10. Concentrations are expressed in volume formality, F, the number of formula weights per liter of solution.

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Clinical Experiment on the Use of Sodium N-Lauroyl Sarcosinate in the Control of Dental Caries

The belief is widely held that dental caries is caused by acids formed on a tooth surface by the enzymic degradation of carbohydrates (*1*). On the basis of this concept, attempts have been made to control dental caries by reducing the availability of the sugar substrate (*2*), by making the tooth more resistant to acids (*3*) or by the prevention of fermentation. One method of control that has been suggested (*4*) makes use of enzyme inhibitors to prevent the glycolysis of the sugars. This method has been clinically tested with the use of a dentifrice containing 2-percent sodium N-lauroyl sarcosinate (*5*), a very effective inhibitor of hexokinase *in vitro* (*6*).

The test subjects were young adults from the Meredith Publishing Company, Des Moines, Iowa, and from the student bodies of the University of Miami, Florida, the University of Florida, Gainesville, and Drake University. The experiment at each geographic location was conducted independently by different investigators, although the procedures used were the same and were formulated by an over-all coordinator.

Each subject was classified in one of the following test groups. *T-1-D*: Subjects used a dentifrice containing 2-percent sodium N-lauroyl sarcosinate morning and night. *T-2-D*: The same dentifrice was used after each meal. *C-1*: Subjects used a dentifrice of their choice in the manner to which they were accustomed. *C-2-D*: Subjects used, morning and night, a dentifrice identical to that used by the T-1-D and T-2-D groups, except that sodium N-lauroyl sarcosinate was replaced by 2-percent sodium salt of sulfated glyceride of coconut fatty acids. In all groups except C-1, instructions were given for proper brushing techniques, and all subjects were issued dentifrice and brushes as needed.

Since it was expected that 30 to 40 percent of the participants in the experiment would drop out each year, the homogeneity of the groups was not determined until the experiment had been completed. Comparisons were made then from data collected before the tests among the various groups and locations in respect to distribution of age; sex; DMF (decayed, missing, filled) surfaces; DF teeth; missing teeth; caries-free surfaces; caries-free teeth; free proximal surfaces; free labial, lingual, or buccal surfaces; and free occlusal surfaces.

At the start of the experiment, the average age of the subjects was 26 at Meredith, 19 at Drake, 22 at Miami, and 20 at Gainesville. In all groups and locations, the males were slightly more numerous than the females. Although within each group the numbers of decayed, missing, and filled surfaces were consistent, the counts were somewhat higher at Meredith and considerably higher at Gainesville. Initial examinations were made on 2543 subjects. At the end of the first year of the test, there were 1883. This number decreased to 1159 subjects who completed the full 2 years of the experiment.

The increment of new carious surfaces was determined by clinical examination and radiographs, as was the involvement of teeth that had been noncarious at the start of the tests. In this regard, test subjects were compared with the controls with the data from each installation. Inasmuch as the group at Gainesville was not strictly comparable with the others

Table 1. Dental caries activity during 2-year test period.

Group	Installation	Condition at start				2-year increment of dental caries			
				Average no.		Teeth		Surfaces	
		No. subjects	Avg. age	DMF surfaces	DF teeth	$\bar{x}$	σ^2	$\bar{x}$	σ^2
C-2-D	Gainesville	41	20.2	51.6	19.7	0.76	0.49	7.51	14.55
	Miami	86	22.1	39.4	16.6	0.57	0.51	3.19	4.91
	Meredith	182	26.6	35.8	16.0	0.39	0.40	1.78	2.72
	Drake	74	18.8	34.1	16.2	0.38	0.43	1.69	3.04
	All groups	383	23.4	38.0	16.6	0.47	0.45	2.69	7.63
T-1-D	Gainesville	60	19.5	44.5	19.0	0.40	0.45	4.25	9.62
	Miami	107	21.5	37.3	16.0	0.18	0.25	1.12	2.09
	Meredith	116	26.7	37.1	16.3	0.15	0.16	0.68	0.95
	Drake	135	19.5	34.6	16.0	0.21	0.26	1.07	1.79
	All groups	418	22.0	37.4	16.5	0.22	0.26	1.43	4.10
T-2-D	Gainesville	36	20.3	55.9	20.1	0.50	1.00	4.22	15.22
	Miami	105	23.0	40.2	16.2	0.24	0.25	1.20	1.78
	Both groups	141	22.3	44.2	17.2	0.31	0.44	1.97	6.90
C-1	Gainesville	19	19.7	56.5	20.5	0.79	1.62	7.00	24.00
	Miami	82	22.1	34.6	15.4	0.62	0.83	2.71	5.48
	Meredith	86	26.6	35.0	16.1	0.44	0.48	1.92	3.18
	Drake	30	19.4	32.0	15.2	0.17	0.14	1.40	3.35
	All groups	217	23.3	36.4	16.1	0.50	0.68	2.60	7.82

