

AN INVESTIGATION INTO THE INHIBITORY POWERS
OF SALIVA AGAINST BACTERIAL GROWTH

by

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Introduction

From the beginning of modern medical knowledge, medical specialists in the area of the mouth have noticed that the tissues of the mouth were highly resistant to infection. And, with the advent of more thorough bacterial knowledge, the idea of such a high degree of resistance to infection became even more surprising since the decaying teeth, food particles, and the constantly sloughing epithelial membranes furnish an excellent environment for bacterial growth. Thus many investigators began to believe that an antibacterial agent was present in the saliva.

Up to the year 1934, the results obtained in investigation in this field were indecisive and quite often conflicting. From that date on, however, much investigation has been carried out, and authorities such as Dold, Weigmann, Lachele, Hsing, Koehn, Noeske, and Thompson have shown that a definite salivary inhibitory effect exists against diphtherial growth. Other investigators such as Cleugh, Taylor, Bibby, Hine, Ball, Berry, and Kesteren have also shown that a definite inhibitive action is present in the saliva that is effective against lactobacilli and other related organisms. Thus the body has a defense against pathological damage to the teeth as it has been shown that the acids produced by lactobacilli are not only harmful to the enamel of the teeth, but are also a predisposing cause of dental caries.

Authorities now believe that the inhibitory effect is due to powers other than those of the bacteria present in the saliva, but results of investigation in this field have been contradictory. "Dold, Lachele, and Hsing (1936) and Weigmann and Noeske (1937) found that the inhibitory agent was removed by filtration, but Weigmann and Koehn (1936) and Casassa (1937) reported that the agent passed both Seitz and Berkefeld filters."¹ However, Kesteren, Bibby, and Berry state that "approximately the first 5-ml. portion of each filtrate was inactive, but thereafter successive portions were active, becoming more active with the passage of more saliva through the candle."²

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1. Thompson, R. and Shibuya, M. 1946. "The Inhibitory Action of Saliva on the Diphtheria Bacillus: The Antibiotic Effect of Salivary Streptococci." Journal of Bacteriology, Vol. 51, p. 671.
 2. Kesteren, M.V., Bibby, B.G., and Berry, G.P. 1942. "Studies on the Antibacterial Factors of Human Saliva." Journal of Bacteriology, Vol. 43, p. 575.

Thus, as filtration would remove the bacteria, it seems probable that the effect is other than a bacterial power. Similar conflicting results have been reported as to the changes created by centrifugation, and by the oligodynamic effect of copper ions.

The saliva contains mucous secretions which also contain low molecular bacteriolytic proteins, the lysozymes. It was at one time believed that these were responsible for the inhibition of bacterial growth, but Thompson has shown that a temperature of 100° C. will destroy the agents responsible for the inhibitions in growth of several organisms but will not destroy the lysozyme.³

Kesteren, Bibby, and Berry conducted experiments with fifteen bacteria which had been shown susceptible to the inhibitory effects of saliva. These organisms were *Micrococcus lysodeikticus*, *Sarcinia lutea*, *Bacillus megatherium*, *Staphylococcus aureus*, *Streptococcus hemolyticus*, *Lactobacillus acidophilus*, *Bacillus subtilis*, *Eberthella typhosa*, *Salmonella paratyphi*, *Salmonella schottmuelleri*, *Proteus morganii*, *Shigella paradysenteriae*, *Escherichia coli*, *Proteus vulgaris*, and *Pseudomonas aeruginosa*. In their work, the following treatments appeared to effect in various degrees the inhibitive effects of saliva.

1. Filtration. The filtration of saliva through a Berkefeld-N candle left the filtrates devoid of action against *Micrococcus lysodeikticus*, *Sarcinea lutea*, and *Bacillus megatherium*. With the other organisms, the first 5-ml. portion of each filtrate was inactive but the remaining filtrate was active.

3. Thompson, R. 1941. "Certain Antibacterial Properties of Saliva and Tears not Due to Lysozyme." Journal of Bacteriology, Vol. 41, p. 77.

