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Demonstration of Rapid Production of Lactic Acid in Oral Cavity.*

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Despite the opposing schools of thought on the origin of dental caries, it is generally agreed that one of the important factors in this condition is a solution of the enamel by acids produced by microorganisms in the oral cavity. Many *in vitro* studies have been made showing the production of acids by mouth organisms over periods of time ranging from an hour to days or more.

Except for the work of Stephan[1] recently reported, we know of no work which shows the quick production of acids in the mouth. Stephan reports that, immediately following rinsing of the mouth with certain carbohydrate solutions, there is a marked fall in the pH of plaques and open tooth cavities in the mouth. What particular acid or acids are responsible for this fall in pH is not stated.

In the work herein reported, the rapid production of lactic acid in the oral cavity is shown as the result of allowing certain carbohydrates to dissolve in the mouth. This same finding is also demonstrated *in vitro,* by incubating such carbohydrate solutions with saliva, but not if the saliva has first been rendered sterile by passing it through a Seitz filter.

Our work was made possible only through having at hand the Miller and Muntz method[2] for estimating ultramicroquantities of lactic acid.† The method was modified to adapt it for use with the

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1 Stephan, R. M., *J. Am. Dent. Assn.*, 1940, **27**, 718.

2 Miller, B. F., and Muntz, J. A., *J. Biol. Chem.*, 1938, **126**, 413.

† Pyruvic acid has recently come into prominence as one of the acids involved in the production of dental caries.[3,4] If the time of heating in the Miller and Muntz method for lactic acid is 5 minutes, pyruvic acid, if present, will interfere. However, this interference is eliminated if the time of heating is 15 minutes. Some preliminary experiments indicate that in our work no pyruvic acid is present in the oral cavity contents when the criterion set up by Miller and Muntz is used—*i. e.*, comparing the findings for lactic acid when the solutions are heated for 15 minutes instead of 5 minutes. However, if the method using 2-4-dinitrophenylhydrazine as given by Lu[5] is used, there is evidence of pyruvic acid in our control and experi-

Summerson photoelectric colorimeter[6] by adding 4 cc of concentrated sulfuric acid before making the readings.

The procedure of our experiments may be briefly stated as follows: In the *in vivo* experiments, the mouth was rinsed 3 times with 25 cc quantities of water, each rinsing lasting 20 seconds. Ten minutes later, the first control sample of saliva was taken, and the second, 10 minutes after the first. A tablet of carbohydrate (containing about 1.5 g carbohydrate) was then allowed to dissolve in the mouth.‡ Ten minutes after the tablet had completely dissolved, the next sample of saliva was obtained. Other samples followed at 10 minute intervals. The period of collecting samples was usually 1½ minutes, the amounts varying from 0.5 cc to 1.7 cc. Analyses for lactic acid were made.

In the *in vitro* experiments, saliva was obtained as for the first control sample noted above and mixed with an equal quantity of approximately 0.1% carbohydrate solution. This mixture was incubated at 38° and samples for analysis taken at 10 minute intervals. In some instances, besides making determinations for lactic acid, sugar was determined by the Benedict method.[7]

Our subjects were laboratory workers, all males except Subject 1. Their ages ranged from 22 to 42 years.

The results of the *in vivo* experiments are shown in Table I. It is quite evident that within 10 minutes after banana or tablets of

mental samples. The amounts found are, however, too small to account for very much of the lactic acid obtained in our work. We are investigating this further.

[3] Fosdick, L. S., and Hansen, H. L., *J. Am. Dent. Assn.*, 1936, **23**, 401.

[4] Fosdick, L. S., Hansen, H. L., and Wessinger, G. D., *J. Am. Dent. Assn.*, 1937, **24**, 1445.

[5] Lu, G. D., *Biochem. J.*, 1939, **33**, 249.

[6] Summerson, W. H., *J. Biol. Chem.*, 1939, **130**, 149.

‡ Tablets of sucrose, glucose, and starch were used. The time for complete solution and disappearance of these from the mouth varied from 4 to 7 minutes. Acacia tablets were also used, but the results obtained with these are not considered reliable. In some of these acacia experiments, slight but significant increases of lactic acid were found. These were due to a peculiar blue color given in the reaction. That these increases were not due to lactic acid is shown by the fact that no such results were obtained when acacia was used in the *in vitro* experiments. The peculiar blue color also occurred when the reaction used in this work was carried out with pure acacia or arabinose solutions.

In contrast to the 1.5 g tablets of the carbohydrates used in the *in vivo* experiments, about 0.7 g of gelatin, in the form of small thin sheets, was used. This was allowed to dissolve in the mouth for about 8 minutes; at this time, not all of the gelatin had completely dissolved.

It took about a minute for 10 g of banana to completely dissolve in the mouth.

[7] Benedict, S. R., *J. Biol. Chem.*, 1931, **92**, 141.

TABLE I.

Production of Lactic Acid in the Oral Cavity.

Results are expressed as mg of lactic acid per 100 cc of saliva. Samples are taken at 10-minute intervals, samples 1 and 2 serving as controls. Sample 3 is taken 10 minutes after the complete solution of the test material in the mouth (see text and footnote ‡).