Table 2. Comparison of control and test results.

Contrast	Installation	Teeth		Surfaces	
		Reduction (%)	t	Reduction (%)	t
C-2-D	Gainesville	47	2.62*	43	4.72†
vs.	Miami	68	4.45†	65	7.82†
T-1-D	Meredith	62	3.67†	62	6.55†
	Drake	45	2.07*	37	2.88‡
	All groups	53	5.88†	47	7.40†
C-2-D	Gainesville	34	1.34	44	3.73†
vs.	Miami	58	3.76†	62	7.65†
T-2-D	Both groups	51	3.83†	57	7.03†
C-1	Gainesville	49	1.74	39	2.90‡
vs.	Miami	71	4.22†	59	5.75†
T-1-D	Meredith	66	3.78†	65	6.33†
	Drake	−24	−0.40	24	1.14
	All groups	56	5.33†	45	6.02†
C-1	Gainesville	37	0.92	40	2.30*
vs.	Miami	61	3.64†	56	5.55†
T-2-D	Both groups	52	3.23‡	44	3.98†

* Significant level 0.05. † Significant level 0.001. ‡ Significant level 0.01.

on the basis of oral conditions at the start of the experiment, the distribution between the test and control subjects was such that it was thought of interest to list each group separately in addition to indicating over-all results.

The results of the 2-year experiment are shown in Tables 1 and 2 and indicate that sodium N-lauroyl sarcosinate in a dentifrice, when it is used either morning and night or after meals, will materially reduce dental caries activity. The percentage reductions in Table 2 are average reductions for the test groups. Individual subjects may have derived more or less benefit than the average. The results from each group, with the exception of a small group at Drake and one at Gainesville, are highly significant, whether the comparisons are made on the basis of teeth involved or tooth surfaces involved.

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Demonstration of Particulate Adhesion of the Rieckenberg Type with the Spirochete of Syphilis

In 1917 Rieckenberg (*1*) described an adhesion reaction between trypanosomes and blood platelets requiring antibody. Microorganism-antibody systems that are not trypanosomal have also shown this reaction, and particulate materials such as white cells, bacteria, and gamboge particles can substitute for platelets (*2*). The reaction, however, has been more difficult to elicit with the spirochete of syphilis, although Krantz in 1930 reported platelet adhesion to pathogenic *Treponema pallidum,* and Turner *et al.* observed that in treponeme-syphilitic serum mixtures tissue debris tended to stick to the organisms (*3*). Recently, doubt has been cast on the existence of the Rieckenberg phenomenon with the syphilis organism in a report of the failure to observe its adhesion with organic or inorganic particles (*4*). We wish to record our experience in being able to observe regularly the Rieckenberg type of adhesion with *T. pallidum* (*5*).

Suspensions of treponemes (Nichols strain) were separated from rabbit testes by the method of Hardy and Nell (*6*). The organisms, which were centrifuged from the citrate solution employed by these authors, were resuspended in fresh citrate solution, recentrifuged, drained carefully to remove the citrate, and finally resuspended and stored in sterile 0.85 percent sodium chloride solution. The temperature for the organisms was maintained at 4°C during each of these procedures.