2. Heat. Heat seemingly inhibits the inhibitory powers in various degrees of intensity according to the degree of temperature attained. After 120° C., the temperature of autoclaving, the property is killed.

3. Ultra-violet radiation. Ultra-violet radiation removed or weakened the inhibitive action as far as *Micrococcus lysodeikticus*, *Sarcinia lutea*, and *Bacillus megatherium* were concerned, but had no effect on the action against other bacteria.

4. Freezing and Thawing. These physical activities had no effect except that the power against *Lactophylus acidophilus* was reduced.

5. Storage. The activity was destroyed completely in 12 days at 20° C.; at 5° C., it was not diminished after two months. After one day at 37° C., the power was destroyed against *Sarcinia lutea* and *Bacillus megatherium*. The potency of the power was weakened against *Micrococcus lysodeikticus*. After 6 days storage at 37° C., the effect on *Staphylococcus aureus*, *Streptococcus hemolyticus*, and *Lactobacillus acidophilus* disappeared.

6. Adsorption. Kaolin removed all activity; charcoal had no effect except to reduce the power against *Staphylococcus aureus*; and the results with aluminum hydroxide were inconclusive.

7. Chemical. Chemical treatment with acetone, acetic acid, and chloroform seemingly destroy the effect of the supernatant fluid against certain organisms but not against others. The resuspended precipitate was active against all organisms tested.⁴

In order to treat the problem in full cognizance of all its

4. Kesteren, Bibby, and Berry. op. cit., 574-576.

aspects, it is necessary to know the composition of saliva. Saliva is defined by Dorland's The American Illustrated Medical Dictionary as:

the spittle; a clear, alkaline, somewhat viscid digestive fluid secreted by the salivary glands. It contains ptyalin, a digestive ferment, and serves to moisten and soften the food, keeps the mouth moist, and converts starch into maltose. The saliva also contains mucin, serum-albumin, globulin, leukocytes, epithelial debris, and potassium thiocyanate. Certain toxins frequently occur in it.

Saliva is produced by the parotid, submaxillary, sublingual, and buccal glands in response to psychic, mechanical, or chemical stimulation. The secretion of the saliva is entirely under the control of the nervous system, there being no hormonal control of salivary secretion. However, the hormone of the parasympathetic nerve endings, acetylcholine, acts as a salivary secretagogue. Salivary secretion is also stimulated by physostigmine and pilocarpine, and is inhibited by atropine. Some 1,500 c.c. of saliva with a normal pH range of 6.3 to 7.0 is secreted by man daily.

The solid components are listed as follows by Israel Kleiner in Human Biochemistry:

The solid constituents of saliva are albumins, globulins, mucin, enzymes, urea, uric acid, and inorganic salts. The inorganic components differ markedly in concentration from those of blood serum, but the non-protein nitrogenous constituents (urea, uric acid, NH₄ salts) appear to bear some relation to the same constituents in blood.

The ions present in saliva are potassium, phosphate, and chloride. These may combine in various forms to create insoluble precipitates. Many authors are of the impression that a foreign body, such as a

blood clot or a clump of bacteria, can establish a nucleus around which the precipitation of these salts occur.

Saliva has primarily a digestive function but it also possesses the task of moistening food to permit easy swallowing. It protects the mucosa of the mouth from acids and cleanses the teeth. It contains the enzymes ptyalin (an amylase), maltase, catalase, urease, protease, and a lipase. There is a possibility that ptyalin could act on the encapsulated forms of bacteria and initiate the breaking down of the capsule wall. Then death of the bacteria would follow.

The saliva contains numerous bacteria as it contains bacteria that have been washed from the mouth. Saliva, however, is not a suitable culture medium. Saliva will have increased numbers of bacteria in times when a respiratory, nasal, or pharyngeal infection is running its course. The main organisms found in the saliva are streptococci and staphylococci, sometimes termed the salivary streptococci and salivary staphylococci.

