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The Effect of Copper on Taste Sensitivity and Caries Activity

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THE EFFECT OF COPPER
ON TASTE SENSITIVITY AND CARIES ACTIVITY

by

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LIFE

John S. Borello, was born in Chicago, Illinois, July 22, 1953. He was graduated from St. Vincent Ferrer grade school in 1966. From September, 1966 to June, 1970, he attended Fenwick High School. Following graduation from Fenwick he entered Loyola University, Chicago, Illinois, in 1970. In 1974, he graduated Loyola University, receiving a Bachelor of Science degree.

In January, 1975, he began his graduate studies in the Department of Oral Biology of Loyola University, School of Dentistry.

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INTRODUCTION

AND

STATEMENT OF THE PROBLEM

In the past seventy-five years much research has been done in two areas, taste sensitivity and dental caries. The results have shown that: 1) taste thresholds towards the four modalities (sweet, sour, bitter and salty) vary among normal individuals and that these thresholds are affected by several endogenous oral factors; 2) dental caries is a common and variable disease with many subjective and objective parameters; 3) caries activity is directly related to sugar consumption; and 4) greater concentrations of sucrose are required by caries resistant persons to perceive a sweet taste, whereas lower concentrations of sucrose are required to perceive a sweet taste by caries active persons.

The purpose of this experiment is to study the effects of salivary copper on taste sensitivity and on caries conduciveness of saliva. The data collected will be analyzed to determine whether there exist any correlation between taste sensitivity and copper concentrations of saliva, and between saliva copper levels and caries conduciveness of saliva.

CHAPTER II

REVIEW OF THE RELATED LITERATURE

Dreizen (12) analyzed human saliva and found the presence of copper in all the samples of saliva. The concentration in stimulated saliva ranged from 10 to 47.5 micrograms per 100ml. with the mean from 23-25 micrograms per 100ml. He also found that the concentration of copper required to inhibit the acid production by microrganisms in human whole saliva greatly exceeds those found to be actually present in this medium. In vitro studies (11) clearly demonstrated that in the maximum quantities avaliable in saliva, copper does not influence dental caries activity, as measured by acid production in saliva and the growth of an oral strain of Lactobacillus Acidophilus.

Giampiccolo (14) studied the effects of ingestion of copper sulfate by albino rats and found a 13.55% reduction in dental caries when compared to control animals fed Ershoff's diet (14) without a copper suppliment. However, the concentration of $CuSO_4$ found to reduce caries also was found to have a marked toxic effect, causing cirrhosis of the liver.

Zengo and Mandel (31) studied sucrose tasting and dental caries in man. The results of this experiment showed that in deciduous dentition greater concentrations of sucrose are required by caries resistant persons to perceive a sweet taste, whereas lower concentrations of sucrose are required by caries active person.

Several types of bacteria have been associated with the cause of dental caries. Most common is Lactobacillus, which is both acidogenic and aciduric and thus is capable of producing a lowered pH in the media and surviving in it. Furthermore, the relationship between high numbers of salivary lactobacilli and low pH minima in plaques, as well as the correlation between the continued presence of relatively high numbers of salivary lactobacilli and caries activity associate this organism with the decay process.

A considerable amount of work has been done to determine whether the concentration of lactobacilli can be related to dental caries. There seems to be a divergence of opinion concerning the nature of the relationship between caries activity and the presence of lactobacilli in the oral cavity. Some researchers believe that lactobacilli represents the main etiological factor in dental caries (24) while others feel that the occurrence of these lactobacilli in the oral cavity is a phenomenon only secondary to the development of dental caries (2).

Koehne, Bunting and Morell (20) found that an increase in the dietary carbohydrate resulted in an increase in the number of lactobacilli as well as an increase in caries activity. Hadley (16), Jay (18) and Becks (1) observed that drastic restriction of the dietary carbohydrates is accompanied, or followed by a decrease in the number of oral lactobacilli and, as a rule, also by a decrease in caries activity.

In this connection it might be useful to mention that Bunting (5) and Jay (17) failed to implant lactobacilli in mouths which were naturally free of them.

Collins (9), Becks (1) and Stralfors (28), found that the number of lactobacilli in the saliva can be used as an index of caries activity. Lactobacilli have been demonstrated in the saliva of some eighty percent of all patients with rampant caries, as against ten to twenty percent of those that are caries free, or caries inactive. It has also been shown by several inverstigators (16,26) that salivary lactobacillus counts in those individuals who are caries immune are very low and may even be non-existant in many of these subjects.

Steinle, Madonia and Bahn (27) have shown that oral lactobacilli are strictly localized to sites on the teeth where caries occur, or have occurred, and the magnitude of the salivary lactobacillus count is directly related to the number of such sites harboring lactobacilli at the time the count was made.

Snyder (26) found that acid conditions which produce caries favor persistence of lactobacillus and it was shown that there is, in general, a correlation between the lactobacillus count and caries activity. Carious dentin is almost always acidic and located within this carious dentin are large quantities of lactobacilli. If all the carious lesions are eliminated, the oral lactobacillus count falls noticably.

It has been shown (26) that a pH of 5.5 or lower is required before enamel will dissolve. It is at a pH of about 5.5 that the streptococci begin to be inhibited whereas the proliferation of lactobacilli is facillitated. Since acidities of this order can arise only in stagnation areas around teeth and it is only in such areas that caries occur,

the link between dental caries and increasing numbers of oral lactobacilli is, surly, self-evident.

Green (15) studied the differences between salivas from caries suseptible and caries immune persons and found that saliva from caries immune persons is more inhibitory to the growth of lactobacilli than is that from suseptible ones. Because of that, Green assumes saliva from immune mouths to contain some factors inhibitory to oral lactobacilli, or to be deficient in some substances necessary for the rapid growth of lactobacilli.

Kenny (19) determined from his initial study, that as plaque bacteria accumulated there was a decrease in the value of the oxidation-reduction potential of the area. He also found that changes in the pH will affect the oxidation-reduction potential by changing the ionic equilibria.

CHAPTER III

METHODS AND MATERIALS

Eighty-nine dental students were selected for this study from the freshman dental class at Loyola University School of Dentistry. This group consisted of eighty-one males and eight females and ranged in age from twenty-one to twenty-five years. Five ml of stimulated saliva were collected from each subject and the samples were immediately refrigerated and frozen. The following afternoon the frozen samples were removed from the refrigerator and allowed to thaw. These samples were then analyzed to determine the concentration of copper and lactobacilli present in each sample.

The lactobacillus counts were made on pourplates utilizing Kulp's Tomato Agar Technique. This agar is prepared as follows:

Tomatoes 250g
Distilled water 500 ml

The tomatoes are minced and steamed in the water for an hour or until they are pulped, then Clarified through gauze and filtered through paper.

Peptone 10g
Peptonized milk 10g
Agar 20g
Tomato juice 400 ml
Distilled water 600 ml

Dissolve the above solids in water by heating. Add tomato juice, mix, and sterilize at 115°C for twenty minutes. The final pH value of this medium should be 6 - 6.2.

This bacterial medium is well known to be selective for oral lactobacilli, when salivary samples are analized. The counts are given as organisms per ml of saliva and groupings are made for four ranges and related to clinical caries activity. The groupings are:

Class I, 0-1000 lactobacilli = Non Caries Active
Class II, 1001-10,000 lactobacilli = Slightly Active
Class III, 10,001-100,000 lactobacilli = Moderately Active
Class IV, over 100,000 lactobacilli = Highly Active

The copper concentrations were determined by the spectrographic method (10), using a Hitachi arc spectrograph, with a Beckman recording attachment. The individual's salivary sample was spun down and from the clear supernatant a 0.1 ml sample was used for analysis. This sample was then placed in the arc cup of the arc spectrometer and the copper emission was measured. The spectrograph vaporizes the copper and produces a color which is logarithmicly related to the amount of copper present in the saliva. The values were obtained from a standard curve prepared from known concentrations of Baker's Analyzed, Analytical grade $CuSO_4$-$5H_2O$ (Lot No. 4844) in the range from 3 to 1250 micrograms of copper per liter of solution. The levels of copper and lactobacilli for each subject was recorded and is found in Table I of the Appendix, which represents controlled values to be used in this study.

