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A Comparative Study of Plaque Acidogenesis in  
Individuals Residing in Communities  
With and Without Fluoridated Water

Second Annual Summary Report

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## FIGURE

- Figure 1: Mean millivolt changes in response to sugar rinse before and one year after fluoridation of Albany's water supply.

There are two major theories dealing with the mechanisms whereby fluoride ions aid in reducing the incidence of dental caries. The most widely accepted theory states that the fluoride reduces enamel solubility in acid solutions, whereas the second theory suggests that the fluoride alters the metabolism of plaque microorganisms so that less acid is produced. The major anti-caries effect appears to be related to the reduction of solubility of dental hard tissues, but this does not negate the possible significance of inhibition of acidogenesis. This research deals with the second theory and has the following objectives:

1. To determine whether, after challenge by a sugar rinse, less free acid (higher pH) is produced in the dental plaques of persons residing in a community with fluoridated water than in a comparable group of individuals residing in a community without fluoride in its water.
2. To determine how soon after fluoridation the maximum depression of acidogenesis is obtained.
3. To determine the modifying effects of fluoride-containing dentifrice on plaque acidogenesis on subjects using fluoridated water and those using non-fluoridated water.
4. To determine whether a decrease occurs in the caries incidence of the newly-fluoridated community and its correlation with the decrease in acid production.

#### BACKGROUND

Bibby and Van Kesteren (1) demonstrated inhibition of acid formation by microorganisms in vitro in the presence of as little as 1 ppm fluoride. Jenkins (2) also demonstrated an inhibitory effect at about 5 ppm when the pH of the mixture was 5.0 or below. Furthermore, Jenkins showed that as the pH of the mixture is lowered in the presence of a given concentration of fluoride, there is increasing inhibition of acidogenesis.

Briner and Frances (3) grew 24-hour cultures of Lactobacilli on various flattened, polished, enamel surfaces and determined the pH and total lactate concentrations of the suspension. They found that acid production in the microsystems was inhibited by mottled enamel surfaces and by enamel surfaces which had been treated with sodium fluoride or calcium fluoride. Similarly Zwemer (4) treated teeth with topical sodium fluoride and then demonstrated inhibition of acidogenesis in microsuspensions of Lactobacilli placed in contact with the treated enamel surfaces in vivo. He found, however, that the ability of these enamel surfaces to inhibit acidogenesis declined rapidly and was lost after one week. He concluded that the rapid decline in fluoride inhibition of acidogenesis precluded it as a factor in the reduction of caries incidence usually observed following topical fluoride treatment, but he suggested that repeated exposures to dilute solutions might confer some protection.

Woolley and Rickles (5) investigated the acid response to a sugar rinse in 42 teenage girls before and after fluoride treatments. No prophylaxis was given. One half of the maxillary arches of all 42 girls were treated with 2% topical sodium fluoride, while the control halves were treated with 2% sodium chloride. They found that there was strong inhibition of acidogenesis on the fluoride sides eight hours after treatment but no inhibition on the control sides. The magnitude of the 8-hour inhibition was about 0.5 pH units. After three to four days, the inhibition was still statistically significant, but the magnitude was less than 0.2 pH units. There were no significant differences after one week. The shortness of the duration of inhibition confirmed Zwemer's findings and suggested the necessity for repeated fluoride exposures if this mechanism was to be useful in caries reduction.

Seeking to discover if repeated exposures to very dilute solutions of fluoride would be sufficient to cause prolonged inhibition of acidogenesis, Woolley and Rickles performed a preliminary study comparing the acid production in response to a sugar rinse in children residing in a fluoridated community with a similar group residing in a non-fluoridated community. Nine\* teenage boys who were long-term residents of Corvallis, Oregon (containing 1 ppm fluoride in its water supply) were compared with nine\* boys of comparable age and economic status residing in Portland, Oregon (without fluoride). The tests were done at the same time of day and all boys received the same instructions. The Corvallis group had a resting mean pH of 6.8 and a sugar-stimulated mean pH of 6.5. The Portland group went from pH 7.0 to pH 6.1. The difference in acidogenesis was statistically significant at the ninety percent level.

The present study is an outgrowth of this pilot study.

#### METHODS

At the beginning of the project, three communities were selected for study as follows:

Albany--An Oregon community which did not have fluoride in its water supply prior to March 15, 1969, but which added fluoride on that date. Albany was to serve as the test city.

Lebanon--An Oregon community similar to Albany and which uses the same river for its water supply. Lebanon does not have fluoride in its water and has no plans to fluoridate in the foreseeable future. It was to be used as a control community without fluoride.

