ACID PRODUCTION IN SUCROSE BY ORAL BACTERIA AS A MEANS OF ESTIMATING CARIES ACTIVITY: A STUDY OF A NEW TEST PROPOSED BY RICKLES

by

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Chairman, Graduate Education Committee

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I. INTRODUCTION

A variety of laboratory tests using saliva have been devised to estimate caries susceptibility or to predict clinical change at periods of six months to a year after sampling. These tests may be divided into those measuring either some form of bacterial activity or some component or property of saliva (pH, NH_3, Ca/P, buffering and CO_2 capacity, and amylase content). The bacteriological tests include the assessment of numbers of lactobacilli per milliliter of saliva[1,2], acid formation in selective culture media[3,4], and rate of acid production in glucose or sucrose[5,6,7]. Rickles[8] in 1953 described a method for the colorimetric determination of acid production in sucrose solutions containing two indicators. The extent of acid formation in 4 hours by salivary action was correlated directly with clinical change measured 12-14 months later: namely, the greater the amount of acid, the greater the increase in new carious surfaces in this time interval. Because of the simplicity and degree of correlation of this test with clinical change, it was deemed sufficiently important to evaluate this test in a clinical program.

Fosdick, Epple, and Hansen[5] in 1937 were the first to introduce a test based on acid production in carbohydrate

alone. It was conducted in the following manner: 25 milliliters of saliva were collected from a patient. Chewing gum with its usual sugar content provided stimulation as well as the carbohydrate needed for the test. A portion of the specimen was analyzed for calcium (mg./100 ml. saliva); powdered human enamel (0.1 gm.) was added to the balance and the mixture shaken 4 hours at 37.5° C. After centrifugation to remove the undissolved enamel, the supernatant was analyzed for calcium. The difference in the calcium content between the control and incubated saliva represented the amount of enamel dissolved by the acid formed in the saliva-glucose mixture. Although the hydrogen-ion concentration of the mixture was measured before and after incubation, it was neither included in the recommended procedure nor suggested to be related to caries activity. Preliminary tests showed the calcium dissolving power of saliva from 10 caries-susceptible cases to be much greater than that of the saliva from 7 caries-immune subjects. On the basis of at least 2 checks for each of 325 patients, the authors concluded there was correlation with clinical evidence in 85 per cent of cases. No data were provided to substantiate this claim.

In 1943 Wach, Kesel, Hine, and O'Donnell[6] described a test which measured the pH and total acidity of a saliva-glucose mixture. Ten milliliters of unstimulated saliva were divided equally into 3 tubes. One was used as the

control; of this, 1 ml. was titrated for total acidity with N/100 NaOH using phenolphthalein as the indicator, while one drop was used to determine pH by means of comparison with colorimetric indicator standards (LaMotte). Glucose (0.4 ml. of a 1 per cent solution) was added to the other tubes which were incubated for 24 hours. The same measurements were made at 2, 4, and 24 hours with 1 ml. and 1 drop of each sample. In preliminary tests the caries-active individuals showed a drop of pH from 6.8 to 4.8 in 2 hours, 4.4 in 4 hours, and 3.9 in 24 hours, while total titratable acid (in terms of ml. of N/100 NaOH) increased from 0.4 ml. to 1.35 ml. in 2 hours, 1.8 ml. in 4 hours, and 2.9 ml. in 24 hours. With the slightly caries-active patients, however, the pH dropped from 6.8 to 5.6 in 2 hours, to 5.1 in 4 hours, and 4.4 in 24 hours, while the total titratable acid rose from 0.2 ml. to 0.6 ml. in 2 hours, 0.8 ml. in 4 hours, and 2.2 ml. in 24 hours. The greatest rate of production in the caries-active cases occurred in the first 2 hours; in the slightly caries-active cases there was a slow increase in acidity for 4 hours, followed by an increase in rate. At the end of 4 hours the acidity produced by the saliva from caries-active patients was approximately twice that by the saliva from slightly caries-active patients. The difference in acidity at 24 hours between the two groups was essentially insignificant. Consequently, the authors selected the

4 hour reading for their study. Tests were made upon 818 saliva samples from 50 young adult dental students carefully followed for caries activity over a period of 2 years or more. From the laboratory standpoint the degree of caries activity was estimated as follows:

DEGREE OF CARIES ACTIVITY

	pH	Total acidity per cc. saliva
Inactive	7.0-6.0	.50 cc. of N/100 acid
Active +	5.9-5.5	.51-.75 " " " "
Active ++	5.4-5.0	.76-1.00 " " " "
Active +++	4.9-4.5	1.0 -1.4 " " " "
Active ++++	4.4-3.9	1.41-up " " " "

"Classification of these 50 patients was made according to clinical observation of new cavities developing and also according to the above scale. In 86 per cent of these individuals the results of the test coincided with the presence or absence of caries. However, the amount of acid formed in the test was not indicative, necessarily, of the extent of clinical caries activity." That is, there was complete agreement between laboratory estimate and clinical measurement in only 11 (22 per cent) of patients, in greater or lesser agreement in 32 (64 per cent) of the group, and no correlation in 7 (14 per cent) of the subjects.

Dewar[7] in 1949 modified the calcium dissolution test by substituting the hydrogen-ion concentration measurement. Her procedure was to determine the pH of the activated saliva with a glass electrode potentiometer and place 10 ml. of the

specimen with 0.4 gram of glucose and 0.1 gram of powdered enamel in a large test tube (1 x 8 inches). This tube was sealed and agitated at 37° C. for 4 hours at which time the pH was measured again. She averaged the pH values, calcium dissolving power, buffering capacity, and lactobacillus counts for 1-12 specimens of saliva (average of 3 specimens) for each of 86 subjects[9]. Arbitrary groupings of the DMF (decayed, missing, and filled) teeth of these people were established. Relatively high pH values (> pH 5.61) and buffering capacity but low lactobacillus counts and calcium dissolving power were associated with low DMF. Conversely, lower pH values and buffering capacity but increasing lactobacillus counts and calcium dissolving power were associated with higher DMF scores. A "good" correlation was obtained in 45 to 58 per cent, "fair" in 35-40 per cent, and "poor" in 9-15 per cent of these cases.

The test described by Rickles[8] in 1953 utilized sucrose instead of glucose, and two indicators to allow comparisons over a greater pH range than by either alone. Equal volumes (0.5 ml.) of saliva and 8 per cent sucrose containing 0.004 per cent each of bromcresol green and bromcresol purple were placed in 100 x 13 mm. culture tubes. After 4 hours incubation at 37° C. the changes in color resulting from acid formation were compared with a series of indicator standards over a range of pH 6.6 to 4.2 in 0.4 pH increments. Rickles evaluated his test clinically by obtaining single samples of

saliva from 129 subjects 18-34 years of age with a mean age of 22 years. These specimens (48-96 hours old) were analyzed for pH change in sucrose, total titratable acidity (in terms of 0.2 N NaOH) and lactobacillus counts. These figures were compared with the increase in new carious surfaces for each individual established through clinical and radiological examinations 12-14 months later by an examiner unaware of the laboratory study. Statistical methods showed a direct correlation, not only between amount of acid formation in sucrose and the lactobacillus count with new caries experience, but also between the tests themselves. However, while the tests were correlated, successful prediction of new caries could be made only with pH change and total titratable acidity; such prediction could not be made with the lactobacillus count alone. Rickles then prepared a classification of this colorimetric test at the 68 per cent confidence level. He emphasized that while this grouping was made with single specimens an average of several tests might be more precise for predictive value. His classification was as follows:

pH*	PREDICTED NEW DENTAL CARIES AFTER ONE YEAR (68 Per Cent Confidence Limit)	DEGREE OF ACTIVITY
6.6	0 (0-3)	LOW
6.2	1 (0-4)	
5.8	3 (0-5)	MODERATE
5.4	4 (1-6)	
5.0	5 (3-8)	HIGH
4.6	7 (4-9)	
4.2	8 (5-11)	

*pH of "saliva-sucrose" mixture after 4 hours of incubation.

The purpose of this thesis was to evaluate the rate of acid formation in sucrose by salivary action as a means of estimating or predicting caries activity, compared with caries experience established by clinical and radiological examinations 1 year after sampling. Another laboratory test, the lactobacillus count, was also included for reference value.

II. MATERIALS AND METHODS

A. Study Subjects

The children used as subjects in this study were located at the Washington School for the Deaf, Vancouver, Washington. The same children were also being followed by periodical clinical and laboratory examinations over a 5 year period for evaluation of lactobacillus count, acid production in selective culture media, and salivary amylase as predictors of caries activity. The members of this school represented about as stable a population as could be obtained for a longitudinal study. The greatest shortcoming was that their residence was limited to the usual school year.