Immediately following the collection of saliva the subjects were given a sucrose taste sensitivity test to determine their sugar taste threshold. The sucrose concentrations ranged from 5 to 50 mM/L and each subject was given increasing concentrations of sucrose solutions with which to sample and their responses were recorded, Table I (Appendix).

The lowest concentration at which an individual was able to just detect a distinct sweet taste was regarded as the taste threshold. Between each sample the subject was required to rinse his mouth with tap water before sampling the next higher concentration. When the threshold was reached, lower levels were again tested to assure no sweet detection at lower levels.

The subject was then instructed to throughly rinse the mouth with a 0.01% copper sulfate solution for thirty seconds and to expectorate the remaining solution.

Following this, the subject was again given the sucrose taste test in order to determine whether or not the copper sulfate solution would alter the sugar taste threshold of the individual. Again the lowest concentration at which the individual just detected a sweet taste was regarded as the threshold. The data obtained from the sucrose taste tests was recorded and is located in Table I of the Appendix.

Seventy-two of the eighty-nine students used for collecting the saliva samples were then given the Reductase Caries Conduciveness Test at the same time periods of the day. This test evaluates the conduciveness of the oral environment to dental caries based on the activity of reductase enzymes in saliva. Reductase activity is high in caries conducive and low in non-conducive mouths. The equipment and procedure for this test is as follows:

Equipment:

a) Saliva stimulator (flavored wax).
b) Collection and Reaction tube. (This is the calibrated vial)
c) Reagent cap. (This is the cap to the vial. It already contains the exact amount of reagent needed for one test, 0.06 mg. Reazurin.)

d) Color Chart (Described Below):

Class I Blue at start, no change in 15 minutes. NONCONDUCIVE
Class II Orchid in 15 minutes. SLIGHTLY CONDUCIVE
Class III Red in 15 minutes. MODERATELY CONDUCIVE
Class IV Red immediately on mixing. HIGHLY CONDUCIVE
Class V White in 15 minutes. (or white and pink) EXTREMELY CONDUCIVE

Procedure:

1) Open the collection tube. Set the reagent cap aside.
2) Chew the saliva stimulator. Do not swallow the saliva, but expectorate directly into the collection tube. Collect saliva until it reaches the calibration mark (5ml). Discard chewed wax.
3) Replace the reagent cap on to the Collection Tube now containing the saliva. Shake for 30 seconds. NOTE THE COLOR.
4) Allow to stand at room temperature for EXACTLY 15 MINUTES.
5) NOTE THE COLOR.

The data collected from the Reductase Fifteen Minute Caries. Test were recorded in Table II of the Appendix.

CHAPTER IV

RESULTS

The results of the tests are shown in Table IX of the Appendix. These findings were divided into two groups. Group I deals with the results of copper on the taste threshold, and Group II consists of those results pertaining to the relationship between copper and the caries conduciveness of saliva.

GROUP I

The sucrose taste threshold for each subject was recorded and a graph was prepared comparing salivary copper levels with sugar taste sensitivity for each individual (Figure 1) using the data found in Table I (Appendix). The subject then rinsed his mouth with copper sulfate solution and after expectorating the remaining solution the subject was again given the sucrose taste test to determine whether the copper sulfate would alter the taste threshold of the individual. The results (Table I, Appendix) were recorded and another graph was prepared comparing the salivary copper level with sugar taste sensitivity after the subject rinsed his mouth with copper sulfate (Figure 2).

An analysis of the data was then conducted to determine if there is any correlation between the salivary copper level and sucrose taste sensitivity. From the statistical analysis of the results it was shown that the correlation (Table III, Appendix) comparing taste thresholds to copper levels before the subject rinsed his mouth with copper sulfate solution,

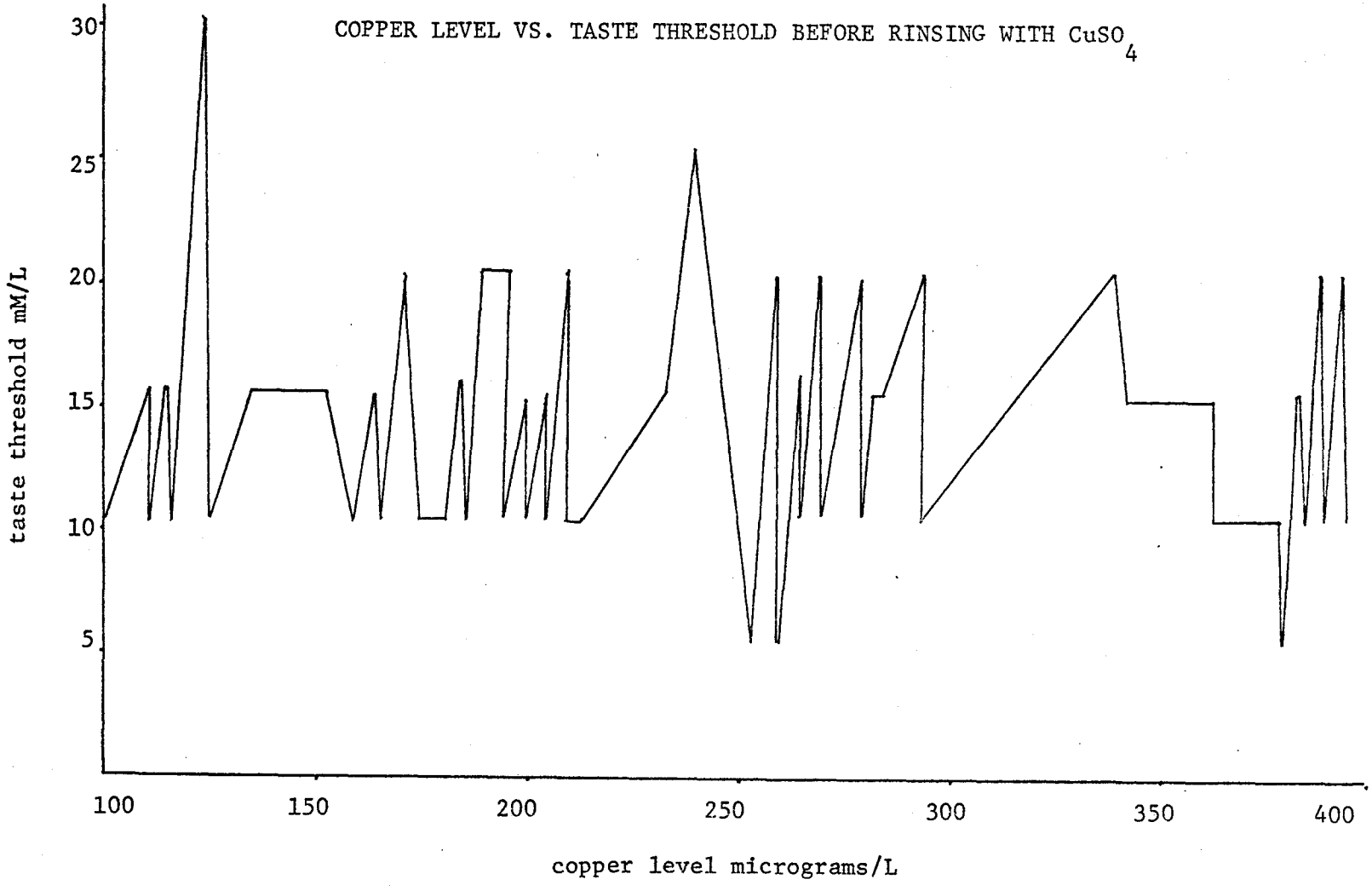

COPPER LEVEL VS. TASTE THRESHOLD BEFORE RINSING WITH $CuSO_4$
taste threshold mM/L
30
25
20
15
10
5
100
150
200
250
300
350
400
copper level micrograms/L