Subject	Test Substance	Sample 1	2	3	4	5	6
1	Glucose	1.0	1.0	19.5	2.9	2.0	1.7
2	"	2.3	1.6	50.0	7.7	1.9	1.4
3	"	3.0	3.8	33.4	8.4	4.7	3.6
4	"	3.4	2.4	14.0	4.3	2.3	2.2
5	"	2.7	3.7	15.4	5.6	4.5	2.3
1	Sucrose	1.3	1.0	15.9	6.0	1.5	1.2
2	"	2.5	1.8	43.0	15.7	3.2	3.2
3	"	2.6	3.2	40.0	10.8	5.0	3.3
4	"	2.0	1.8	16.6	4.8	3.5	2.0
5	"	2.8	2.5	26.7	10.4	5.8	5.1
1	Starch	1.6	1.3	4.2	1.4	1.2	1.3
2	"	1.9	1.7	13.6	3.1	2.0	1.5
3	"	2.4	2.2	10.2	3.7	2.6	1.8
4	"	3.0	2.5	12.0	3.9	2.3	2.5
5	"	2.3	2.1	13.1	3.3	3.3	2.8
1	Gelatin	2.0	1.9	1.7	1.6	1.6	1.7
2	"	2.7	2.1	2.8	2.4	2.1	1.8
3	"	3.2	2.9	3.0	2.8	2.8	2.6
4	"	3.6	3.3	3.3	3.2	2.4	2.8
5	"	2.9	2.4	2.5	2.8	2.8	3.0
1	Banana	1.0	1.4	10.0	2.2	1.8	1.1
2	"	1.8	1.5	31.6	3.7	1.6	1.8
3	"	2.2	2.5	15.3	5.7	3.9	3.7
4	"	3.2	2.6	11.7	4.0	2.9	2.6
5	"	2.8	2.4	24.2	5.8	4.1	4.1

sucrose, glucose or starch have been completely dissolved in the mouth, there is a marked increase of lactic acid in the contents of the oral cavity. After this, the concentration of lactic acid falls off sharply. The increases noted cannot be ascribed to changes in the rate of salivary secretion.

Table II illustrates the effect on lactic acid production of incubating mixtures of saliva and various substances. As shown in the *in vivo* experiments, increases in lactic acid are shown with starch and sucrose, but not with gelatin. Negative findings in these experiments are also given with inulin, arabinose and acacia.

That the production of lactic acid from carbohydrates in the *in vitro* experiments is accompanied by a loss of carbohydrates is shown in Table III.

Where saliva has been rendered sterile by passing it through a

TABLE II.

Production of Lactic Acid *in vitro*.

Results are expressed as mg of lactic acid per 100 cc of mixture, composed of equal parts of saliva and 0.1% test substance solution. Samples are taken at 10-minute intervals, sample 1 being the control taken immediately after making the mixture.

Saliva from Subject	Test Substance	Sample 1	2	3	4	5	6
1	Sucrose	1.2	3.3	6.7	10.2	14.8	17.8
2	"	6.6	9.1	12.6	16.1	21.0	26.4
1	Starch	1.0	1.7	3.7	6.0	8.2	11.2
2	"	2.2	3.2	4.4	6.5	9.2	12.3
1	Inulin	1.9	1.7	1.5	1.4	1.3	1.2
5	Acacia	5.5	5.5	5.3	5.0	4.7	4.4
2	Arabinose	2.0	1.6	1.3	1.0	0.8	0.6
1	Gelatin	1.2	1.0	0.8	0.8	0.8	0.8

TABLE III.

Production of Lactic Acid *in vitro*.

Results are expressed as mg of lactic acid and mg of the carbohydrate (shown in parenthesis) per 100 cc of mixture, composed of equal parts of saliva and 0.1% carbohydrate solution. Samples are taken at 10-minute intervals, sample 1 being the control taken immediately after making the mixture.

Saliva from Subject	Test Substance	Sample 1	2	3	4	5	6
4	Xylose	1.2(50)	1.2(50)	1.3(49)	1.3(51)	1.3(50)	1.2(51)
1	Glucose	0.9(53)	1.9(49)	4.0(44)	6.9(38)	9.6(32)	13.2(25)
2	"	2.9(53)	5.6(47)	8.8(38)	11.6(28)	15.2(19)	19.4(10)

Seitz filter, and then incubating such saliva with sucrose or glucose solutions, no production of lactic acid is obtained. The Seitz filtrates contain ptyalin. The production of lactic acid in our experiments must therefore be ascribed to the action of microorganisms.§

Summary. The rapid production (within 10 minutes) of lactic acid in the oral cavity and *in vitro* is demonstrated as the result of the action of oral microorganisms on certain carbohydrates.

§ The point may be raised that the presence of certain carbohydrates in the mouth may stimulate the salivary glands to increased lactic acid production. However, the negative findings with acacia (and gelatin) in the *in vivo* experiments and with sterile saliva in the *in vitro* experiments tend to negate such a contention. It may be pointed out here that in connection with another problem being carried out in collaboration with Dr. W. H. Summerson at Cornell University Medical College, it has been found that there is a marked increase in lactic acid in paraffin-stimulated saliva as compared to lactic acid in resting saliva. However, if paraffin is chewed vigorously for 5 or 6 minutes, and a sample of saliva is obtained 10 minutes later, no increases in lactic acid as reported in this paper are found. The findings on lactic acid content of stimulated and non-stimulated or resting salivas will be reported shortly.