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## Changes in the Reaction of Resting Saliva Produced by Different Micro-Organisms.*

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Dental literature contains many references to the deleterious effects of acids in the saliva, especially after ingestion of excessive amounts of carbohydrate. Those who believe that caries is a process which begins primarily on the external part of the tooth structure and extends inward, stress the solvent action of acids in saliva upon tooth enamel, (largely inorganic) and, subsequently upon the inorganic constituents of the dentine.

Since the reaction of saliva obtained immediately after expectoration as well as that obtained by cannulation is only slightly acid, pH6.6,[1, 2] the combined secretions and excretions of the salivary glands possess too low a concentration of hydrogen-ions to have even the slightest solvent action upon the tooth structure. However, to the actual secretions and excretions of the salivary glands we must add "mouth sweepings," comprising the desquamated cells, food particles, dead bacteria, etc., together with a large variety of living micro-organisms. The importance of the type of food residues, etc., in relation to the predominant micro-organisms in the oral cavity, and, the reaction of the saliva has been emphasized by Bunting.[3]

During the last three years we have been studying the inorganic constituents of the saliva. It seemed desirable to carry out experiments to determine the extreme variations in the reaction that could be brought about by various organisms and with various types of food residues.

### METHODS.

Samples of Resting Saliva collected from 3 to 4 adults were combined, and, after removing a small portion for the controls, the main portion was sterilized in Erlenmeyer flasks by autoclaving at 20 pounds pressure for 15 minutes. Except for *B. acidophilus,* the organisms used were obtained by aspiration from the gingival tis-

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TABLE I.
Changes in the Reaction of Incubated Saliva by Various Micro-organisms.

| Micro-organisms used | pH OBSERVATIONS | | | | | |
|---|---|---|---|---|---|---|
| | Saliva Sample No. 1 | | | | | |
| | May 4 | May 5 | | | | |
| | 8 p. m. | 8 a. m. | 11 a. m. | 2 p. m. | 5 p. m. | 8 p. m. |
| Mixed oral (control) | 7.1 | 6.8 | 6.9 | 7.0 | 6.9 | 6.9 |
| *Strep. salivarius* | 7.1 | 7.9 | 8.0 | 7.8 | 7.8 | 8.0 |
| *Strep. mitis* | 7.1 | 7.7 | 7.5 | 7.3 | 7.3 | 7.6 |
| *Staph. albus* | 7.1 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| | Saliva Sample No. 2 | | | | | |
| | May 6 | May 7 | | | | |
| | 9 a. m. | 9 a. m. | 1 p. m. | 5 p. m. | 9 p. m. | |
| Mixed oral (control) | 7.0 | 7.1 | 7.1 | 7.1 | 7.2 | |
| *B. acidophilus* (milk culture) | 6.0 | 5.0 | 5.0 | 5.1 | 5.0 | |
| | Saliva Sample No. 3 | | | | | |
| | May 18 | May 19 | | | | May 20 |
| | 8 a. m. | 8 a. m. | 11 a. m. | 3 p. m. | 8 p. m. | 8 a. m. |
| Mixed oral (control) | 7.1 | 6.8 | 6.8 | 7.2 | 7.3 | 7.5 |
| *Strep. mitis* | 7.1 | 6.8 | 7.2 | 7.3 | 7.4 | 7.6 |
| *Strep. salivarius* | 7.1 | 6.7 | 7.0 | 7.2 | 7.3 | 7.3 |
| *Staph. albus* | 7.1 | 7.5 | 7.5 | 7.8 | 7.8 | 8.0 |
| | Saliva Sample No. 4 | | | | | |
| | May 19 | May 20 | | | | May 21 |
| | 9 a. m. | 9 a. m. | 1 p. m. | 5 p. m. | 9 p. m. | 9 a. m. |
| Mixed oral (control) | 7.3 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| *B. acidophilus* (milk culture) | 6.6 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| | Saliva Sample No. 5 | | | | | |
| | May 20 | May 21 | | | | May 22 |
| | 9 p. m. | 9 a. m. | 1 p. m. | 5 p. m. | 9 p. m. | 9 a. m. |
| Mixed oral (control) | 7.3 | 6.0 | 6.2 | 6.4 | 6.6 | 6.8 |
| *Strep. mitis* | 7.3 | 6.1 | 6.5 | 6.6 | 6.6 | 6.6 |
| *Strep. salivarius* | 7.3 | 6.7 | 6.8 | 7.0 | 7.5 | 7.8 |
| *Staph. albus* | 7.3 | 8.0 | 8.0 | 8.0 | 8.0 | |
| | Saliva Sample No. 6 | | | | | |
| | May 24 | May 25 | | | | May 26 |
| | 9 a. m. | 9 a. m. | 1 p. m. | 5 p. m. | 9 p. m. | 9 a. m. |
| Mixed oral (control) | 6.8 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| *B. acidophilus* | 6.4 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| | Saliva Sample No. 7 | | | | | |
| | May 24 | May 25 | | | | May 26 |
| | 9 p. m. | 9 a. m. | 1 p. m. | 5 p. m. | 9 p. m. | 9 a. m. |
| Mixed oral (control) | 7.6 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| *Strep. mitis* | 7.6 | 5.8 | sample lost | | | |
| *Strep. salivarius* | 7.6 | 7.6 | 6.8 | 7.2 | 7.2 | — |
| *Staph. albus* | 7.6 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |

PROTOCOLS.