The children were housed in dormitories and ate in a common dining room. Their diet appeared adequate and there was excellent supervision both for health and education. Of a total of 175 children in the study group, only 134 were included in this phase of the investigation. They ranged in age from 5 years and 8 months to 16 years and 11 months. The mean age was 11 years and 4 months. The others were eliminated on the following grounds: (1) not being present for the three successive clinical examinations on which this

study was based, and (2) not having a specimen of saliva collected within 60 days of the initial clinical examination.

B. Clinical Examinations

Clinical examinations were performed at 6 month intervals by one examiner who dictated his findings to a recorder. Bite-wing roentgenograms were taken by a technician. The films were developed each night during the examination period for recall of faulty results. The dentist also examined the bite-wing radiographs and read his findings to the same recorder. The recorder prepared all records, summarized the results on standardized record sheets, and transcribed the results to IBM punch cards for listing and analysis.

New carious surfaces were considered those which, between examination periods, changed from sound to carious, doubtful to carious, and not present (tooth not yet erupted) to erupted and carious. Other possibilities, such as a sound surface to filled, and sound to filled and carious, were not included.

C. Test Material

1. Saliva Samples. Since specimens of saliva were routinely being collected at monthly and semi-monthly intervals as part of a longitudinal program, this investigation

was designed to obtain a series of values for acid formation in sucrose and lactobacillus counts over a 6 month period with new caries experience observed 12 and 18 months later. The base line clinical examination selected was that of September 1954. The last was the one scheduled for March 1956 (18 months). Unfortunately, the last examination was delayed by illness in the school; this data is not available for correlation purposes in this thesis. Therefore, the predictive value of acid formation in sucrose and the lactobacillus count of a single specimen of saliva in respect to new caries experience 12 months later was studied in the manner described by Rickles[8]. As the predictive value of any test diminishes as the time of clinical measurement approaches, correlation was limited to the first specimen of saliva collected within 60 days after the September 1954 examination.

2. Collection of Saliva. Saliva was collected semi-monthly and at a constant time (1-3 hours) after breakfast, since it was impossible to obtain pre-breakfast samples. Each child had been instructed to chew on a pellet of paraffin for 3-5 minutes and expectorate all the saliva stimulated into a labeled sterile bottle. The specimens were placed in an insulated box previously cooled to 5° C. with frozen plastic gel. The temperature of this box did not exceed 10° C. before it reached the laboratory some 30

minutes after all specimens were collected. Storage in the laboratory was maintained at 5° C. All laboratory tests were completed within 24 hours.

D. Tests

1. The Lactobacillus Count. Lactobacillus counts were done after the method of Hadley[2] as modified by Jay[10]. In the present study the tomato juice agar was adjusted to pH 5.0 with lactic acid after being brought to pH 7.0 with 40 per cent sodium hydroxide. This procedure assured a lactate content within the limits of 3.3-5.6 grams per liter, considered by Dewar[11] as desirable for this medium. Yeast-like organisms of the oral flora were inhibited by a 1:20,000 concentration of sodium azide. This medium was almost completely selective for lactobacilli when freshly prepared[12]. The routine inoculum for each plate was 0.1 ml. of a 1:10 dilution of the specimen of saliva in distilled water. It was spread over the surface of a tomato juice peptone agar plate by means of a sterile bent glass rod while the plate was being rotated on a turntable[13]. Colony counts were made after 4 or 5 days of incubation at 37° C. with a binocular dissecting microscope (7x) and a Frost counting card (No. 4099). The results were expressed as the number of lactobacilli per milliliter of the specimen of saliva. This count was recorded as a 3 place logarithm to the nearest 5 on IBM punch cards; that is, a lactobacillus

count of 1300/ml. saliva was recorded as the logarithm of 1500, and so forth. Because the final dilution of saliva was 1:100, any count below 100 per ml. of saliva was impossible to determine. The log of 0 is infinity; the log of 1 is 0; and the log of 10 is 1. Thus, all negative counts were arbitrarily given a lactobacillus count of 10 so that the log 1 would be in consecutive order for comparison.

2. The Colorimetric Test. This test at first was conducted precisely as Rickles described: "0.5 cc. of the saliva was added to 0.5 cc. of a sucrose-indicator solution in a precipitation tube, bringing the concentration of sucrose to approximately 3.9 per cent. The sucrose-indicator solution was made up by adding to 1000 cc. of distilled water, 80 gm. of sucrose, 10 cc. bromcresol purple, and 10 cc. bromcresol green (both were 0.4 per cent indicator solutions made up with 95 per cent ethyl alcohol); the pH of the solution was adjusted to 6.6 with 4 cc. N/20 NaOH. This made sufficient sucrose available for degradation, yet did not produce a great enough osmotic pressure to inhibit the metabolism of these microorganisms. Then the saliva-sucrose-indicator mixture (hereafter referred to as the "saliva-sucrose" mixture) was incubated at 37° C. for four hours and after gentle agitation of the tube contents, the pH was determined by comparison with standard buffer-indicator solutions (pH values 6.4 and 6.0 were estimated by

interpolation). These standard phosphate and phthalate-HCl buffer solutions were made up at 0.4 pH intervals, ranging from pH 6.6 to pH 4.2. One cubic centimeter of each of these buffers was placed into small precipitation tubes to which 0.1 cc. of bromcresol purple and 0.1 cc. of bromcresol green were added (both 0.04 per cent indicator solutions made up with 95 per cent ethyl alcohol; then the standard buffer solutions ranged in color from deep blue to a bright yellow). Sharp color changes in these standard solutions at the 0.4 intervals resulted."[8]

Two difficulties were encountered. The first was the formation of an excessive amount of sediment which obscured comparison with the color standards. It was learned later that Rickles allowed his tubes to set 10 minutes after mixing before final pH comparisons were made with the supernatant. The second problem was that the 100 x 13 mm. culture tubes required inversion for satisfactory mixing; this was both cumbersome and time-consuming with a large series of readings. To meet the first objection, a small amount of agar (0.2 per cent) was added to the sucrose solution. This gel was sufficiently fluid to allow ready mixing of the saliva with the sucrose, yet was viscous enough to prevent objectionable sedimentation. Color changes in the solutions containing agar not only permitted more accurate comparisons than the original, but the rate of acid formation was accelerated. To overcome the need for inversion of the tubes

resort was made to larger tubes which could be twirled instead of inverted. The final volume selected was 2.0 ml., of which one milliliter was 8 per cent sucrose with the double indicator system and the other milliliter saliva. The ratio of components remained the same at 1:1. No difference was noted in pH change per hour with tests conducted in Wassermann (100 x 13 mm.) and standard 150 x 16 mm. culture tubes for 35 specimens of saliva. Therefore, all subsequent tests were made with 2.0 ml. volumes in 150 x 16 mm. culture tubes incubated 4 hours at 37° C. The buffered standard solutions with indicators in 5 ml. volumes were in similar tubes.

All specimens of saliva were tested in sucrose solutions with and without agar. Changes in the indicators were read hourly to obtain maximum amount of information on rate of acid formation within the 4 hour limit commonly specified for these tests[5,6,7]. Color comparison was best made by reflected light with the tubes slanted over a white background. Changes estimated to be halfway between a 0.4 pH increment were not interpolated but recorded at the next higher value. The hourly readings for the saliva-sucrose mixtures (with and without agar) were recorded in terms of pH and transcribed to IBM punch cards.

E. Statistical Analysis

Standard methods of calculation were applied for evaluation of the test results. They were as follows:

1. Correlation Coefficients. These were computed as a measure of the fit of grouped data to a straight line[14]. Those values for the degree of linear relationship in a range of less than -0.4 to 0, and 0 to less than +0.4 indicated that the comparisons were of no consequence. Coefficients of -0.4 to -1.0 and of +0.4 to +1.0 were desired for validity of correlation.

2. Straight Line of Best Fit. This was computed for the given hour for which the graphical comparisons appeared to be close to a straight line, or was the closest comparison of a group of graphs to a straight line. This yielded the value of predicted caries for each of the 7 pH values at a given hour. The computation was done according to the criterion of least squares[14].

III. RESULTS

A. Acid Formation (pH) in Sucrose Solutions by Salivary Action

The distribution of pH change per hour for 4 hours induced in sucrose solutions with and without agar by single specimens of saliva from 134 children is given in Table I (page 17). This table illustrates that acid formation, as reflected by pH change, increased steadily with time. The rate was markedly accelerated by the addition of agar to the sucrose solution. Thus, 76.1 per cent of the specimens failed to alter the pH of sucrose solutions containing agar at the end of the first hour and only 7.5 per cent in four hours, compared to 97.8 per cent and 18.7 per cent, respectively, for the same specimens in sucrose solutions without agar. This effect was even more striking in respect to pH 5.0. Approximately the same number of specimens, ~~18.9~~ 19.4 per cent, were observed to be at pH 5.0 or lower in 2 hours in the presence of agar as in 4 hours without agar (23.9 per cent).