Other organisms which are present in the mouth and appear transiently in the salivary secretions are Pneumococci; Micrococcus candidus; Micrococcus catarrhalis; Micrococcus flavus; Micrococcus pharyngis siccus; Meningococci; Bacillus Hoffmannii; Diphtheroids; Proteus vulgaris; Aerogenes; Freidlander's bacillus; the fusiform bacillus of Vincent's disease; Spirocheta vincentii; Treponema macrodentium; Treponema microdentium; and Buccalis maximus buccalis. These organisms will be present in the mouth in greater or smaller numbers according to whether or not pathological processes are present in the mouth; if they are present in the mouth, they will be present in the saliva. The incidence of their appearance will also be governed by the use of antiseptic mouth washes.

Excessive salivation is frequently produced by duodenal ulcer, oesophageal lesions, oral lesions, gastritis, pregnancy, and by the introduction of a gastric or duodenal tube for forced feeding. Aptyalinism, the complete lack of salivation, is rarely found in human beings.

The method which I used to attempt to prove the presence of inhibitive powers present in saliva was the same in essentials as that employed by Dr. Thompson of the Bacteriology Department of the University of Colorado School of Medicine. The organisms were taken from the stock cultures available in the bacteriology department and were transferred to slants composed of the various medias necessary for their growth. The organisms were incubated on these slants for forty-eight hours and then were ready for utilization.

Respective medias for plating were made up and then cooled to a temperature just above their hardening point. The organisms then were transferred from the test tube slants to the agar plates by means of sterile saline. Once the organisms were placed in the respective media, the plates were agitated to provide for the even distribution of the organisms in the media. The agar plates were then allowed to harden.

Two exceptions were utilized in regard to the above method. This was done in the case of *Pseudomonas fluorescens* and the yeasts and molds. The *Pseudomonas fluorescens* was inoculated into the media by streaking and was incubated at room temperature as it does not grow above 36° C. The molds and yeasts were grown on agar slants and then a drop of saliva was allowed to run down the slants. In

a control for this method, a drop of saliva was allowed to run down a slant of *Sarcina lutea* and a zone of inhibition was present where the saliva had coursed.

Following the hardening of the media, the plates were marked off into five sections. Upon each of these five sections, a drop of one of the salivas being used was placed on by means of a capillary pipette. The salivas used were ordinary, undiluted saliva; saliva diluted 1:4; centrifuged saliva; centrifuged saliva diluted 1:4; and centrifuged saliva diluted 1:8. The saliva used for the centrifugation results was centrifuged at 2500 rpm for two minutes. The plates were respectively inoculated with the saliva and then were left outside in the room until the saliva had dried upon the media. The plates were then placed in the incubator for a period of 48 hours and then were read for results. The best method for reading was holding the plates against a strong light with a dark background. The zones of inhibition could be clearly seen by this method.

The organisms which were utilized were as follows: *Mycobacterium smegmatis*; *Eberthella typhosa*; *Salmonella enteritidis*; *Salmonella schottmuelleri*; *Salmonella typhimurium*; *Brucella abortus*; *Staphylococcus aureus*; *Streptococcus lactis*; *Pseudomonas fluorescens*; *Sarcina lutea*; *Serratia marcescens*; *Bacillus subtilis*; *Escherichia coli*; *Proteus vulgaris*; *Alcaligenes faecalis*; and *Alcaligenes viscosus*. Four molds and yeasts were utilized as the author could find no data as to whether or not any investigation had been done on the question as to whether or not yeasts and molds were susceptible to the inhibitive agent in saliva. These four organisms were *Penicillium notatum*;

Saccharomyces cerevisiae; *Aspergillus niger*; and *Rhizopus nigricans*.

