-	-	-
3	2	2	1	1	-	-	1	-	2	-	1	-
4	2	4	2	1	-	1	1	1	1	1	-	-
5	2	-	1	-	-	-	1	2	-	1	1	1
6	5	-	-	-	3	-	-	-	-	1	-	-

[a]Case: C = control, T = treated, U = untreated

[b]Series = A, B, C or D

FREQUENCY DISTRIBUTION OF PREFERRED

SUCROSE SOLUTION BY CASE AND SOLUTION NUMBER

Solution Number	Case		
	Controls	Treated	Untreated
1	8	5	1
2	10	7	4
3	4	3	2
4	1	0	3
5	0	0	1
6	1	2	1

APPENDIX C

REGRESSION EQUATIONS FOR SALIVARY FLOWRATE AND TOTAL STIMULATED PAROTID SALIVARY PROTEIN

APPENDIX C1

REGRESSION EQUATIONS BY GROUP FOR SPS ZINC
ON SALIVARY FLOWRATE

Group	Regression Equation[a]	r
Untreated	Y = 28.80 + 7.70 (X)	0.158
Treated	Y = 64.21 - 27.42 (X)	0.308
Controls	Y = 53.42 - 23.28 (X)	0.386

[a] Y = a + b (X) where Y = zinc concentration (ng/mL) and X = flow rate (mL/min)

APPENDIX C2

REGRESSION EQUATIONS FOR SPS ZINC ON
TOTAL SPS PROTEIN BY GROUP

Group	Regression Equation[a]	r
Untreated	Y = 27.44 + 0.05 (X)	0.148
Treated	Y = 16.33 + 0.16 (X)	0.381
Controls	Y = 24.15 + 0.10 (X)	0.381

[a] Y = a + b (X) wjere Y = zinc concentration (ng/mL) and X = protein (mg/dL).

APPENDIX D

COMPARISON OF AQUEOUS STANDARDS AND STANDARDS CONTAINING TCA

ANALYSIS OF ZINC CONCENTRATION IN

STIMULATED PAROTID SALIVA BY TWO METHODS

Sample	Varian Techtron AA5[1]	Perkin Elmer 4000[2]
	ng/mL	ng/mL
1	62	77
2	17	28
3	17	23
4	19	26
5	22	30
6	178	188
7	24	29
8	19	19
9	19	20
10	18	22
11	71	76
12	101	125
13	51	57
14	18	18
15	61	70

[1] Using aqueous standards, acidified with HNO_3

[2] Using standards containing TCA and distilled water (2:5)