Although the correlation of laboratory data with clinical change was limited to the results obtained with single specimens of saliva, it was thought of interest to

TABLE I

Acid formation per hour for 4 hours at 37° C. in 8% sucrose solutions with and without agar (0.2%) by single specimens of saliva from 134 children

	8% Sucrose-Saliva Mixtures								8% Sucrose-Saliva Mixtures with Agar (0.2%)							
	Hours								Hours							
	1		2		3		4		1		2		3		4	
pH	No.	%*	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
6.6	131	97.8	91	67.9	52	38.8	25	18.7	102	76.1	56	41.8	19	14.2	10	7.5
6.2	0		24	17.9	32	23.9	20	14.9	18	13.4	22	16.4	19	14.2	3	2.2
5.8	1	0.8	12	9.0	19	14.2	18	13.4	5	3.7	15	11.2	15	11.2	13	9.7
5.4	1	0.8	5	3.7	23	17.2	39	29.1	6	4.5	15	11.2	25	18.7	17	12.7
5.0	1	0.8	2	1.5	8	6.0	31	23.1	2	1.5	23	17.2	49	36.6	51	38.1
4.6	0		0		0		1	0.8	1	0.8	3	2.2	7	5.2	39	29.1
4.2	0		0		0		0		0		0		0		1	0.8

*Calculated to nearest 0.1 per cent

tabulate the hourly pH change for all specimens (1076) of saliva to allow comparison with similar readings for the single specimens as given in Table I. The distribution of these pH changes for the total is listed in Table II (page 19). This listing shows there were no significant differences between pH recordings per hour for 4 hours in sucrose solutions with and without agar by all and those read for single specimens. Thus, the arbitrary selection of the first specimens of saliva for correlative studies actually gave a representative sample of the whole in terms of acid-forming potential.

A few tests became discolored with a muddy brown hue which made color comparison impossible. Because the majority of these were observed to reach pH 5.0 before this occurred, they were all recorded below this level.

Since pH 5.0 is held to be a critical value for the decalcification of enamel[15,16,17,18,19], it seemed of interest to compare the rate of time necessary to reach this acidity in sucrose solutions by the group of single specimens and the total. The percentages of both sets reaching pH 5.0 or lower in 8 per cent sucrose solutions with and without agar is shown graphically in Figure 1 (page 20).

Figure 1 shows that for all practical purposes the small amount of agar in the sucrose solutions resulted in halving the time of incubation; that is, just about the same

TABLE II

Acid formation per hour for 4 hours at 37° C. in 8 per cent sucrose solutions with and without agar (0.2%) by 1076 specimens from 134 children.

8% Sucrose

	1 hour		2 hours		3 hours		4 hours	
pH	No.	%	No.	%	No.	%	No.	%
6.6	1001	93.0	670	62.3	344	32.0	160	14.9
6.2	53	4.9	215	20.0	248	23.1	152	14.1
5.8	12	1.1	111	10.3	187	17.4	169	15.7
5.4	6	0.6	66	6.1	249	23.1	371	34.5
5.0	2	0.2	6	0.6	39	3.6	208	19.3
4.6							4	0.4
4.2								
Discolored	2	0.2	8	0.7	9	0.8	12	1.1

8% Sucrose with 0.2% Agar

	1 hour		2 hours		3 hours		4 hours	
pH	No.	%	No.	%	No.	%	No.	%
6.6	825	76.7	358	33.3	156	14.5	74	6.9
6.2	110	10.2	154	14.3	93	8.6	48	4.5
5.8	73	6.8	150	13.9	113	10.5	55	5.1
5.4	47	4.4	219	20.4	195	18.1	130	12.1
5.0	17	1.6	176	16.4	467	43.4	584	54.3
4.6	1	0.1	9	0.8	40	3.7	167	15.5
4.2							1	0.1
Discolored	3	0.3	10	0.9	12	1.1	17	1.6

Figure 1

(a). Percentage of specimens recorded as positive (pH 5.0 and below) per hour in 8 per cent sucrose solutions with and without 0.2 per cent agar for single saliva samples from 134 children.

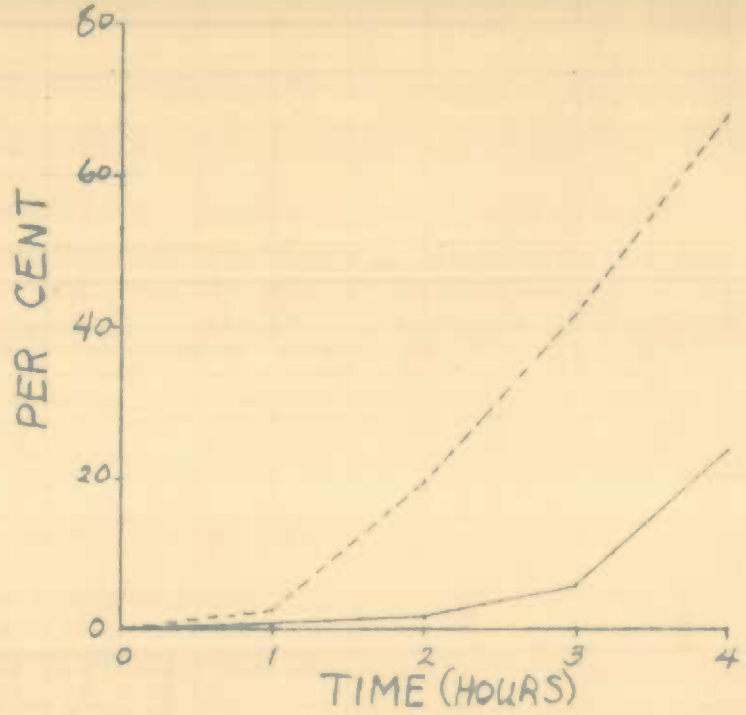
—— without agar

- - - with agar

(b). Percentage of specimens recorded as positive (pH 5.0 and below) per hour in 8 per cent sucrose solutions with and without 0.2 per cent agar for a total of 1076 saliva samples from 134 children.

—— without agar

- - - with agar


80
60
40
20
0
PER CENT
0
1
2
3
4
TIME (HOURS)

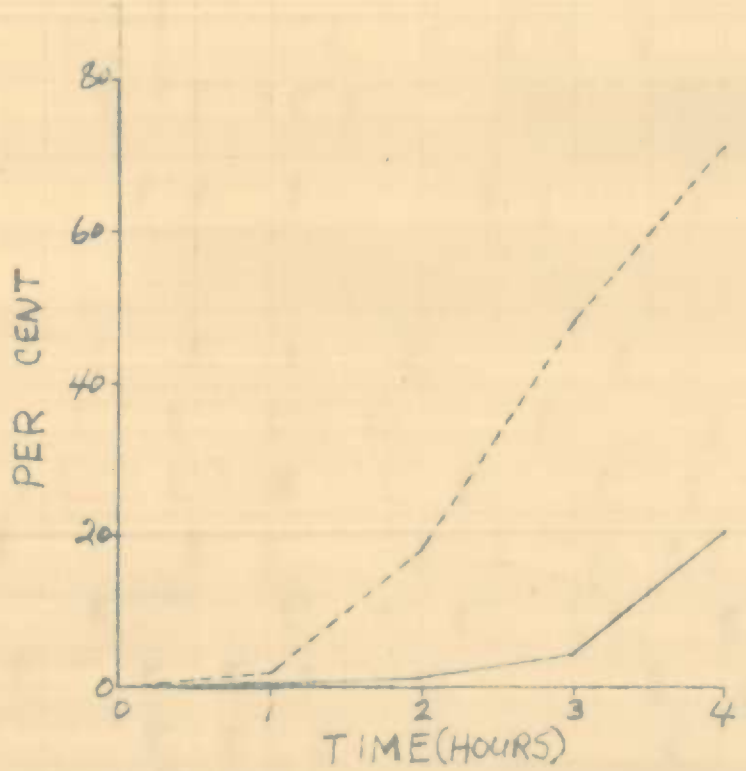

80
60
40
20
0
PER CENT
0
1
2
3
4
TIME (HOURS)

Figure 1

percentages were positive (pH 5.0 and below) in 2 hours as at the conventional reading time of 4 hours. However, on an hour for hour basis, the incidence of positive recordings after the first hour increased at a steady three to four fold rate in sucrose solutions without agar, in contrast to the nine fold jump from the first to the second hour in sucrose solutions containing 0.2 per cent agar. The precise role of agar was not determined; it was thought that it probably acted by dispersing clumps of bacteria, which in turn rapidly formed local acid, instead of prompt sedimentation in the liquid medium with slower diffusion of the acid. It did not seem these alterations could be ascribed to variations in numbers of bacteria in the constant volume of the inoculum (1.0 ml.) of saliva.