Saliva sample No. 1; collected May 4, 1925, from two women and one man.

Saliva sample No. 2; collected May 6, from same subjects as above.

Saliva sample No. 3; collected May 18, from three women.

Saliva sample No. 4; collected May 19, from two women and one man. Each subject chewed taffy prior to collecting the saliva.

Saliva sample No. 5; collected May 20, from three women. Taffy candy eaten prior to collecting the saliva.

Saliva sample No. 6; collected May 24, from two women and one man. Each subject ate one soda cracker just prior to collecting saliva.

Saliva sample No. 7; collected May 24, from two women and one man. Each subject ate one soda cracker just prior to collecting the saliva.

sues of human subjects. (We are indebted to Dr. T. D. Beckwith of the Department of Bacteriology for these cultures, as well as for many helpful suggestions.) After inoculation, the samples and the controls were incubated at 37.5° C. Small samples were removed aseptically at various times. The pH determinations were made colormetrically, using appropriate phthalate or phosphate buffer solutions with one of the sulphonephthalein indicators. These buffer solutions were carefully prepared from recrystallized salts and stored in paraffined pyrex flasks. (We are indebted to Mr. K. L. Carter for the preparation of these buffers and also subsequent checking against a hydrogen electrode.) The results of these experiments are presented in the following table:

From the above data it can be seen that only in the controls (mixed organisms of unsterilized saliva) and in the *B. acidophilus* cultures did the changes in the hydrogen-ion concentration become at all significant, coming to a standstill at pH of 5.0. Since we had to resort to mass inoculation (from milk culture), with acidophilus, we cannot definitely attribute the change to the action of the organisms upon the constituents of the saliva. The amount of milk introduced would markedly change the type of the media. In saliva samples 4, 5, 6, and 7, where an excess of carbohydrate was present, it can be seen that the mixed organisms of the mouth are also capable of reaching a pH of 5.0. These changes appear within twelve hours incubation.

It does not seem reasonable to assume that the saliva (excreted and secreted) can ever reach a hydrogen-ion concentration sufficient to have an appreciable solvent action upon the inorganic constituents of the tooth. However, through the action of micro-organisms upon the food particles carried in the saliva, there may be inaccessible places (between the teeth, carious areas, etc.) where the hydrogen-ion concentration would be sufficient to cause solution of

the tooth. Direct determinations of the reaction of the saliva, and, *in vitro* experiments such as we have reported would not give any information as to the reaction in such localized areas.

[1] Starr, H. E., *J. Biol. Chem.*, 1922, liv, 43, 55.
[2] Bunzell, H. H. Bulletin No. 1, Colgate & Co., 1923.
[3] Bunting, R. W., *J. Am. Dent. Assn.*, 1924, xi, 64.

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## Occurrence of Schick Negative Reactions in Absence of Free Antitoxin in Blood Stream.

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The object of this communication is to present some observations concerning the relationship of antitoxic immunity to the Schick test.

The Schick test, consisting of the intra-dermal injection of 1/50 M. L. D. of diphtheria toxin, shows under proper conditions, a reaction at the site of injection in the non-immune (positive), and a complete absence of reaction in those who are immune (negative). The generally accepted explanation of a negative Schick test is that antitoxin is present in the blood serum, the circulating antitoxin reaching and neutralizing the toxin before tissue damage results.

This explanation is of course plausible, especially since we know of no product of the tissues capable of neutralizing toxin, except antitoxin. There was no ground for dispute of this hypothesis, so long as the presence of antitoxin in the blood was predicated, but it now develops as a result of a series of comparative observations with the Schick and the Kellogg[1] tests that there are individuals who give absolutely conflicting results, in that a negative Schick is accompanied by a lack of antitoxin in the blood as shown by the Kellogg test. These persons have all, with one exception, been found in the group of those originally Schick positive and who have become Schick negative by the administration of toxin-antitoxin. This may have a bearing on the explanation of the phenomenon, and points the way toward a line of investigation.

Attention was first directed to this matter, when some children in the California School for the Deaf and Blind, who had been immunized and later found to be Schick negative, were inadvertently included in a group that was having blood samples taken for the Kel-