FIGURE 1

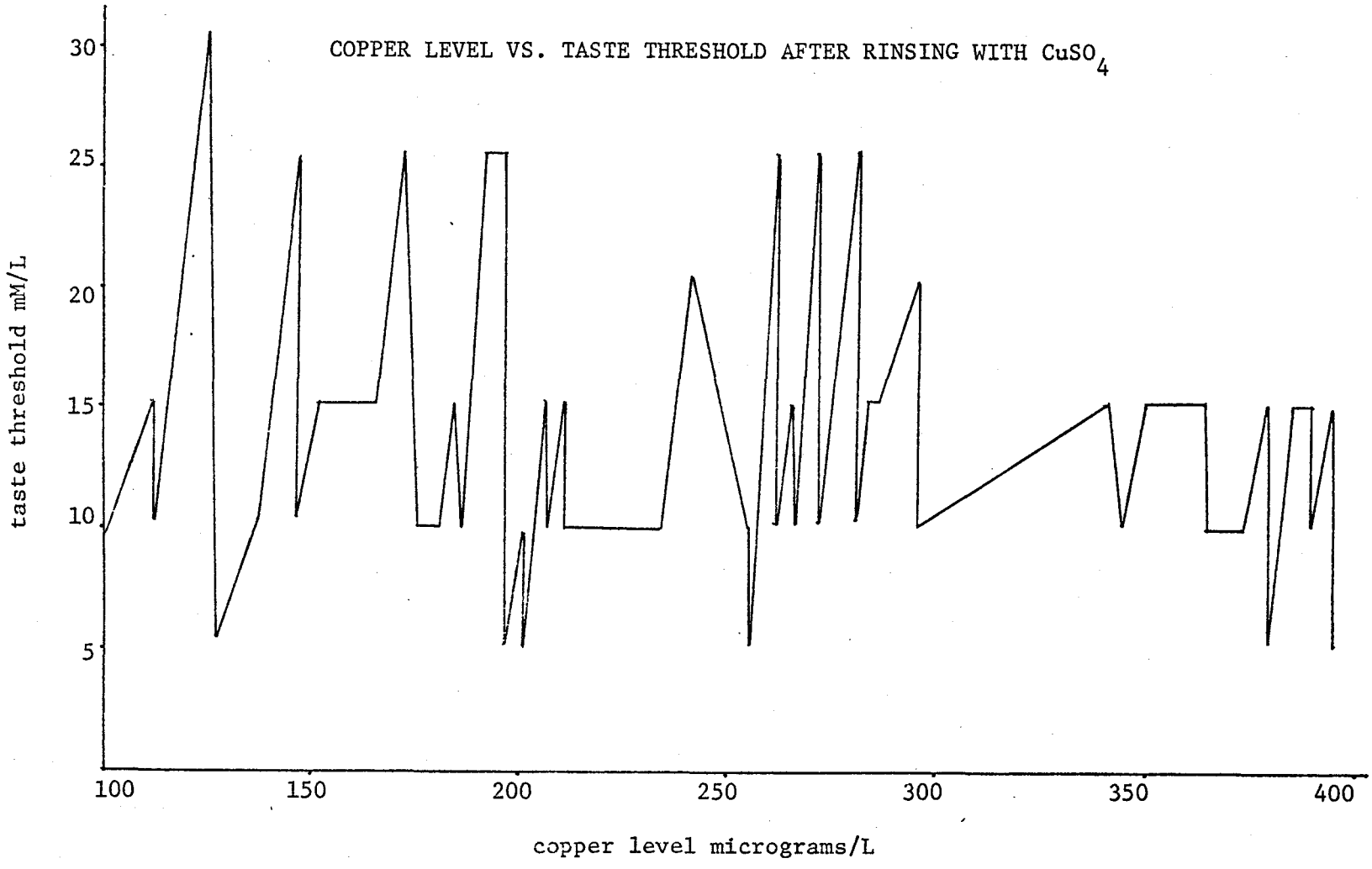

COPPER LEVEL VS. TASTE THRESHOLD AFTER RINSING WITH $CuSO_4$
taste threshold mM/L
30
25
20
15
10
5
100
150
200
250
300
350
400
copper level micrograms/L

FIGURE 2

has a coefficient of correlation of -0.04. The analysis with 87 df (degrees of freedom) at a p of less than 0.05, (t=0.373) shows that the copper level of saliva and the taste threshold are not related.

The correlation comparing taste thresholds and copper levels after the subject rinsed his mouth with copper sulfate has a coefficient of correlation of -0.11 and with 87 df at a p of less than 0.05, (t=1.028) also shows that there is no relation between copper levels and the taste thresholds (Table IV, Appendix).

Following this, the subjects were divided into three groups based upon the copper content in their saliva. The groups are as follows:

Group I = High Copper Content = 301-415 micrograms/L
Group II = Medium Copper Content = 186-300 micrograms/L
Group III = Low Copper Content = 70-185 micrograms/L

The percentage of students from each group whose thresholds were either raised, lowered or remained the same after rinsing their mouths with copper sulfate was determined, and the results are found in Table VIII (Appendix).

When dealing with the sample as a single group it was found that after the subjects rinsed their mouths with copper sulfate solution, 24.7% of the subject's thresholds were raised, 27.0% were lowered and 48.3% remained the same. Next the percentage of those thresholds raised, lowered, and remaining the same were determined for each of these three groups. In Group I (Cu^{++}level 300-415 micrograms/L) it was found that 14% of the subject's thresholds were raised after rinsing with the copper sulfate solution, 45% were lowered and 41% remained the same.

A "Paired t test" was then run on group I to determine if there was a difference in the subject's taste threshold after rinsing with the copper sulfate solution. The results from this test (Table V, Appendix) showed that with 28 df and (t=-2.53) there was a significant difference at the 0.05 level.

In Group II (Cu^{++}level 185-300 micrograms/L) it was determined that 24.3% of the subject's thresholds were raised, 21.6% were lowered and 54.1% remained the same. The t test performed on this group showed that with 36 df and a p value of less than .05, (t=0.25) there was no difference in the taste threshold after rinsing with copper sulfate solution.

In Group III (Cu^{++}level 70-185 micrograms/L) it was shown that after rinsing with copper sulfate, 39.1% of the subjects taste thresholds were raised, 13.1% were lowered and 47.8% remained the same. The results from the t test showed that with 22 df, (t=1.90) there was no significant difference at the .05 level, but at the .10 level a difference does exist.

By running these tests on the individual groups it allows us to see that, while there is no significant change when dealing with the group as a whole, there was a significant difference at higher levels of copper concentration at the 0.05 level and at lower levels of copper content at the 0.10 level. Therefore: it appears that the copper content of the saliva does have an effect on the taste threshold of an individual by lowering the taste threshold at high copper levels and raising the taste threshold at low copper levels.

GROUP II

From a statistical point of view, two tests which showed definitly significant results are as follows: first, the "Between-Within Analysis of Variance," which was used to determine if there is a difference between the salivary copper levels in the five classes of Reductase Activity with regard to the caries conduciveness of the saliva. Note: the classes are based on the activity of Reductase enzymes in saliva, this activity is high in caries conducive and low in non-conducive mouths (Table VII, Appendix) at a p of less than 0.05 and with 71 df, (f=239.98), distinctly shows that there is a difference in the copper level with regard to the caries conduciveness of the saliva.