Corvallis--An Oregon community near Albany and Lebanon which has had fluoride in its water for more than fifteen years. Corvallis was to serve as the control community with fluoride.

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\* These were the total number of boys out of about thirty in each group with sufficient plaque present to afford reliable measurements with the pH meter.

During our study in Lebanon, we found that many individuals residing there use well water and that some of this water contains significant amounts of fluoride. We, therefore, added a fourth community to the study.

Canby--An Oregon community without fluoride in its water and with no plans to fluoridate.

In each community a group of 75-150 seventh and eighth grade students was selected so that the students belonged to families of similar socioeconomic status. They were screened to make sure that they fulfilled the various qualifications for inclusion in the study. These qualifications were:

The students had to be residents of their respective communities for at least five years and any previous residencies had to have been in communities with similar fluoride characteristics. In each instance where a student listed a previous residence, we corresponded with appropriate individuals in that community to ascertain the fluoride content of its water.

The students were not to have had topical fluoride treatments for at least one year prior to their initial examination.

There had to be good assurance that the students would remain residents of their respective communities during the period of the study.

The following examinations were made:

Caries Experience - All posterior and maxillary anterior teeth were clinically evaluated for caries and intra-oral roentgenograms were made. A second similar evaluation for caries will be performed at the end of the experimental period.

pH Measurements are being made by use of an antimony electrode attached to a Corning Model 7 pH meter. The electrode is shaped in such a manner that embrasure plaque potentials can be measured. All measurements are made between 1:00 and 3:30 p.m., and the time of measurement for each student is recorded. Those measurements made between 1:00 and 1:30 have shown residual effects of the students' lunch.

The procedure for measuring plaque pH is as follows:

1. The maxillary posterior embrasure plaques on the right or left side are first measured with the antimony electrode-pH meter assembly by reading the negative millivolt scale. The embrasure plaques of the contralateral teeth were removed and frozen for fluoride analysis.
2. The student was given a 25 percent sugar solution and directed to rinse for ten minutes.

3. The embrasure plaques were again measured. The difference between the before-sugar and after-sugar rinse measurements constitute the statistic for comparison. Data was collected in this manner in all communities prior to and one year following fluoridation in Albany.

### RESULTS TO DATE

The results of the project to date are given in Tables 1-6 and are illustrated in Figure 1. Measurements taken by the antimony electrode are read on the negative millivolt scale of the pH meter. These millivoltage values have a linear relationship with pH in the range of our measurements. All statistical computations were made using millivolt readings, without first converting them to pH. Such conversions of millivolts to pH units are easily performed, however, since each 10 millivolt increment corresponds to 0.18 pH unit according to the standardization of our equipment.

We have had continual difficulty with the Lebanon phase of the study due to various factors. Several wells in the area of Lebanon have fluoride in them, which possibly accounts for some of the difficulty. Data from Lebanon is being collected but will not be included in the statistical evaluations until further investigation of the difficulties can be made.

The data contained in Tables 1-6 are based upon sample sizes somewhat smaller than those reported in previous reports. The reasons for this reduction in sample size are as follows:

1. Some subjects were lost to the experiment during the first year of investigation.
2. If the data was incomplete for a particular subject (such as the loss of a deciduous tooth), the computer eliminated his card for purposes of the statistical comparisons. Additional programming will recover all pertinent data.
3. If the values exceeded or were less than certain confidence limits, the cards were pulled for further checking before being included in the computations.

When the data is more complete and checked for possible errors, the sample sizes will probably be larger.

### DISCUSSION

It can be seen from Table 6 and Figure 1 that less acid was produced in the test communities which had fluoridated water (Corvallis and Albany after fluoride) than in those towns without fluoride (Conby and Albany before fluoride). Statistical comparisons of the means of individual fluoride vs. non-fluoride communities show that the difference between such areas are significant both initially and after the first year of the study. However, analysis of variance studies of the combined data from the three communities at the one-year, post-fluoride interval

suggests that the differences between communities are not significant. This disparity of statistical significance is partly related to the differences of precision of the two statistical methods. It is also related to the large size of the statistical variances ( $S^2$ ) of the data collected when it is difficult to adequately control the co-variables. Such difficulty in the control of co-variables often characterizes clinical research problems and has been a problem in our experiment. For instance, it is very difficult to control the types of food or beverages used by the students prior to their appointment. Even the chewing of gum can affect the millivoltage readings.