B. Comparison of Acid Formation in Sucrose with Caries Experience

The raw data from which subsequent correlations and comparisons were made with clinical findings are listed in Appendix I (page 44). Here the reader will find the results of acid formation (pH change) per hour for 4 hours in 8 per cent sucrose solutions with and without agar, the lactobacillus count, and the caries experience (new carious surfaces) observed one year after the initial clinical examination for each of the 134 subjects.

The first step in the computation of correlation coefficients was to tabulate the data in order to obtain the statistical units needed. These were as follows, for each pH value per hour: total number of new carious surfaces (c), total number of individuals (N), and mean caries experience ($\overline{X} = \frac{c}{N}$). These were calculated for reactions in sucrose solutions with and without agar. The results are to be found in Appendix II (page 48). An example of this tabulation for the acid formed in 8 per cent sucrose at the end of the first hour is given in Table III (page 23).

The lack of correlation between pH change and mean caries experience as shown in Table III was of course to be expected, since 98.7 per cent of the specimens of saliva were negative at the end of the first hour.

The comparison of hourly pH changes for each test method with mean caries experience as given in Appendix II is portrayed graphically in Figures 2 and 3 (pages 24 and 25). It is clear from these plotted values that little or no relationship can be found between extent and rate of acid formation by salivary action in 8 per cent sucrose with and without agar and the mean caries experience in the children under study. The sharp rise to a mean caries value of 11 at 1 hour in Figure 2 is of no consequence since it was plotted for one of the only 3 pH changes of the 134 tests. These results were of interest since they were contrary to the theory normally accepted; that is, increased caries

TABLE III

Tabulation of data for statistical units. Listing of pH changes at end of 1 hour in 8 per cent sucrose and new caries experience in 134 subjects over a 10-12 month period.

No. of New Dental Caries	pH at End of 1 Hour Incubation							No. of Individuals with Given Number of Caries
	6.6	6.2	5.8	5.4	5.0	4.6	4.2	
0	7							7
1	15							15
2	13							13
3	14							14
4	15				1			16
5	21			1				22
6	14							14
7	8							8
8	12							12
9	4							4
10	4							4
11			1					1
13	1							1
15	1							1
16	1							1
17	1							1
c	621		11	5	4			
N	131		1	1	1			
X	4.74		11.0	5.0	4.0			

Figure 2

Comparison of pH values (1, 2, 3, and 4 hours) induced by single specimens of saliva in 8 per cent sucrose compared with the mean caries experience of 134 children 10-12 months after sampling.

*Linear relationship of the values which Rickles[8] obtained at 4 hours.

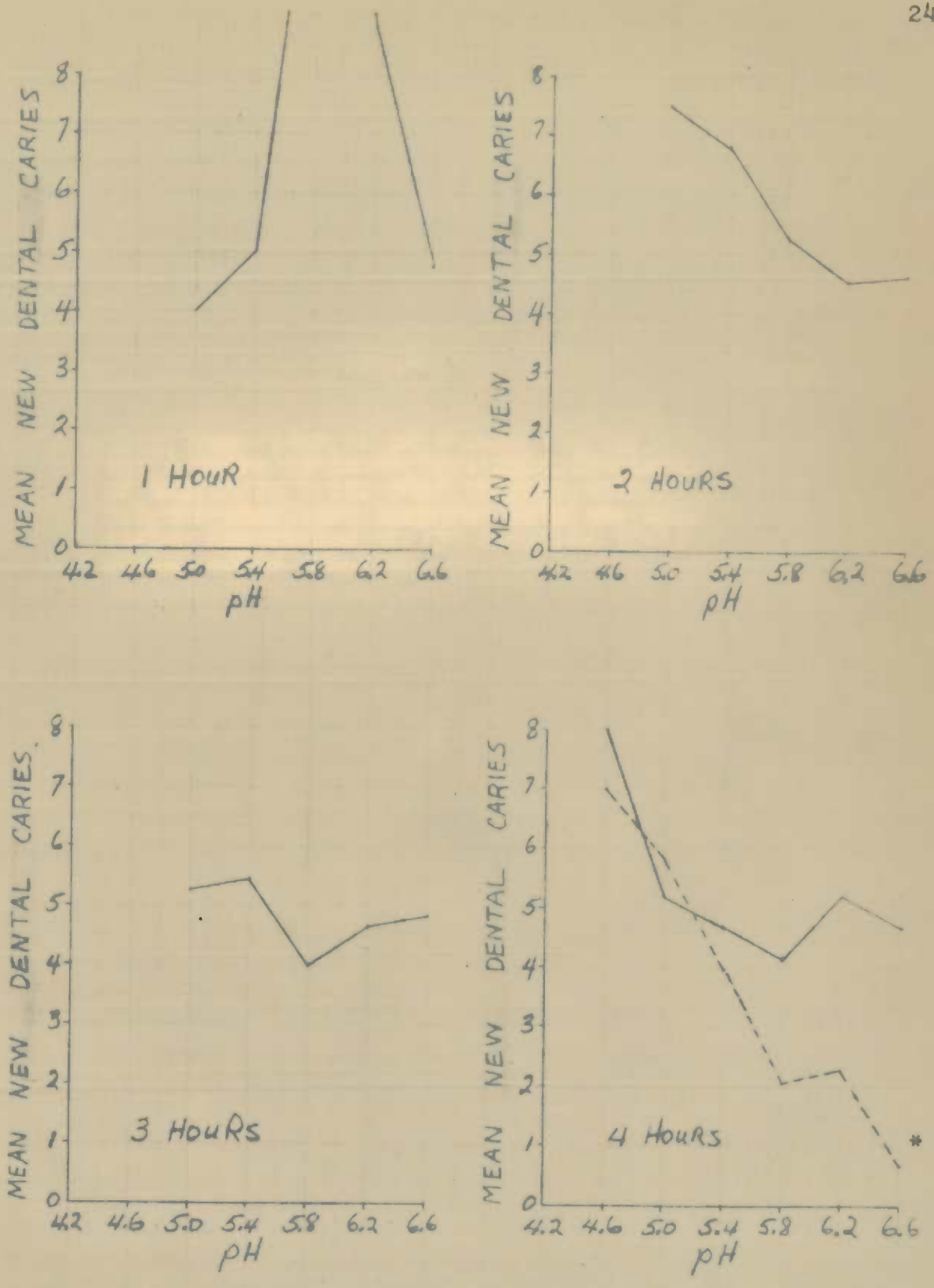

MEAN NEW DENTAL CARIES
0
1
2
3
4
5
6
7
8
4.2
4.6
5.0
5.4
5.8
6.2
6.6
pH
1 HOUR
2 HOURS
3 HOURS
4 HOURS
*

Figure 2

Figure 3

Comparison of pH values (1, 2, 3, and 4 hours) induced by single specimens of saliva in 8 per cent sucrose with 0.2 per cent agar compared with the mean caries experience of 134 children 10-12 months after sampling.