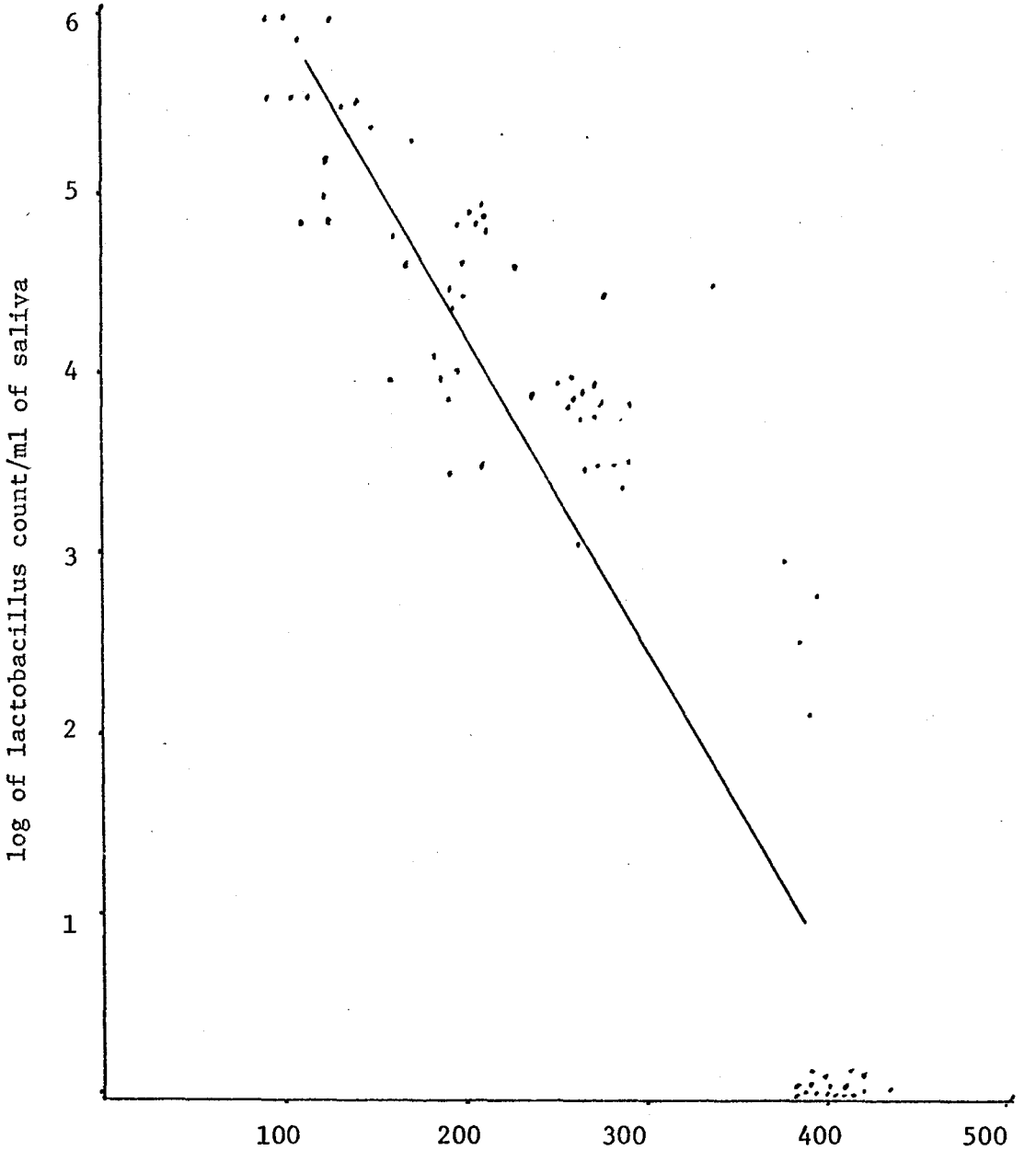
A "K test", was also performed in Table VII of the Appendix to determine if there is a difference between the five classes. The results from this test are as follows:

Classes	I&II	are different
Classes	I&III	are different
Classes	I&IV	are different
Classes	I&V	are different
Classes	II&III	are different
Classes	II&IV	are different
Classes	II&V	are different
Classes	III&IV	are different
Classes	III&V	are different
Classes	IV&V	show no difference between the copper levels with regard to the caries conduciveness of the saliva.

The data collected from the Reductase Fifteen Minute Caries Test was recorded in Table II (Appendix). Figure 3, page 17 is a semi- logarithmic graph describing the relationship between the individual's lactobacillus count and the salivary copper level.

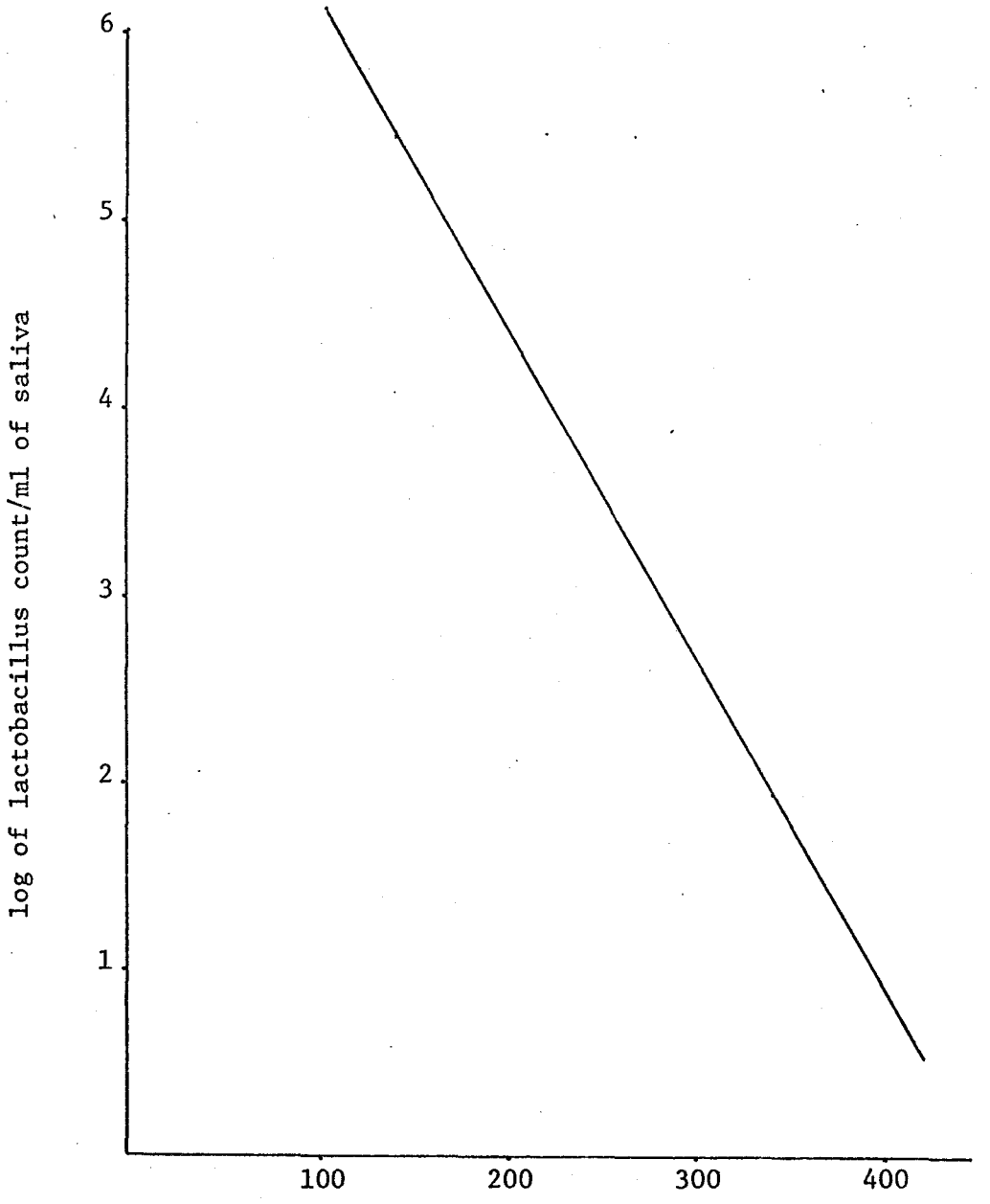
Next, the data from Table II in the Appendix and the subject's copper levels (Table I, Appendix) was used to draw a regression line of the graph in Figure 3. This regression line occurs in Figure 4, and the statistical results are found in Table VI of the Appendix.

The last test run was to test for a logarithmic correlation between the salivary copper levels and the lactobacillus count (Table VI, Appendix). The results of this test proved to be significant at a p of less than 0.01 with 70 df. With the table value for t being 2.00 and ($t=18.44$) we reject the null hypothesis and this shows that the salivary copper level and the lactobacillus counts are logarithmically related. The coefficient of correlation is -0.91, and thus, as the copper level in saliva increases the lactobacillus count decreases.