In spite of such difficulty, our data suggests that it is probable that the addition of fluoride to the water supply results in sustained depression of acidogenesis. Since this depression is only of the magnitude of 0.2 pH units, it is unlikely that by itself it is sufficient to prevent cariogenic acidity. The fact that there is a sustained depression of acidogenesis is very encouraging, however. Furthermore, we have not yet evaluated the data for possible selective effects--such as a differential effect dependent upon the pre-fluoride pH drop after sugar rinse. As previously noted, when a 2% solution of NaF is applied to dental plaque in situ, the depression of acidogenesis is about 0.5 pH unit. This is probably sufficient to decrease cariogenic acidity if it can be maintained for prolonged periods of time. Daily use of a mouthwash which could decrease cariogenic acidity would be of great value. The possibilities for the development of such a mouthwash should be studied. In this regard, Torrell and Ericsson (6) have shown that a 0.2% NaF mouthwash used daily results in a greater reduction in DMF than routine topical fluoride applications.

#### MILITARY SIGNIFICANCE

That topical application of two percent NaF can inhibit acidogenesis in dental plaques seems well established. Furthermore, the inhibitory effect seems to be sufficient to prevent the development of pH's below 6.0 where the caries process probably begins. On the other hand, the inhibitory effect following a single treatment persists for only about one week, making it unlikely that inhibition of acidogenesis is significant in the overall prevention of tooth decay by the single treatment method.

It seems probable from this experiment that dilute solutions of NaF can cause sustained depression of acidogenesis. The magnitude of such depression which occurs after water fluoridation (0.2 pH unit) is probably not sufficient by itself to prevent cariogenic acidity. The development of a fluoride mouthwash, to be used in conjunction with water fluoridation is a distinct possibility. Such a mouthwash, once developed, would be of great worth to man in the field.

#### WHERE DO WE GO FROM HERE?

During the final year of this study, we will make our final measurements of acidogenesis and DMF. We will also complete our analysis of plaque fluoride. A pilot study for the development of a fluoride mouthwash will also be carried out. Application for a grant to develop the mouthwash will also be submitted.

TABLE 1: Mean millivolt changes in response to a sugar rinse at beginning of study.

<u>City</u>	<u>Sample Size</u>	<u>Mean</u>	<u>Standard Deviation</u>
Albany	58	27.7	22.2
Corvallis	75	19.4	16.1
Canby	56	29.8	21.0

TABLE 2: Mean millivolt changes in response to a sugar rinse one year following fluoridation of Albany's water supply.

<u>City</u>	<u>Sample Size</u>	<u>Mean</u>	<u>Standard Deviation</u>
Albany	73	14.8	18.1
Corvallis	104	17.7	18.5
Canby	59	21.9	17.3

TABLE 3: DMF plus def (surfaces) in communities at beginning of the study.

<u>City</u>	<u>Sample Size</u>	<u>DMF + def (surfaces)</u>
Albany	65	13.4
Corvallis	72	10.1
Canby	45	12.1

TABLE 4: DMF plus def (teeth) in communities at beginning of the study.

<u>City</u>	<u>Sample Size</u>	<u>DMF + def (teeth)</u>
Albany	65	7.8
Corvallis	72	6.4
Canby	45	7.9

TABLE 5: Statistical comparisons between cities at the beginning of the study.

<u>Cities Compared</u>	<u>F.<sub>.95</sub></u>	<u>t.<sub>.95</sub></u>
Albany vs. Corvallis	1.93 (Sig.)	2.50 (Sig.)
Albany vs. Canby	1.12 (Not Sig.)	-0.54 (Not Sig.)
Corvallis vs. Canby	0.59 (Not Sig.)	3.23 (Sig.)

ANALYSIS OF VARIANCE:

	Degree of Freedom	
Among	3	F. <sub>.95</sub> 3.70 (Sig.)
Within	247	

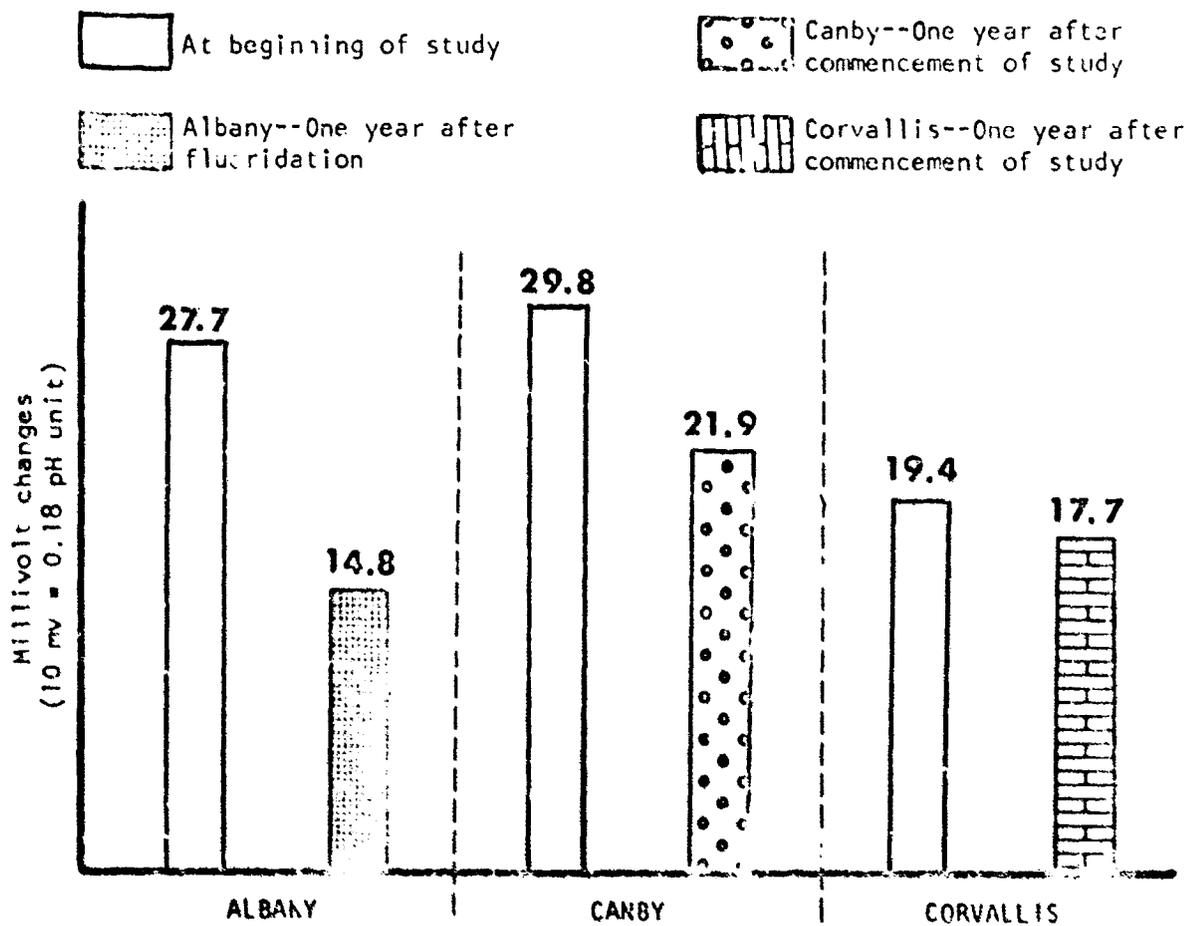
TABLE 6: Statistical comparisons between cities one year after fluoridation.

<u>Cities Compared</u>	<u>F.<sub>.95</sub></u>	<u>t.<sub>.95</sub></u>
Albany vs. Corvallis	0.97 (Not Sig.)	-1.01 (Not Sig.)
Albany vs. Canby	1.10 (Not Sig.)	-2.28 (Sig.)
Corvallis vs. Canby	1.14 (Not Sig.)	-1.44 (Not Sig.)

ANALYSIS OF VARIANCE:

	Degree of Freedom	
Among	3	F. <sub>.95</sub> 1.64 (Not Sig.)
Within	304	

FIGURE 1: Mean millivolt changes in response to sugar rinse before and one year after fluoridation of Albany's water supply.



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13. ABSTRACT

The ability of dental plaques to produce acid in response to a sugar rinse in fluoridated and non-fluoridated communities was indirectly evaluated by measuring plaque pH with an antimony electrode attached to a Corning pH meter.

144 seventh and eighth grade students were evaluated in Albany, Oregon shortly before this city fluoridated its water supply and again three months after fluoridation. 131 similar students were evaluated in Corvallis, Oregon, a city which has had fluoride in its water for about fifteen years. 181 students were evaluated in Lebanon and Canby, Oregon, communities without fluoride.

In general it was found that students residing in fluoridated communities produced less acid than those living in the other communities. The magnitude of the depression was about 0.2 pH units, which was statistically significant (t.95) It is unlikely that this degree of inhibition of acidogenesis is adequate to prevent cariogenic acidity; however, if supplemented by a daily mouthwash containing fluoride, it may. Once developed, such a mouthwash would be of great value to men in the field.