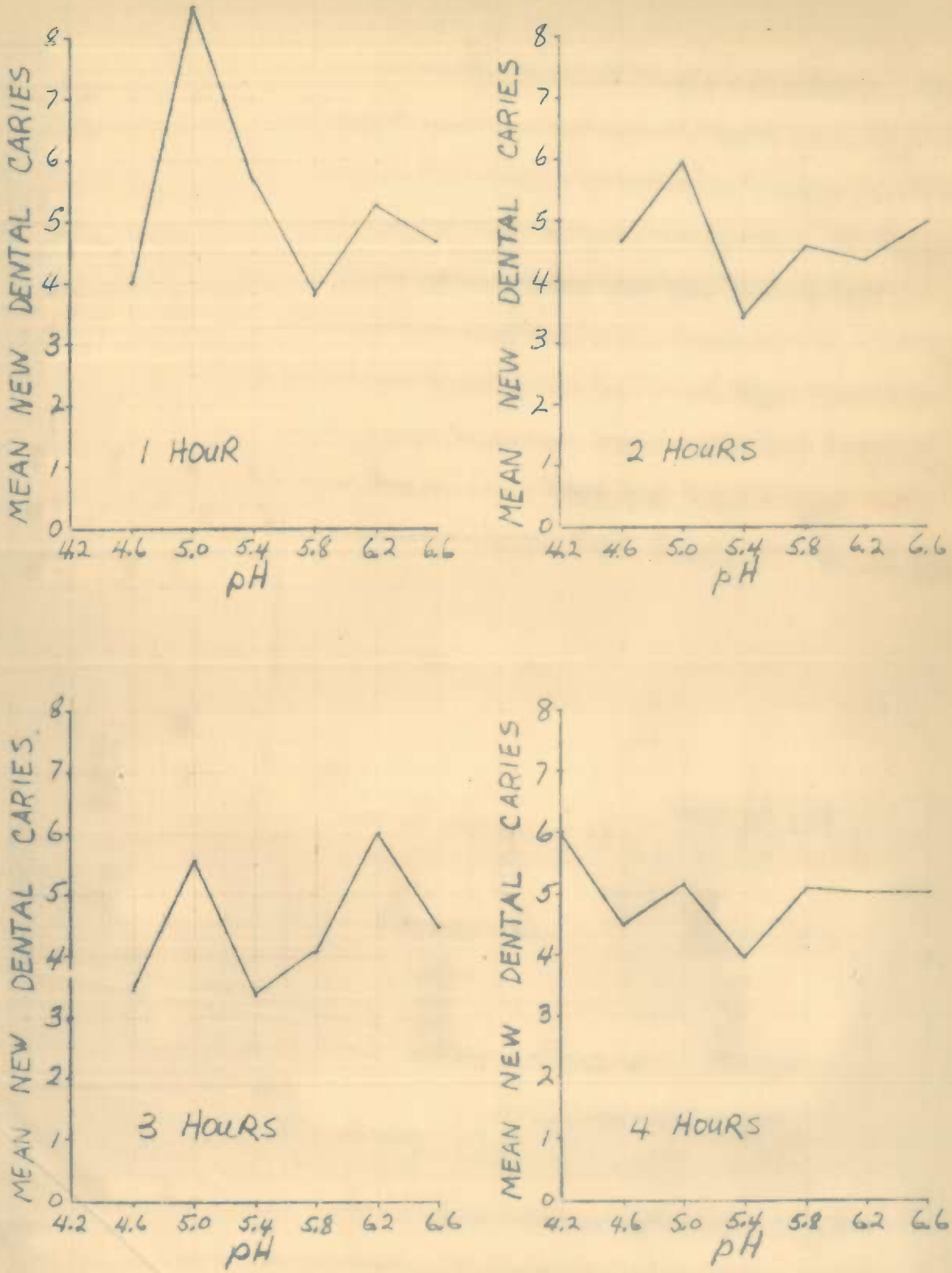

MEAN NEW DENTAL CARIES
0
1
2
3
4
5
6
7
8
1 HOUR
4.2 4.6 5.0 5.4 5.8 6.2 6.6
pH
MEAN NEW DENTAL CARIES
2 HOURS
pH
MEAN NEW DENTAL CARIES
3 HOURS
pH
MEAN NEW DENTAL CARIES
4 HOURS
pH

Figure 3

activity is associated with increased acid production. In no case was there less than a mean caries experience of 4.5 surfaces with completely negative laboratory findings. Similarly, the plotted values show slight linearity. No satisfactory explanation can be provided for the marked discrepancy between these results and those recorded by Rickles[8] at the end of the 4 hour incubation in 8 per cent sucrose.

The disparity between clinical experience and laboratory data was further emphasized when the correlation coefficients for these relationships were computed. These are given in Table IV.

TABLE IV

Correlation coefficients for hourly acid formation in 8 per cent sucrose with and without agar compared with mean caries experience in 134 children (from data in Appendix II).

TIME (hours)	8% SUCROSE WITHOUT AGAR		8% SUCROSE WITH AGAR
1	-0.05		-0.02
2	-0.14		-0.03
3	-0.05		-0.002
4	-0.02	(-0.56)*	+0.02

*Correlation coefficient obtained by Rickles[8]

The correlation coefficients listed in Table IV are well within the zone of insignificance; therefore, it was obvious that acid formation in sucrose by salivary action in this study was not correlated with an increased number

of new carious surfaces.

Since Rickles[8] obtained an excellent lineal response (see 4 hour graph in Fig. 2, page 24), it was thought of interest to compare the line of best fit for the data recorded in this study at the same time and in the same medium. The line of best fit for the 2 hour reading in sucrose solutions with agar was also included. The comparative predictive values for the 3 lines are given in Table V.

TABLE V

Comparison of predictive values of lines of best fit for acid production by salivary action in sucrose solutions at 4 hours (both by Rickles[8] and present study) and 2 hours in sucrose solutions with agar, with mean caries experience.

	Predicted Caries (New Surfaces) Present Study		Predicted Caries (New Surfaces) Rickles' Study
pH	Without Agar	With Agar	
6.6	4.68	4.69	0 (0-3)*
6.2	4.78	4.75	1 (0-4)
5.8	4.79	4.81	3 (0-5)
5.4	4.84	4.88	4 (1-6)
5.0	4.90	4.94	5 (3-8)
4.6	4.95	5.00	7 (4-9)
4.2	5.01	5.07	8 (5-11)

*Range of caries prediction after calculation of standard errors of prediction.

It is clearly apparent that the slopes derived for the present study, while close to each other, differ sharply from that computed by Rickles[8]. The spread of observed lesions developing over a year in the children under study

was essentially the same at pH 6.6 and pH 4.2 (0-10 surfaces). Therefore, pH change in sucrose solutions in this study had no predictive importance. For this reason, further statistical computations, such as standard errors of prediction, were not made.

C. Comparison of Lactobacillus Count with Caries Experience

Comparison was made in a manner similar to that already described; the logarithms of the lactobacillus counts for each of the specimens of saliva from the 134 children were tabulated in respect to total caries experience observed 10-12 months later. New mean caries values were then calculated. The results are given in Table VI (page 29).

Analysis of these data was made by plotting the logarithms of the lactobacillus counts against the mean caries experience as computed in Table VI. The resultant graph is shown as Figure 4 (page 30).

The curve line in Figure 4 again indicated little relationship between lactobacillus count and mean caries experience. The correlation coefficient or degree of linear relationship was +0.27 which, while considerably better than any computed value for acid formation in sucrose, was still short of a significant coefficient (0.4) for group correlation. Nevertheless, a line of best fit to a straight line was computed; the calculated values are listed in Table VII (page 31).

TABLE VI

Tabulation of data for statistical units. Listing of logarithms of lactobacillus count and new caries experience in 134 subjects over a 10-12 month period.

No. of New Dental Caries	Logarithm of Lactobacillus Count					No. of Individuals with Given Number of Caries
	1	2	3	4	5	
0	4		1		2	7
1	6	2	3	3	1	15
2	7	3		1	2	13
3	6		3	5		14
4	5		2	8	1	16
5	7	2	2	8	3	22
6	2	3	3	5	1	14
7	2	1	2	3		8
8	3	2	1	5	1	12
9	1	1		2		4
10			1	1	2	4
11					1	1
13				1		1
15				1		1
16				1		1
17				1		1
c	152	68	80	272	69	
N	43	14	18	45	14	
$\bar{X}$	3.5	4.9	4.4	6.04	4.93	

Figure 4

Comparison of logarithms of lactobacillus counts from single specimens of saliva with the mean caries experience of 134 children determined 10-12 months after sampling.