COPPER LEVEL VS. (LOG) OF LACTOBACILLUS COUNT

FIGURE 3



REGRESSION LINE: COPPER VS. LOG OF LACTOBACILLUS

FIGURE 4

CHAPTER V

DISCUSSION

In an attempt to understand the fundamental nature of dental caries, one is confronted by the question, "What is the agency by which enamel and dentin are dissolved from teeth, under the conditions that exist in the mouth."

Its been found by Fosdick (13) that dental caries develop through an acid decalcification of the mineral portion of the tooth, followed or accompanied by degradation of its organic matrix. The acid is produced by enzymatic conversion of fermentable carbohydrates to lactic and other acids. And if acid does form in protected aspects of the tooth surface faster than it can be neutralized, destroyed or removed, a carious lesion will ultimately result.

The saliva in the oral cavity is saturated with respect to calcium and phosphate ions, and also contains, in addition to these ions, Na^{+}, K^{+}, Cl^{-}, HCO_3^{-}, $SO_4^{=}$, Cu^{++} and a host of trace elements. When the oral cavity is at rest, saliva from it is neutral or slightly acidic and the pH ranges from 6.5 to 7.0 with an average of about 6.8. When the oral cavity is stimulated the salivary pH rises. Maximum stimulation produces a pH of 7.5 to 8.4.

Dental caries ordinarily do not occur on tooth surfaces most readily accessible to saliva. Its occurrence is most frequent in those areas where access to saliva is limited and dental plaque is constantly present.

Green (15) found that approximately one in one hundred persons will be found to be caries immune. It was also shown that these caries immune persons have comparatively few lactobacilli in their saliva.

The purpose of this experiment was to investigate the effects of copper levels in saliva on taste sensitivity and on the caries conduciveness of saliva. In table VIII of the Appendix it can be seen that at "Higher" copper levels, a larger percent of the population's taste thresholds will be lowered, and at "Lower" copper levels a larger percent of the population will raise their threshold after rinsing their mouth with a copper sulfate solution. These conclusions were substantiated by t tests and were significant at a p of less than 0.05 for the former and a p of less than 0.10 for the latter. However, when viewing the population as a whole, as was seen in Chapter IV (Results), an overall significant correlation between the copper level in saliva and the sucrose taste threshold of an individual was not observed.

A positive relation was found between the copper level in saliva and the caries conduciveness test performed on it. Considering the information shown from this experiment it may be concluded that copper is capable of influencing the enzymatic activity of the reductase enzyme and thus limiting reactions in the formation of the products hazardous to the tooth substance. This is shown in Table VII (Appendix) where those with high copper levels are found in Class I of the Reductase caries suseptibility.

As has been shown by numerous investigators, there is a general correlation between the salivary lactobacillus count and caries activity.

In this experiment a correlation has been shown between the salivary copper content and the salivary lactobacillus count. This is seen in Table VI of the Appendix. This is important because when all the data is studied the results show that, at either high or low salivary copper levels one's taste threshold is altered and as the copper content of saliva is increased, the salivary lactobacillus count is decreased and the reductase activity is lowered. Therefore, at elevated salivary copper levels caries conduciveness is decreased.

These results seem to contradict those found by Dreizen (12), where even though he suggested that copper is a normal constituent of human whole saliva, he found that the concentration of copper required to inhibit acid production in human saliva greatly exceeds those quantities actually present in this medium, as indicated by the acid production in saliva and by the growth of an oral strain of lactobacillus.

The results of the present experiment show that there are sufficiently high levels of copper in the saliva to influence dental caries activity and the concentration of lactobacilli. The apparent contradiction may be due to the fact that Dreizen's experiment was performed <u>in vitro</u> whereas the present experiment was performed <u>in vivo</u>. Many biochemical reactions which are constantly occurring <u>in vivo</u> might not be present <u>in vitro</u> and thus obscure the results.

CHAPTER VI

SUMMARY

The purpose of this study was to investigate the relationship between taste sensitivity and caries activity. The experimental procedures were designed to demonstrate a possible relationship between the salivary copper level and taste sensitivity, as well as the salivary copper content and the caries conduciveness of saliva. Some relationships were established on the basis of each individual test. The following summary seems to be justified on the basis of the results of this study:

1) Copper is a salivary constituent present in most humans.
2) Copper seems to affect the sucrose taste threshold at "High" copper concentrations and also at "Low" copper levels.
3) At "High" initial copper levels rinsing the mouth with the copper sulfate solution lowers the sweet taste threshold, whereas at "low" initial copper levels rinsing with a copper sulfate solution raises the sweet taste threshold.
4) The higher the copper level in the saliva the less caries conducive the saliva is.
5) The higher the copper content in the saliva the lower the lactobacillus count is.
6) The higher the copper content in the saliva the lower the reductase activity is.

7) Lactobacillus counts and the reductase activity are low in non-conducive mouths.

8) Non-conducive saliva is found when the salivary copper content is high.

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APPENDIX

TABLE I

ACTUAL ANALYTICAL DATA

SUBJECT	COPPER LEVEL micrograms/L	REDUCTASE CLASS	LACTOBACILLUS COUNT /ml of saliva	TASTE THRESHOLD BEFORE mM/L	TASTE THRESHOLD AFTER mM/L
1	375	1	800	10	10
2	260	2	6,520	15	20
3	200	3	45,350	10	10
4	210	3	73,600	20	15
5	280	2	2,450	20	25
6	295	2	5,200	20	20
7	125	4	165,000	45	50
8	410	1	0	15	15
9	395	1	525	15	10
10	260	-	4,670	20	25
11	405	1	0	30	30
12	275	2	7,520	15	15
13	283	2	3,210	15	15
14	255	2	5,800	5	10
15	390	1	0	15	10
16	205	3	86,050	10	10
17	145	4	254,000	15	25
18	365	-	0	10	15
19	175	2	3,520	10	10

TABLE I (CONTINUED)

SUBJECT	COPPER LEVEL micrograms/L	REDUCTASE CLASS		LACTOBACILLUS COUNT /ml of saliva	TASTE THRESHOLD BEFORE mM/L	TASTE THRESHOLD AFTER mM/L
20	190	2		9,500	20	25
21	350	-		285	15	15
22	390	1		0	20	15
23	110	5		305,150	15	15
24	410	1		0	20	15
25	395	1		150	20	15
26	210	3		86,500	15	15
27	385	1		0	15	15
28	115	5		605,100	15	15
29	195	3		12,510	20	25
30	125	-		91,100	10	15
31	160	2		8,360	10	15
32	265	2		1,500	15	15
33	240	-		6,550	25	20
34	95	5		345,000	10	10
35	380	1		0	10	10
36	195	2		2,610	20	15
37	410	1		0	10	5
38	110	4		73,600	10	10
39	360	1	OUT	0	15	15

TABLE I (CONTINUED)

SUBJECT	COPPER LEVEL micrograms/L	REDUCTASE CLASS	LACTOBACILLUS COUNT /ml of saliva	TASTE THRESHOLD BEFORE mM/L	TASTE THRESHOLD AFTER mM/L
40	385	1	350	10	15
41	115	-	870	10	15
42	265	2	7,600	10	10
43	210	-	3,210	15	15
44	270	2	8,050	15	15
45	100	5	855,000	10	10
46	150	4	215,500	15	15
47	235	-	45,100	15	10
48	210	3	51,500	10	10
49	285	2	6,600	15	15
50	295	2	2,500	10	10
51	195	3	20,200	10	5
52	270	2	5,660	20	25
53	200	3	86,500	10	5
54	380	1	0	5	5
55	390	1	0	10	10
56	185	3	15,500	10	15
57	280	2	31,150	10	10
58	135	4	280,500	15	10
59	205	-	2,660	15	15
60	175	-	205,500	10	15

TABLE I (CONTINUED)

SUBJECT	COPPER LEVEL micrograms/L	REDUCTASE CLASS	LACTOBACILLUS COUNT /ml of saliva	TASTE THRESHOLD BEFORE mM/L	TASTE THRESHOLD AFTER mM/L
61	165	-	97,500	15	15
62	145	-	66,500	15	15
63	260	-	3,550	5	10
64	410	1	0	10	15
65	415	1	0	10	10
66	390	1	0	15	10
67	125	4	110,100	15	15
68	185	2	6,500	15	15
69	390	1	0	15	10
70	110	4	355,000	15	15
71	170	3	43,250	20	25
72	185	3	16,400	15	10
73	380	1	0	10	15
74	165	3	56,600	10	15
75	345	-	0	15	10
76	270	2	3,450	10	10
77	180	-	123,400	10	10
78	340	2	25,450	20	15
79	395	-	0	10	5
80	410	1	0	15	15
81	270	2	8,500	10	10