Inasmuch as occasionally it was necessary to differentiate the colonies of the organisms from colonies of the saliva, the following colonial characteristics were found in A Manual of Determinative Bacteriology by David Bergey:

1. *Mycobacterium smegmatis*: Convex, glistening, with entire margins, at first smooth but after 10 to 14 days growth folded or wrinkled. Opaque, at first white, after two or three days growth becomes yellow.
2. *Eberthella typhosa*: Grayish, transparent to opaque.
3. *Salmonella enteritidis*: Circular, gray, translucent, moist, smooth, entire.
4. *Salmonella schottmuelleri*: Small, circular, bluish-gray, transparent, homogenous, entire to undulate.
5. *Salmonella typhimurium*: Small, circular, grayish, entire to undulate.
6. *Brucella abortus*: Small, circular, convex, amorphous, smooth, glistening, entire.
7. *Staphylococcus aureus*: Circular, smooth, yellowish to orange, glistening, butyrous, entire.
8. *Streptococcus lactis*: Small, round, or oval, gray, entire, slightly raised. Streak culture tends to remain as definite colonies throughout, confluent in parts.
9. *Pseudomonas fluorescens*: (by personal observation) circular, small.
10. *Sarcina lutea*: Yellow, coarsely granular, circular, raised, moist, glistening, entire margin.

11. *Serratia marcescens*: Circular, thin, granular, gray, becoming red. R and S colonies with mucoid variants.
12. *Bacillus subtilis*: Small, grayish, circular to amoeboid, margins crenate.
13. *Escherichia coli*: Usually white, sometimes yellowish-white, rarely yellow, yellow-brown, golden-brown, reddish-orange, or red; entire to undulate, moist, homogenous. Atypical forms occur frequently.
14. *Proteus vulgaris*: Opaque, gray, spreading.
15. *Alcaligenes faecalis*: Transparent with opaque center, undulant margin.
16. *Alcaligenes viscosus*: After 3 to 4 days, circular, 4 to 6 mm. in diameter, white, viscid, shining, edge entire.

The examination of the plates gave the results listed below. To be considered an area of inhibition, a clear space was required with no colonial growth in it. The clear areas varied from 1 to 6 mm. in diameter.

1. *Mycobacterium smegmatis*

a. Saliva	no inhibition
b. Saliva 1:4	no inhibition
c. Centrifuged saliva	no inhibition
d. Centrifuged saliva 1:4	no inhibition
e. Centrifuged saliva 1:8	no inhibition

2. *Eberthella typhosa*

a. Saliva	no inhibition
b. Saliva 1:4	inhibition
c. Saliva, centrifuged	no inhibition
d. Saliva, centrifuged, 1:4	inhibition
e. Saliva, centrifuged, 1:8	inhibition

3. *Salmonella enteritidis*
- a. Saliva inhibition
 - b. Saliva 1:4 inhibition
 - c. Saliva, centrifuged, 1:4 inhibition
 - d. Saliva, centrifuged inhibition
 - e. Saliva, centrifuged, 1:8 no inhibition
4. *Salmonella schottmuelleri*
- a. Saliva inhibition
 - b. Saliva 1:4 inhibition
 - c. Saliva, centrifuged inhibition
 - d. Saliva, centrifuged, 1:4 inhibition
 - e. Saliva, centrifuged, 1:8 no inhibition
5. *Salmonella typhimurium*
- a. Saliva inhibition
 - b. Saliva 1:4 inhibition
 - c. Saliva, centrifuged no inhibition
 - d. Saliva, centrifuged, 1:4 no inhibition
 - e. Saliva, centrifuged, 1:8 no inhibition
6. *Brucella abortus*
- a. Saliva no inhibition
 - b. Saliva 1:4 no inhibition
 - c. Saliva, centrifuged, inhibition
 - d. Saliva, centrifuged, 1:4 inhibition
 - e. Saliva, centrifuged, 1:8 inhibition
7. *Staphylococcus aureus*
- a. Saliva no inhibition
 - b. Saliva 1:4 no inhibition