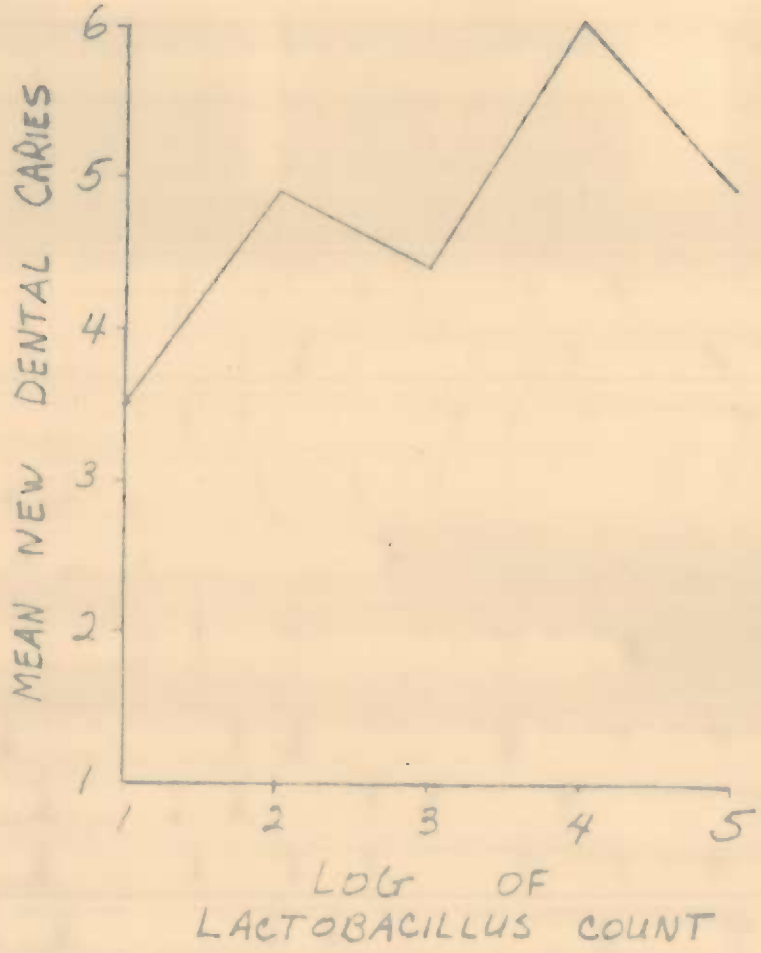

MEAN NEW DENTAL CARIES
6
5
4
3
2
1
1
2
3
4
5
LOG OF
LACTOBACILLUS COUNT

Figure 4

TABLE VII

Comparison of line of best fit to a straight line for the logarithm of the lactobacillus count with mean caries experience.

Logarithm of Lactobacillus Count	Predicted Caries (New Surfaces)
1	3.71
2	4.31
3	4.90
4	5.49
5	6.09

The slope of the line in Table VII, derived from the statistical analysis of observed mean caries, has no practical application in prediction of new carious surfaces. This was in agreement with the coefficient of low significance for group correlation.

D. Correlation of Both Laboratory Tests

The correlation of laboratory tests with each other was done. The hourly pH values of single specimens of saliva in 8 per cent sucrose with and without agar were tabulated against the logarithms of lactobacillus counts of respective specimens from 134 children. Calculations for the mean logarithmic values were made for each hourly pH level. These values were plotted against the pH increments for the hourly determinations in 8 per cent sucrose with and without agar.

The mean logarithmic value for the negative reactions (pH 6.6) in 8 per cent sucrose with and without agar was 2.05 or slightly more than 100 lactobacilli/ml. of saliva; the same index for the lowest pH reading (pH 4.2) was 4.0 or 10,000 lactobacilli/ml. of saliva. Although there were higher values for some pH changes in the range of the test, the curve was considered to show the tests to have little relationship. This was borne out by the correlation coefficients calculated from these data. These ranged from -0.19 at the first hour to a maximum of -0.28 at 3 hours for sucrose solutions without agar and -0.24 at 2 hours for sucrose with agar. Thus, acid formation in sucrose was not held to be significantly correlated with numbers of lactobacilli in the saliva. In view of these results, further statistical analysis was not made.

IV. DISCUSSION

Before discussing acid formation by saliva in sucrose solutions as a predictor of caries experience, some comment should be made about the test itself. It is a very simple test. There are only four variables: the sucrose, the indicators, the saliva proper, and the bacteria of the saliva. As part of preliminary experimentation, it was found that sucrose was superior to glucose as it was more stable both to autoclaving and to storage. Thus, autoclaving 8 per cent glucose in the presence of indicators lowered the pH below 6.6, while 8 per cent sucrose remained unchanged. Unsterilized sucrose solutions in tubes with metal caps could be kept in the refrigerator for weeks without showing evidence of alteration, whereas molds were soon found in the glucose solutions. Perhaps it should be mentioned here that in a test completed in 4 hours, growth and multiplication of bacteria as contaminants of the glassware and reagents were of little consequence; so sterilization of glassware and reagents was not done. In other words, sterility was of no importance.

The introduction of a double indicator system by Rickles[8] was an innovation to laboratory tests in this field.

Both Snyder[3] and Rogosa and Wiseman[4] used only single indicators, bromcresol green and alizarin red S, respectively, which were satisfactory for the range used. However, in the present test the two indicators, bromcresol green and bromcresol purple, gave a wider range of pH than either alone. The colors went from bluish-purple at pH 6.6 to blue, blue-green, green, green-yellow, and yellow at pH 4.2. Efforts were made to standardize the readings by means of a colorimeter. Between sedimentation in the sucrose solutions, effect of agar on light transmission, the necessity of using sufficient volumes in standardized tubes for reading, and two wave lengths of maximum absorption (one each for the pH range of greatest influence for each indicator), it proved impractical to use such an instrument. Therefore, all observations were visual. The shortcomings of this type of observation are sufficiently well known to need no comment. Suffice it to say, every effort was made to keep the estimation of color change as constant as possible by using the same light source and background.

Some experiments were done with the sediments and supernatants of centrifuged saliva. The sediment produced acid in 8 per cent sucrose at a fairly rapid rate, whereas the supernatant of saliva produced absolutely no change in the pH of sucrose solutions in 4 hours, which is in agreement with what is known: there are no enzymes in the saliva <u>per se</u> which are capable of hydrolysing sucrose[20]. It would

seem, therefore, that saliva plays no active role in the utilization of sucrose, but rather acts as a carrier of bacteria removed from the teeth and mucous membranes of the oral cavity.

The role of bacteria in this test was investigated only on a small scale. However, the findings were interesting. Strains of lactobacilli, streptococci, staphylococci, gram-negative diplococci, and yeast-like organisms selected at random from cultures of saliva were added to sucrose solutions in the form of washed cells to give approximately 5 per cent by volume. Despite some disparity in the cell/volume ratio, it was obvious that streptococci produced acid in the sucrose at a faster rate than any of the others. Thus, the prompt acid formation in sucrose by specimens of saliva could be attributed to streptococcal action rather than that of other forms; this is in line with the fact that sucrose-fermenting streptococci (S. salivarius) are the dominant culturable aerobic forms in the saliva[21]. It would also help to explain why there was no correlation with caries activity since no specific streptococcus type has been convincingly demonstrated to be associated with human caries. The test as conducted reflects acid formation by all the viable bacteria in the specimen, and as the time of incubation continues it is probably greatly influenced by symbiotic and synergistic relationships amongst these cells.

The purpose of this study was, however, not to study acid formation in sucrose solutions as a problem, but what it meant in respect to estimating or predicting caries activity. Rickles[8] obtained a reasonably clear cut relationship which supported the generally accepted theory of increased caries activity with increased acid formation. We were unable to confirm his findings; there was no relation between rate and extent of acid formation in sucrose and new caries experience in the group of children under study.

In seeking explanations for the markedly different correlations between the two investigations, an analysis of the factors was made. There were three apparent discrepancies: the test procedure, the age of the subjects, and age of the test material (saliva). Although we modified the procedure in respect to volume and tube size, enough tests (35) were done to convince us that the rate of acid formation was the same per hour in the larger volume and tube as in the smaller. The ratio of saliva to sucrose was the same. Therefore, the test itself would not appear to be involved. The mean age of the group studied by Rickles[8] was 22 years, compared to the mean age of 11 years and 4 months in the present study. This meant that the older group had permanent dentition and the younger one mixed dentition. Although only 29 of our 134 children were 14 years or older and with all permanent dentition, correlation coefficients computed for this group were no different than those for the total.

Thus it would appear that the state of dentition was not a determining factor. This would apparently reduce the problem to the age of the specimen of saliva. Rickles[8] used specimens of saliva 48-96 hours of age, while all our tests were completed within 24 hours. This discrepancy missed our attention until it was too late to evaluate it, but it was a point about which a few tests had been done early in the investigation. In our hands we observed no difference in specimens held 24 and 48 hours at either room or refrigerator temperatures. The number of the specimens tested was admittedly too few (17) to be convincing, but in view of the rapid deterioration (putrefaction) of saliva at room temperature it was held preferable to perform the test as rapidly as possible after collection. Since we did not conduct tests with specimens of saliva older than 48 hours, we cannot provide any information on this point. It may be of critical importance.

In both studies clinical examinations were carried out by independent personnel so that bias would be reduced to a minimum. Both were measured in terms of new carious surfaces, but there may be a difference in the ratio between new carious surfaces and the total number of surfaces exposed. While an index based on this relationship, which allows for the deciduous dentition, has been developed for the major program, it was not used in this study because the computations based on the recording of the most recent clinical

examination were incomplete. Still, it would not appear that this method of clinical measurement will account in a major way for the lack of correlation observed.