Adhesion in the presence of serums was observed by dark-field microscopy. Involvement varied from an occasional treponeme to almost 100 percent, and the number of particles that adhered to different treponemes varied considerably. One particle or innumerable particles might be adherent to a treponeme. When the number of particles that adhered was small, the particles tended to accumulate at the tip of the treponeme. Treponemes to which many particles became attached were often difficult to distinguish.

Materials that were shown to adhere to treponemes were the following: *Streptococcus pyogenes, Streptococcus lactis, Escherichia coli, Alkaligenes fecalis, Spirillum rubrum, Saccharomyces cerevisiae, Schizosaccharomyces pombe,* collodion particles, blood platelets (man, rabbit, guinea pig), and red-cell ghosts (sheep, rabbit, guinea pig, chicken). Blood platelets were collected by differential centrifugation from whole blood with 0.1 percent sodium ethylenediaminetetracetate (EDTA) and, for use in adhesion tests, were washed and resuspended in saline. The yeasts were least effective. *Streptococcus pyogenes* proved quite satisfactory. This organism, in saline, could be stored in an icebox for more than a month. However, the amount of adhesion to treponemes was sometimes increased by centrifugation and resuspension in fresh saline a few days or a week before use.

The adhesion did not occur with heat-inactivated rabbit and human serums in the absence of added guinea pig serum or when the added guinea pig serum was first diluted 10 times. Furthermore, either the application of heat or the addition of a chelating agent such as EDTA caused guinea pig serum to lose its capacity to support adhesion. The effect of the chelating agent was reversed by the addition of a mixture of calcium and magnesium ions. These cations themselves were inhibitory at high concentrations. These suggestions of a role for complement are not unique, since others have previously implicated complement in the Rieckenberg reaction (*7*).

Table 1. Comparison of adhesion reactions with standard serologic tests (STS) and treponemal immobilization tests (TPI) in 80 human serums.

STS	TPI	No. of serums	Adhesion reaction with streptococci*		
			+	±	0
+	+	12	12		
0	+	11†	6	1	4
+	0	7‡	1		6
0	0	13	1		12
+	Not done	26	26		
0	Not done	11	1§		10

* Positive reaction (+) denotes more than 10 percent of 50 to 100 treponemes counted showing adherent cocci. Doubtful reaction (±) denotes adhesion consistently to about 10 percent of treponemes, usually with only one or two cocci adherent to one end of a treponeme. Negative reaction (0) denotes less than 10 percent adhesion.
† Eleven serums from old, treated cases of syphilis.
‡ Seven serums from clinically diagnosed biologic false positive (BFP) reactors.
§ This serum tested positive with *S. lactis* and negative with *S. pyogenes.*

Serums from five rabbits that were infected with *T. pallidum* gave the adhesion phenomenon, while five normal rabbit serums did not. Reactions with human serums (Table 1) revealed a correlation of the adhesion reaction with traditional flocculation tests for syphilis and the treponemal immobilization test. These tests were performed by mixing 0.1 ml of serum with 0.1 ml each of guinea pig serum, saline, and saline suspensions of treponemes and cocci and then incubating the mixture at 34°C for 2 hours. As a control for each serum, in another tube guinea pig serum was replaced by saline. The stock suspension of cocci had a turbidity corresponding to the first tube of a MacFarland nephelometer, and the treponemes had a concentration of 80 million organisms per milliliter. Group A *S. pyogenes* (strain C203S) was employed except for 13 serums tested with *S. lactis* (ATCC7963).

The findings permit the conclusion that antibody responsible for adhesion is a result of *T. pallidum* infection. They do not define either the sensitivity or the specificity of the reaction. There also remains to be elucidated what roles the kinds of treponeme preparations and particles chosen for demonstrating adhesion may play in determining the specificity of the reaction.

Two antiserums that were prepared against cardiolipin in presumably normal rabbits by A. G. Osler gave positive ad-