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| c. | Saliva, centrifuged | no inhibition |
| d. | Saliva, centrifuged, 1:4 | no inhibition |
| e. | Saliva, centrifuged, 1:8 | no inhibition |
| 8. Streptococcus lactis | | |
| a. | Saliva | no inhibition |
| b. | Saliva 1:4 | no inhibition |
| c. | Saliva, centrifuged | no inhibition |
| d. | Saliva, centrifuged, 1:4 | no inhibition |
| e. | Saliva, centrifuged, 1:8 | no inhibition |
| 9. Pseudomonas fluorescens | | |
| a. | Saliva | no inhibition |
| b. | Saliva 1:4 | no inhibition |
| c. | Saliva, centrifuged, | no inhibition |
| d. | Saliva, centrifuged, 1:4 | no inhibition |
| e. | Saliva, centrifuged, 1:8 | no inhibition |
| 10. Sarcina lutea | | |
| a. | Saliva | inhibition |
| b. | Saliva 1:4 | inhibition, 6 mm. area |
| c. | Saliva, centrifuged, | inhibition |
| d. | Saliva, centrifuged, 1:4 | inhibition |
| e. | Saliva, centrifuged, 1:8 | inhibition |
| 11. Serratia marcescens | | |
| a. | Saliva | no inhibition |
| b. | Saliva 1:4 | no inhibition |
| c. | Saliva, centrifuged | no inhibition |
| d. | Saliva, centrifuged, 1:4 | no inhibition |
| e. | Saliva, centrifuged, 1:8 | no inhibition |

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| 12. <i>Bacillus subtilis</i> | |
| a. Saliva | slight inhibition |
| b. Saliva, 1:4 | slight inhibition |
| c. Saliva, centrifuged | no inhibition |
| d. Saliva, centrifuged, 1:4 | no inhibition |
| e. Saliva, centrifuged, 1:8 | no inhibition |
| 13. <i>Escherichia coli</i> | |
| a. Saliva | inhibition |
| b. Saliva 1:4 | inhibition |
| c. Saliva, centrifuged | inhibition |
| d. Saliva, centrifuged, 1:4 | inhibition |
| e. Saliva, centrifuged, 1:8 | inhibition |
| 14. <i>Proteus vulgaris</i> | |
| a. Saliva | inhibition |
| b. Saliva 1:4 | inhibition |
| c. Saliva, centrifuged | inhibition |
| d. Saliva, centrifuged, 1:4 | inhibition |
| e. Saliva, centrifuged, 1:8 | inhibition |
| 15. <i>Alcaligenes faecalis</i> | |
| a. Saliva | inhibition, around 2 mm. |
| b. Saliva 1:4 | inhibition, 4 mm. |
| c. Saliva, centrifuged | slight inhibition |
| d. Saliva, centrifuged, 1:4 | inhibition |
| e. Saliva, centrifuged, 1:8 | no inhibition |
| 16. <i>Alcaligenes viscosus</i> | |
| a. Saliva | no inhibition |
| b. Saliva 1:4 | no inhibition |
| c. Saliva, centrifuged | no inhibition |

d. Saliva, centrifuged, 1:4	no inhibition
e. Saliva, centrifuged, 1:8	no inhibition
17. <i>Penicillium notatum</i>	no inhibition
18. <i>Saccharomyces cerevisiae</i>	no inhibition
19. <i>Aspergillus niger</i>	no inhibition
20. <i>Rhizopus nigricans</i>	no inhibition

On a percentage basis, the results obtained showed a fair degree of inhibitive action in the saliva. These results are shown below.

<u>Saliva</u>	<u>Percentage of inhibition</u>
Saliva	45%
Saliva 1:4	45%
Saliva, centrifuged	35%
Saliva, centrifuged, 1:4	40%
Saliva, centrifuged, 1:8	25%

Conclusion

Saliva does possess the power to inhibit the growth of various organisms. The inhibitory agent seems to be most effective against those organisms that are not a part of the natural flora of the mouth and pharynx.

Saliva does not seem to possess an antibiotic effect against yeasts and molds.

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