It was also interesting to note that there was no predictive value for the lactobacillus count in estimating new caries experience observed one year after sampling. This finding is in agreement with Rickles[8]. It is contrary to studies which have shown caries activity increases with increasing numbers of lactobacilli[9,22,23,24,25], although the most ardent supporters of this theory claim best correlation at the extremes; namely, no caries with negative counts and active caries with the highest counts. None has ventured to predict or estimate the actual numbers of surfaces to be involved or for longer than 6 months in advance. Glass[26] found absolutely no relationship between the lactobacillus count of specimens of saliva and the number of surfaces determined one year later in a group of children. At the present time, we would appear to be in agreement with Glass[26].

Some comment should be made on the use of single specimens. Both studies were based on the use of single specimens of saliva for predicting caries experience. Despite the recognized shortcomings of a single sampling, for any predictive purpose, it was found the percentage of these specimens forming acid in sucrose per hour was not significantly different for those of the total 1076 samples. There were

marked variations in the capacity of the saliva of the individual to form acid with specimens collected semi-monthly. Whether averages of several successive samples would be better predictors of caries activity than the single specimen remains to be computed as a phase of the larger investigation of which this is a part.

We can provide no rational explanation at the present time for our results diverging so from those of Rickles[8] and from those of many investigators[9,22,23,24,25] who support the important role of the lactobacilli in dental caries.

V. CONCLUSION

Evaluation of acid formation in 8 per cent sucrose by salivary action and lactobacillus counts of the same specimens as predictors of new caries experience led to the following conclusion:

1. No relationship existed between the rate and amount of acid formation by salivary action of single specimens in 8 per cent sucrose with and without agar and new caries experience of 134 children 10-12 months later.

2. No relationship existed between the lactobacillus count of single specimens of saliva and new caries experience of 134 children 10-12 months later.

3. No relationship existed between the rate and extent of acid formation by salivary action of single specimens in 8 per cent sucrose with and without agar with the lactobacillus count of respective specimens.

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APPENDIX I

Listing of acid formation (pH) in 8 per cent sucrose solution with and without 0.2 per cent agar and lactobacillus counts of specimens of saliva with new caries experience for each of 134 children 10-12 months after laboratory tests completed.

Case No.	pH Values in 8% Sucrose-Saliva Mixtures (Hours)				pH Values in 8% Sucrose(0.2% Agar)-Saliva Mixtures (Hours)				L.A. Count	New Dental Caries (Surface)
	1	2	3	4	1	2	3	4		
2	6.6	6.6	6.6	6.6	6.6	6.6	6.2	5.8	23,000	10
3	6.6	6.6	6.6	6.6	6.6	6.6	6.2	5.8	300	5
4	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.2	0	5
5	6.6	6.6	6.6	6.2	6.6	6.6	5.8	5.4	56,000	6
9	5.0	5.0	5.0	5.0	4.6	4.6	4.6	4.6	86,000	4
10	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	1,500	3
11	6.6	6.6	6.6	6.6	6.6	6.6	6.6	5.8	87,000	8
12	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	0	1
14	6.6	6.6	6.6	6.6	6.6	6.6	6.6	5.8	0	3
16	6.6	6.6	6.2	5.8	6.2	5.8	5.0	4.6	42,000	4
18	6.6	6.6	5.8	5.4	6.6	6.2	5.0	5.0	0	3
19	6.6	6.2	5.4	4.6	6.2	5.0	5.0	4.6	98,000	8
20	6.6	6.2	5.4	5.0	6.2	5.0	5.0	4.6	27,000	3
22	6.6	6.6	5.8	5.4	6.6	5.8	5.0	5.0	108,000	2
27	6.6	6.6	6.6	6.2	6.6	6.6	6.6	5.8	500	2
28	6.6	6.6	6.6	6.2	6.6	6.6	6.2	5.4	68,000	15
30	6.6	6.2	5.4	5.0	6.2	5.0	5.0	4.2	61,000	6
32	6.6	6.6	6.6	6.6	6.6	6.6	6.2	5.8	33,000	4
34	6.6	6.2	5.4	5.4	6.6	5.8	5.0	5.0	700	7
35	6.6	6.6	6.6	6.2	6.6	6.2	5.8	5.0	3,500	0
36	6.6	6.6	6.6	5.4	6.6	6.2	5.4	4.6	13,000	4
37	6.6	5.8	5.4	5.0	5.0	4.6	4.6	4.6	400	6
38	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	3,400	6
39	6.6	6.6	6.6	6.6	6.6	6.6	6.2	5.8	70,000	9
43	6.6	6.6	6.2	5.4	6.6	6.2	5.4	5.0	5,000	5
44	6.6	5.8	5.0	5.0	5.4	5.0	4.6	4.6	700	1
45	6.6	6.2	5.8	5.4	6.2	5.8	5.0	5.0	37,000	5
46	6.6	6.6	6.2	5.8	6.6	6.2	5.8	5.0	400	8
49	6.6	6.2	5.8	5.4	6.6	5.8	5.4	4.6	1,500	1

(continued)

Appendix I continued

Case No.	pH Values in 8% Sucrose-Saliva Mixtures (Hours) 1	2	3	4	pH Values in 8% Sucrose(0.2% Agar)-Saliva Mixtures (Hours) 1	2	3	4	L.A. Count	New Dental Caries (Surface)
50	6.6	6.6	6.2	5.4	6.6	6.2	5.4	5.0	50,000	8
51	6.6	5.8	5.4	5.4	5.8	5.0	5.0	4.6	30,000	16
53	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.2	600	1
57	6.6	6.6	5.8	5.0	6.6	6.2	5.0	4.6	0	3
64	6.6	6.6	6.6	6.6	6.6	6.6	5.8	5.0	11,000	1
66	6.6	5.8	5.0	5.0	5.4	4.6	4.6	4.6	32,000	4
77	6.6	6.6	6.6	6.2	6.6	5.8	5.4	5.0	0	5
78	6.6	6.2	5.8	5.4	6.2	5.0	5.0	5.0	0	6
79	6.6	6.6	6.2	5.4	6.6	6.6	6.2	5.0	0	5
81	6.6	6.2	5.8	5.0	6.2	5.4	5.0	4.6	72,000	6
83	6.6	6.6	6.6	5.8	6.6	6.6	6.2	5.0	30,000	13
88	6.6	6.2	5.8	5.0	6.2	5.0	5.0	4.6	14,000	5
90	5.8	5.0	5.0	5.0	5.0	5.0	5.0	5.0	120,000	11
91	6.6	6.6	6.6	6.2	6.6	6.6	6.2	5.4	36,000	3
92	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	900	8
93	6.6	5.8	5.4	5.0	5.8	5.0	5.0	5.0	7,000	3
95	6.6	6.2	5.4	5.0	6.6	5.8	5.0	4.6	140,000	5
97	6.6	6.6	6.2	5.4	6.6	6.6	5.4	5.0	480,000	1
99	6.6	6.2	5.8	5.4	6.6	5.4	5.4	5.0	0	2
100	6.6	6.6	6.6	6.2	6.6	6.6	6.2	5.4	0	8
102	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	34,000	5
103	6.6	6.6	6.6	6.2	6.6	6.6	6.2	5.8	3,600	8
104	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	0	2
107	6.6	6.6	6.6	6.2	6.6	6.6	6.2	5.4	85,000	8
108	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	100	5
110	6.6	6.6	6.2	5.8	6.6	6.6	5.4	5.4	200	2
111	6.6	5.8	5.4	5.4	5.4	5.0	5.0	5.0	320,000	10
114	6.6	5.8	5.4	5.0	6.6	5.0	5.0	5.0	300,000	10
116	6.6	6.2	5.4	5.4	6.6	5.4	5.0	4.6	68,000	1
117	6.6	6.6	6.2	5.4	6.6	6.2	5.4	4.6	0	3
118	6.6	6.6	6.2	5.4	6.6	6.2	5.4	5.0	0	3
119	6.6	5.8	5.4	5.0	6.2	5.0	5.0	4.6	120,000	0
121	6.6	6.2	5.4	5.4	6.6	5.4	5.0	4.6	1,800	1
123	6.6	6.6	6.6	5.8	6.6	6.6	5.8	5.4	0	4
124	6.6	6.6	6.2	5.4	6.6	6.6	5.8	5.0	15,000	7
125	6.6	6.6	6.2	5.4	6.6	6.6	5.8	5.0	21,000	6
128	6.6	6.6	6.2	5.4	6.6	6.2	5.4	5.0	76,000	3
130	6.6	6.6	6.6	6.2	6.6	6.6	6.2	5.4	0	1
131	6.6	6.6	6.2	5.8	6.6	6.6	5.8	5.4	1,200	1
132	6.6	6.6	6.2	6.2	6.6	6.6	5.8	5.4	1,000	4