TABLE I (CONTINUED)

SUBJECT	COPPER LEVEL micrograms/L	REDUCTASE CLASS	LACTOBACILLUS COUNT /ml of saliva	TASTE THRESHOLD BEFORE mM/L	TASTE THRESHOLD AFTER mM/L
82	90	5	855,500	10	15
83	385	1	0	15	15
84	255	2	8,500	5	5
85	410	1	0	20	15
86	215	3	73,000	10	10
87	200	3	27,500	15	10
88	365	-	0	15	10
89	405	1	0	15	15
90	125	5	675,000	10	5

TABLE II

DATA FROM THE REDUCTASE FIFTEEN MINUTE CARIES TEST

CLASS I NON-CONDUCIVE

SUBJECT NO.	COPPER LEVEL micrograms/L	LACTOBACILLUS COUNT per ml of saliva
1	375	800
8	410	0
9	395	525
11	405	0
15	390	0
22	390	0
25	395	150
27	385	0
35	380	0
37	410	0
40	385	350
54	380	0
55	390	0
64	410	0
65	415	0
66	390	0
69	390	0
73	380	0
80	410	0

TABLE II (CONTINUED)

SUBJECT NO.	COPPER LEVEL micrograms/L	LACTOBACILLUS COUNT per ml of saliva
83	385	0
85	410	0
89	405	0

CLASS II SLIGHTLY CONDUCIVE

SUBJECT NO.	COPPER LEVEL micrograms/L	LACTOBACILLUS COUNT per ml of saliva
2	260	6,520
5	280	2,450
6	295	5,200
12	275	7,520
13	283	3,210
14	255	5,800
19	175	3,520
20	190	9,500
31	160	8,360
32	265	1,500
36	195	2,610
42	265	7,600
44	270	8,050
49	285	6,600
50	295	2,500
52	270	5,660

TABLE II (CONTINUED)

SUBJECT NO.	COPPER LEVEL micrograms/L	LACTOBACILLUS COUNT per ml of saliva
57	280	31,150
68	185	6,500
76	270	3,450
78	340	25,450
81	270	8,500
84	255	8,500

CLASS III MODERATELY CONDUCIVE

SUBJECT NO.	COPPER LEVEL micrograms/L	LACTOBACILLUS COUNT per ml of saliva
3	200	45,350
4	210	73,600
16	205	86,050
26	210	86,500
29	195	12,510
48	210	51,500
51	195	20,200
53	200	86,500
56	185	15,500
71	170	43,250
72	185	16,400
74	165	56,600
86	215	73,000

TABLE II (CONTINUED)

SUBJECT NO.	COPPER LEVEL micrograms/L	LACTOBACILLUS COUNT per ml of saliva
87	200	27,500

CLASS IV HIGHLY CONDUCIVE

SUBJECT NO.	COPPER LEVEL micrograms/L	LACTOBACILLUS COUNT per ml of saliva
7	125	165,000
17	145	254,000
38	110	73,600
46	150	215,500
58	135	280,500
67	125	110,100
70	110	355,000

CLASS V EXTREMELY CONDUCIVE

SUBJECT NO.	COPPER LEVEL micrograms/L	LACTOBACILLUS COUNT per ml of saliva
23	110	305,150
28	115	605,100
34	95	345,000
45	100	855,000
82	90	855,500
90	125	675,000

TABLE III

TASTE VS. COPPER LEVEL BEFORE RINSING WITH $CuSO_4$

REGRESSION AND CORRELATION

COPPER LEVEL		TASTE THRESHOLD		
X	X^2	Y	Y^2	$\Sigma XY = 322,716$
$\Sigma X = 23,128$		$\Sigma Y = 1250$		
$\Sigma X^2 = 6,942,314$		$\Sigma Y^2 = 20,300$		
$\bar{X} = 259.87$		$\bar{Y} = 14.04$		
$N = 89$		$N = 89$		

$$b = \frac{\Sigma XY - (\Sigma X)(\Sigma Y)/N}{\Sigma X^2 - \frac{(\Sigma X^2)}{N}}$$

$$b = \frac{322,716 - (23,128)(1250)/89}{6,942,314 - (23,128)^2/89}$$

$$b = \frac{-2115.46}{932,152.5}$$

$$b = -0.0023$$

$$a = \bar{Y} - b\bar{X}$$

$$a = 14.04 - (-0.0023)(259.87)$$

$$a = 14.64$$

I) REGRESSION LINE

$$Y = 14.64 + (-.0023)(X)$$

TABLE III (CONTINUED)

II) LINEAR CORRELATION

$$r = \frac{\sum XY - (\Sigma X)(\Sigma Y)/N}{\sqrt{(\Sigma X^2 - (\Sigma X)^2/N)(\Sigma Y^2 - (\Sigma Y)^2/N}}$$

$$r = \frac{322,716 - (23,128)(1,250)/89}{\sqrt{\left[6,924,314 - \frac{(23,128)^2}{89}\right]\left[20,300 - \frac{(1,250)^2}{89}\right]}}$$

$$r = \frac{-2,115.46}{50,573}$$

$r = -0.04$ no correlation

$df = N-2$
$=89-2$
$=87$

$$t = \sqrt{\frac{r^2 (N-2)}{1-r^2}}$$

$$t = \sqrt{\frac{(-.04)^2 (87)}{1-(-.04)^2}}$$

$t = 0.373$

Table value for t at a $p < .01$ equals 2.63 $\therefore$ X and Y are not related.

TABLE IV

TASTE VS. COPPER LEVEL AFTER RINSING WITH $CuSO_4$

REGRESSION AND CORRELATION

COPPER LEVEL — TASTE THRESHOLD — $\Sigma XY = 317,195$

X — X^2 — $Y = 1245$

$\Sigma X = 23,128$ — $\Sigma Y^2 = 20,800$

$\Sigma X^2 = 6,942,314$ — $\Sigma \bar{Y} = 13.99$

$\bar{X} = 259.87$ — $N = 89$

$N = 89$

$$b = \frac{\Sigma XY - (\Sigma X)(\Sigma Y)/N}{X^2 - (\Sigma X)^2/N}$$

$$b = \frac{317,195 - (23,128)(1,245)/89}{6,942,314 - (23,128)^2/89}$$

$$b = \frac{-6337.13}{932,152.5}$$

$$b = -0.0068$$

$$a = \bar{Y} - b\bar{X}$$

$$a = 13.99 - (-0.0068)(259.87)$$

$$a = 15.75$$

I) REGRESSION LINE

$$Y = 15.75 + (-0.0068)(X)$$

TABLE IV (CONTINUED)

II) LINEAR CORRELATION

$$r = \frac{\Sigma XY - (\Sigma X)(\Sigma Y)/N}{\sqrt{(\Sigma X^2 - (\Sigma X)^2/N)(\Sigma Y^2 - (\Sigma Y)^2/N)}}$$

$$r = \frac{-6337.13}{\sqrt{[932,152.5][20,800 - (1,245)^2/89}}$$

$$r = \frac{-6337.13}{56,100}$$

$r = -0.11$ no correlation

$df = N-2$

$= 89-2$

$= 87$

Table value for t at a $p < .01$ equals 2.63 $\therefore$ X and Y are not related.