(continued)

Appendix I continued

Case No.	pH Values in 8% Sucrose-Saliva Mixtures (Hours) 1	2	3	4	pH Values in 8% Sucrose(0.2% Agar)-Saliva Mixtures (Hours) 1	2	3	4	L.A. Count	New Dental Caries (Surface)
134	6.6	6.6	6.2	5.8	6.6	6.6	5.0	5.0	4,400	8
135	6.6	6.6	6.6	6.2	6.6	6.6	5.8	5.4	84,000	4
136	6.6	6.6	5.8	5.4	5.8	5.4	5.4	5.0	14,000	2
137	6.6	6.6	6.6	6.6	6.6	6.6	6.2	5.8	11,000	5
140	6.6	6.6	6.6	6.2	6.6	6.6	6.2	5.0	2,800	5
141	6.6	6.6	6.2	5.4	6.6	6.6	5.0	5.0	12,000	17
142	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	0	9
144	6.6	6.6	6.2	5.4	6.6	6.2	5.0	5.0	0	4
146	6.6	6.6	6.6	6.2	6.6	6.6	6.6	5.8	0	8
148	6.6	6.2	5.8	5.4	6.6	5.4	5.0	5.0	0	7
149	6.6	5.8	5.0	5.0	5.4	5.0	4.6	4.6	180,000	4
156	6.6	6.6	5.8	5.4	6.6	5.8	5.4	5.0	138,000	2
501	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	0	2
502	6.6	6.6	6.6	6.6	6.6	6.6	6.6	5.4	17,000	3
503	6.6	6.6	6.6	6.2	6.6	6.2	5.8	5.0	10,000	7
504	6.6	6.6	6.2	5.8	6.6	5.4	5.4	5.0	80,000	1
508	6.6	5.8	5.4	5.4	6.2	5.0	5.0	4.6	0	1
510	6.6	6.2	5.4	5.4	6.6	5.4	5.4	5.4	6,000	4
511	6.6	6.6	6.6	5.8	6.2	5.4	4.6	4.6	100	2
513	6.6	6.6	6.6	6.6	6.6	6.6	6.2	5.8	0	0
515	6.6	6.6	6.2	5.8	6.6	6.2	5.4	5.0	0	3
525	6.6	6.6	6.6	6.2	6.6	5.8	5.4	5.0	0	2
527	6.6	6.2	5.4	5.0	6.6	5.8	5.0	5.0	0	5
532	6.6	6.6	6.6	6.2	6.6	6.2	5.8	5.0	0	7
533	6.6	6.6	5.8	5.0	6.6	5.4	5.0	4.6	500	6
534	6.6	6.2	6.2	5.4	6.2	5.8	5.0	5.0	0	8
542	6.6	5.4	5.4	5.0	5.8	5.0	5.0	5.0	600	6
543	6.6	6.6	6.6	5.8	6.6	6.2	6.2	5.4	0	6
544	6.6	6.6	6.6	5.8	6.6	6.6	6.2	5.4	0	0
545	6.6	6.6	6.6	6.6	6.6	6.6	6.6	5.8	0	4
546	6.6	6.2	6.2	5.0	6.6	5.4	5.0	4.6	15,000	5
547	6.6	6.2	5.8	5.0	6.2	5.4	5.0	4.6	0	2
551	6.6	6.6	6.2	6.2	6.6	6.6	5.8	5.4	22,000	4
552	6.6	6.6	6.6	6.2	6.6	6.2	5.8	5.4	0	0
553	6.6	6.6	6.6	5.8	6.6	5.8	5.4	4.6	9,000	7
554	6.6	6.6	6.2	5.8	6.6	5.8	5.0	4.6	1,700	6
555	6.6	6.6	6.2	5.4	6.6	5.4	5.0	5.0	0	5
556	6.6	5.8	5.0	5.0	5.8	5.0	5.0	4.6	80,000	3
557	6.6	6.6	5.8	5.4	6.6	5.8	5.0	4.6	20,000	4
558	6.6	6.6	6.6	5.8	6.6	6.6	5.8	5.4	0	0

(continued)

Appendix I continued

Case No.	pH Values in 8% Sucrose-Saliva Mixtures (Hours) 1	2	3	4	pH Values in 8% Sucrose(0.2% Agar)-Saliva Mixtures (Hours) 1	2	3	4	L.A. Count	New Dental Caries (Surface)
559	6.6	5.4	5.0	5.0	5.4	5.0	5.0	5.0	1,900	10
561	6.6	6.2	5.8	5.4	6.6	5.4	5.0	5.0	0	2
565	6.6	5.4	5.4	5.0	5.8	5.0	5.0	4.6	22,000	5
567	6.6	5.8	5.4	5.0	6.2	5.0	5.0	5.0	48,000	5
568	6.6	6.6	5.8	5.0	6.6	6.6	5.0	4.6	0	4
570	5.4	5.4	5.0	5.0	5.0	5.0	5.0	5.0	216,000	5
571	6.6	6.6	6.6	6.2	6.6	6.2	5.4	4.6	1,500	7
573	6.6	6.6	6.2	5.4	6.6	6.2	5.4	5.0	360,000	0
578	6.6	6.6	6.6	6.6	6.6	6.6	6.2	5.8	0	4
583	6.6	6.2	5.4	5.0	6.6	5.8	5.0	4.6	0	5
584	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	16,000	9
587	6.6	6.6	6.6	6.6	6.6	6.6	6.2	5.8	200,000	5
589	6.6	6.2	5.8	5.0	6.6	5.4	5.0	4.6	30,000	5
590	6.6	6.6	6.2	5.4	6.6	6.2	5.0	4.6	0	5
592	6.6	6.6	6.2	5.8	6.6	6.6	5.8	5.0	0	2
593	6.6	6.6	6.2	5.4	6.6	6.6	5.4	5.0	0	1
594	6.6	6.2	5.4	5.0	6.2	5.0	5.0	5.0	87,000	7
595	6.6	5.4	5.4	5.0	6.2	5.0	5.0	4.6	108,000	8
596	6.6	6.6	6.6	5.8	6.6	6.6	6.6	5.0	0	1
598	6.6	6.6	6.2	5.4	6.6	6.2	5.0	5.0	0	1
599	6.6	6.6	5.8	5.4	6.6	6.2	5.0	5.0	600	9
602	6.6	6.6	6.2	5.8	6.6	6.6	5.4	4.6	8,000	6
606	6.6	6.6	6.2	5.4	6.6	6.6	5.4	4.6	180,000	6
607	6.6	6.2	5.4	5.0	6.2	5.0	4.6	4.6	2,100	3
609	6.6	6.6	6.2	5.4	6.6	6.2	5.4	4.6	77,000	6

APPENDIX II

Acid production (pH) of single specimens of saliva from 134 children summarized in respect to statistical units employed for calculation of correlation coefficients.

Time	Statistical Units*	Medium**	pH 6.6	6.2	5.8	5.4	5.0	4.6	4.2
1 hr.	c	S	621		11	5	4		
		S/A	472	95	19	34	17	4	
	N	S	131		1	1	1		
		S/A	102	18	5	6	2	1	
	$\bar{X}$	S	4.7		1.1	5.0	4.0		
		S/A	4.6	5.3	3.8	5.7	8.5	4.0	
2 hrs.	c	S	420	109	63	34	15		
		S/A	277	95	68	51	136	14	
	N	S	91	24	12	5	2		
		S/A	56	22	15	15	23	3	
	$\bar{X}$	S	4.6	4.5	5.3	6.8	7.5		
		S/A	5.0	4.3	4.5	3.4	5.9	4.7	

*c = total number of caries at a given pH.
N = total number of individuals at a given pH
$\bar{X}$ = mean number of new caries per individual at a given pH

**S = 8 per cent sucrose
S/A = 8 per cent sucrose with 0.2 per cent agar

APPENDIX II (continued)

Time	Statistical Units	Medium	6.6	6.2	5.8	5.4	5.0	4.6	4.2
	c	S	250	148	76	125	42		
		S/A	85	114	61	84	273	24	
3 hrs.	N	S	52	32	19	23	8		
		S/A	19	19	15	25	49	7	
	$\bar{X}$	S	4.8	4.6	4.0	5.4	5.3		
		S/A	4.5	6.0	4.1	3.4	5.6	3.4	
	c	S	117	104	74	183	155	8	
		S/A	50	15	66	67	262	175	6
4 hrs.	N	S	25	20	18	39	31	1	
		S/A	10	3	13	17	51	39	1
	$\bar{X}$	S	4.7	5.2	4.1	4.7	5.2	8.0	
		S/A	5.0	5.0	5.1	3.9	5.1	4.5	6.0