$$t = \sqrt{\frac{r^2 (N - 2)}{1 - r^2}}$$

$$t = \sqrt{\frac{(-.11)^2(87)}{1 - (-.11)^2}}$$

$t = 1.028$

TABLE V

TASTE THRESHOLD

"t" TEST, BEFORE VS. AFTER RINSING WITH $CuSO_4$

No.	Before	After	Diff. X	Diff.^{2}
1	10	10	0	0
2	15	20	5	25
3	10	10	0	0
4	20	15	-5	25
5	20	25	5	25
6	20	20	0	0
7	45	50	5	25
8	15	15	0	0
9	15	10	-5	25
10	20	25	5	25
11	30	30	0	0
12	15	15	0	0
13	15	15	0	0
14	5	10	5	25
15	15	10	-5	25
16	10	10	0	0
17	15	25	10	100
18	10	15	5	25
19	10	10	0	0
20	20	25	5	25
21	15	15	0	0
22	20	15	-5	25
23	15	15	0	0
24	20	15	-5	25
25	20	15	-5	25
26	15	15	0	0
27	15	15	0	0
28	15	15	0	0
29	20	25	5	25
30	10	15	5	25
31	10	15	5	25
32	15	15	0	0
33	25	20	-5	25
34	10	10	0	0
35	10	10	0	0
36	20	15	-5	25
37	10	5	-5	25
38	10	10	0	0

TABLE V (CONTINUED)

No.	Before	After	Diff. X	Diff.^{2}
39	-	-	-	-
40	10	15	5	25
41	10	15	5	25
42	10	10	0	0
43	15	15	0	0
44	15	15	0	0
45	10	10	0	0
46	15	15	0	0
47	15	10	-5	25
48	10	10	0	0
49	15	15	0	0
50	10	10	0	0
51	10	5	-5	25
52	20	25	5	25
53	10	5	-5	25
54	5	5	0	0
55	10	10	0	0
56	10	15	5	25
57	10	10	0	0
58	15	10	-5	25
59	15	15	0	0
60	10	15	5	25
61	15	15	0	0
62	15	10	-5	25
63	5	10	5	25
64	10	15	5	25
65	10	10	0	0
66	15	10	-5	25
67	15	15	0	0
68	15	15	0	0
69	15	10	-5	25
70	15	15	0	0
71	20	25	5	25
72	15	10	-5	25
73	10	15	5	25
74	10	15	5	25
75	15	10	-5	25
76	10	10	0	0
77	10	10	0	0
78	20	15	-5	25
79	10	5	-5	25
80	15	15	0	0
81	10	10	0	0

TABLE V (CONTINUED)

No.	Before	After	Diff. X	Diff.^{2}
82	10	15	5	25
83	15	15	0	0
84	5	5	0	0
85	20	15	-5	25
86	10	10	0	0
87	15	10	-5	25
88	15	10	-5	25
89	15	15	0	0
90	10	5	-5	25
			$\sum X = -5$	$\sum \text{diff.}^2 = 1{,}225$

$N = 89$

$d = -5/89 = -0.056$

$(\sum X)^2 = 25$

$df = 88$

$$s = \sqrt{\frac{X^2 - (\sum X)^2/N}{N-1}}$$

$$\sqrt{\frac{1{,}225 - 25/89}{89-1}}$$

$s = 3.73$

$$t = \frac{d - 0}{Sd/\sqrt{N}}$$

$$t = \frac{056-0}{3.73/\sqrt{89}}$$

$t = -0.1418$

$-1.99 < -0.1418 < 1.99$ no difference

TABLE V (CONTINUED)

TASTE THRESHOLD

"t" TEST, BEFORE VS. AFTER CuSO

GROUP I, HIGH COPPER CONTENT, 301-415 mg/L

No.	Before	After	Diff. X	Diff.^{2}
1	10	10	0	0
8	15	15	0	0
9	15	10	-5	25
11	30	30	0	0
15	15	10	-5	25
18	10	15	5	25
21	15	15	0	0
22	20	15	-5	25
24	20	15	-5	25
25	20	15	-5	25
27	15	15	0	0
35	10	10	0	0
37	10	5	-5	25
40	10	15	5	25
54	5	5	0	0
55	10	10	0	0
64	10	15	5	25
65	10	10	0	0
66	15	10	-5	25
69	15	10	-5	25
73	10	15	5	25
75	15	10	-5	25
78	20	15	-5	25
79	10	5	-5	25
80	15	15	0	0
83	15	15	0	0
85	20	15	-5	25
88	15	10	-5	25
89	15	15	0	0
			X=-45	diff.^{2} =425

TABLE V (CONTINUED)

GROUP I, HIGH COPPER CONTENT

$N = 29$

$d = -45/29 = -1.55$

$(\Sigma X)^2 = 2.025$

$df = 28$

$$s = \sqrt{\frac{X^2 - (\Sigma X)^2/N}{N-1}}$$

$$= \sqrt{\frac{425 - 2,025/29}{28}}$$

$$= \sqrt{12.26}$$

$s = 3.56$

$$t = \frac{\bar{d} - 0}{Sd/\sqrt{N}}$$

$$= \frac{-1.55 - 0}{3.65/\sqrt{29}}$$

$$= \frac{-1.55}{3.65/5.4}$$

$t = -2.35$

$df = 28$

$\alpha = 0.05$

$-2.04 > -2.35 < 2.04$ There is a difference in the subjects taste threshold, after rinsing with $CuSO_4$.

TABLE V (CONTINUED)

TASTE THRESHOLD

"t" TEST, BEFORE VS. AFTER $CuSO_4$

GROUP II, MEDIUM COPPER CONTENT, 186-300 mg/L

No.	Before	After	Diff.X	Diff.^{2}
2	15	20	5	25
3	10	10	0	0
4	20	15	-5	25
5	20	25	5	25
6	20	20	0	0
10	20	25	5	25
12	15	15	0	0
13	15	15	0	0
14	5	10	5	25
16	10	10	0	0
20	20	25	5	25
26	15	15	0	0
29	20	25	5	25
32	15	15	0	0
33	25	20	-5	25
36	20	15	-5	25
42	10	10	0	0
43	15	15	0	0
44	15	15	0	0
47	15	10	-5	25
48	10	10	0	0
49	15	15	0	0
50	10	10	0	0
51	10	5	-5	25
52	20	25	5	25
53	10	5	-5	25
56	10	15	5	25
57	10	10	0	0
59	15	15	0	0
63	5	10	5	25
68	15	15	0	0
72	15	10	-5	25
76	10	10	0	0
81	10	10	0	0
84	5	5	0	0
86	10	10	0	0
87	15	10	-5	25

TABLE V (CONTINUED)

GROUP II, MEDIUM COPPER CONTENT

$\Sigma X = 5$

$\bar{d} = 5/37 = 0.14$

$\Sigma X^2 = 425$

$(\Sigma X)^2 = 25$

$df = 36$

$$s = \sqrt{\frac{X^2 - (\Sigma X)^2/N}{N-1}}$$

$$= \sqrt{\frac{425 - 25/37}{36}}$$

$s = 3.43$

$$t = \frac{\bar{d} - 0}{Sd/\sqrt{N}}$$

$$\frac{0.14}{3.43/\sqrt{37}}$$

$$= \frac{0.14}{3.43/6.08}$$

$t = 0.25$

$df = 36$

$\alpha = 0.05$

$-2.03 < .25 < 2.03$ There is no difference in the subjects taste threshold after rinsing with $CuSO_4$.

TABLE V (CONTINUED)

"t" TEST, BEFORE VS. AFTER CuSO

GROUP III, LOW COPPER CONTENT; 70-185 mg/L

No.	Before	After	Diff. X	Diff.^{2}
7	45	50	5	25
17	15	25	10	100
19	10	10	0	0
23	15	15	0	0
28	15	15	0	0
30	10	15	5	25
31	10	15	5	25
34	10	10	0	0
38	10	10	0	0
41	10	15	5	25
45	10	10	0	0
46	15	15	0	0
58	15	10	-5	25
60	10	15	5	25
61	15	15	0	0
62	15	10	-5	25
67	15	15	0	0
70	15	15	0	0
71	20	25	5	25
74	10	15	5	25
77	10	10	0	0
82	10	15	5	25
90	10	5	-5	25
			$\Sigma X = 35$	Σ diff.$^2 = 375$

$N = 23$

$\bar{d} = 35/23 = 1.52$

$(\Sigma X)^2 = 1,225$

$df = 22$

TABLE V (CONTINUED)

GROUP III, LOW COPPER CONTENT

$$s = \sqrt{\frac{X^2 - (\Sigma X)^2/N}{N - 1}}$$

$$s = \sqrt{\frac{375 - 1,225/23}{22}}$$

$$s = \sqrt{14.62}$$

$$s = 3,83$$

$$t = \frac{\bar{d} - 0}{Sd/\sqrt{N}}$$

$$t = \frac{1.52}{3.83/\sqrt{23}}$$

$$t = \frac{1.52}{0.80}$$

$t = 1.90$

$df = 22$

$\alpha = 0.10$

$-1.72 < 1.90 > 1.72$ There is a difference in the subjects taste threshold after rinsing with $CuSO_4$, at the 0.10 level but not at the .05 level.

TABLE VI

COPPER LEVEL VS. LACTOBACILLUS COUNT

REGRESSION AND CORRELATION

COPPER LEVEL		LACTOBACILLUS COUNT (logarithmic)		
X	X^2	Y	Y^2	$\Sigma XY = 46,309.82$
$\Sigma X = 18,993$		$\Sigma Y = 229.22$		
$\Sigma X^2 = 5,804,039$		$\Sigma Y^2 = 1,033.52$		
N = 72		N = 72		

$$b = \frac{\Sigma XY - (\Sigma X)(\Sigma Y)/N}{\Sigma X^2 - (\Sigma X)^2/N}$$

$$b = \frac{46,309.82 - (18,993)(229.22)/72}{5,804,039 - (18,993)^2/72}$$

$$b = -0.018$$

$$a = \bar{Y} - b\bar{X}$$

$$a = 3.18 - (-4.75)$$

$$a = 7.93$$

I) REGRESSION LINE

$$\text{Log } Y = a + bX$$

$$\text{Log } Y = 7.93 + (-0.018)(X)$$

TABLE VI (CONTINUED)

II) LINEAR CORRELATION

$$r = \frac{\Sigma XY - (\Sigma X)(\Sigma Y)/N}{(\Sigma X^2 - (\Sigma X)^2/N}$$

$$r = \frac{-14,156.51}{[5,804,039 - (18,993)^2/72][1,033.52 - (229.22)^2/72]}$$

$$r = \frac{-14,156.51}{15,528.87}$$

$r = -0.91$ A negative correlation

$df = N - 2$

$= 72 - 2$

$= 70$

$$t = \sqrt{\frac{r2\ (N-2)}{1-r^2}}$$

$$t = \sqrt{\frac{-.91^2\ (70)}{1 - (-0.91)^2}}$$

$t = 18.84$

Table value for t at a $p < .05$ equals 2.00 $\therefore$ X and Y are related

TABLE VII

BETWEEN-WITHIN, ANALYSIS OF VARIANCE

Copper Levels in Micrograms/L

CLASS I	CLASS II	CLASS III	CLASS IV	CLASS V
375	260	200	125	110
410	280	210	145	115
395	295	205	110	95
405	275	210	150	100
390	283	195	135	90
390	255	210	125	125
410	175	195	110	
395	190	200		635
385	160	185	900	
380	265	170		68075
410	195	185	117200	
385	265	165		403225
380	270	215	810000	
390	280	200		67204.17
410	185		115714.28	
415	270	2745		
390	340			
390	270	541175		
380	255			
410	285	7535025		
385	295			
410	270	538216.07		
405				
	5618			
Xm =9095				
	1477664			
X^2m=3599925				
	31561924			
$(\Sigma X)^2$=82719025				
	1434632.9			
$\frac{(\Sigma X)^2}{N}$=3596479.3				

C1 - C5 TOTALS

X =18993

Xm^2=5804039

$(\Sigma X)^2$=123029199

$\frac{(\Sigma X)^2}{N}$=5752246.5

TABLE VII (CONTINUED)

$$\text{Total SS} = \Sigma X^2 - \frac{(\Sigma X)^2}{N}$$

$$= 5804039 - (18993)^2/72$$

$$= 793,844$$

$$\text{Between SS} = \frac{(\Sigma X)^2}{Nm} - \frac{(\Sigma X)^2}{N \text{ total}}$$

$$= \frac{82719025}{23} + \frac{31561924}{22} + \frac{7535025}{14} + \frac{810000}{7} + \frac{403225}{6} - 5010195$$

$$= 742,051.50$$

Within SS = Total SS - Between SS

= 793,844 - 742,051.5

= 51,792.5

SOURCE	DF	SS	MS	F
BETWEEN	4	742,051.5	185512.87	239.98*
WITHIN	67	51,792.5	773.02	
TOTAL	71	793,844.0	186,285.89	

$p < .01$

$F = 3.65$ $\quad 239.98 > 3.65$ Reject Ho: There is a difference between copper levels with regard to saliva conduciveness.

TABLE VII (CONTINUED)

"K" TEST

No. of samples = 5

W/in sample df = 67

From K table, K*= 4.95

Nm = 14.40

Ve = 773.02

$K = K^* \frac{Ve}{Nm}$

$= 4.95 \sqrt{\frac{773.02}{14.40}}$

= 36.23 36.23 is the minimum significant difference between means.

MEANS IN ASCENDING ORDER

CLASS I	CLASS II	CLASS III	CLASS IV	CLASS V
105.83	128.57	196.07	255.36	395.43

CLASS I	&	CLASS II	ARE DIFFERENT
CLASS I	&	CLASS III	ARE DIFFERENT
CLASS I	&	CLASS IV	ARE DIFFERENT
CLASS I	&	CLASS V	ARE DIFFERENT
CLASS II	&	CLASS III	ARE DIFFERENT
CLASS II	&	CLASS IV	ARE DIFFERENT
CLASS II	&	CLASS V	ARE DIFFERENT
CLASS III	&	CLASSIV	ARE DIFFERENT
CLASS III	&	CLASS V	ARE DIFFERENT
CLASS IV	&	CLASS V	ARE NOT DIFFERENT

There is no difference between the copper levels of classes IV & V with regard to caries conduciveness.

TABLE VIII

PERCENT OF SUBJECTS WHOSE THRESHOLDS CHANGED

AFTER RINSING THE ORAL CAVITY WITH $CuSO_4$

(All subjects)

No. raised = 22 = 24.7%

No. lowered = 24 = 27.0%

No change = 43 = 48.3%

(Copper level 301-415 micrograms/L)

No. raised = 4 = 13.8%

No. lowered = 13 = 44.8%

No change = 12 = 41.4%

(Copper level 186-300 micrograms/L)

No. raised = 9 = 24.3%

No. lowered = 8 = 21.6%

No change = 20 = 54.1%

(Copper level 70-185 micrograms/L)

No. raised = 9 = 39.1%

No. lowered = 3 = 13.1%

No change = 11 = 47.8%

TABLE IX

DESCRIPTION AND RESULTS OF THE TESTS PERFORMED

Table I, Actual Analytical Data.

Table II, Data from the Reductase 15 Minute Caries Test.

Table III, Taste Vs. Copper before rinsing with copper sulfate. The results showed that at a p of less than .01 Copper and sugar taste thresholds are not related.

Table IV, Taste Vs. Copper after rinsing with copper sulfate. The results showed that at a p of less than .01 Copper and sugar taste thresholds are not related.

Table V, "t" Test, Before Vs. After rinsing with copper sulfate. The results for the group as a whole showed that at a p of less than .05 there is no difference in the subjects taste threshold after rinsing with copper sulfate. The results of the "t" Test performed on the subjects with high copper content and low copper content showed that at high and low copper levels there is a difference in the subjects taste thresholds.

Table VI, Copper level Vs. Lactobacillus count. The results of this test showed that as the copper level increases the lactobacillus count decreases logarithmically.

Table VII, Copper level Vs. Saliva conduciveness. The results of this test showed that as the copper level increases the caries conduciveness of the saliva decreases.

TABLE IX (CONTINUED)

Table VIII, Percent change after rinsing the oral cavity with copper sulfate. The results of this test showed that at high copper levels the sweet taste threshold was lowered after rinsing with copper sulfate. And at low copper levels the taste thresholds were raised after rinsing with copper sulfate.

APPROVAL SHEET

The thesis submitted by John S. Borello 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 (Oral Biology)

May 18, 1976
Date

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