7-1938

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Recommended Citation

Hayes, Raymond L. (1938) "The Immunology of Dental Caries," The Dentoscope: Vol. 18: Iss. 1, Article 1.
Available at: http://dh.howard.edu/dentoscope/vol18/iss1/1

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THE IMMUNOLOGY OF DENTAL CARIES*

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O F ALL the diseases which affect civilized human beings, none has the prevalence and widespread distribution of dental caries. Dental caries, commonly known as decay of the teeth, is found in all ages and racial groups, in both sexes; in fact, wherever the soft carbohydrate diet of civilized man has been introduced.

This disease is important not only because it is widely distributed throughout the civilized world; it is also most important because of its ability to completely destroy the dental apparatus. Again, it has been a most difficult disease for us to understand, and because of this, it has defied successful treatment in some cases.

Dental caries is in no way similar to other necrotic and inflammatory changes. Here, there is no toxin production and no apparent resistance to the disease in the tooth itself. Instead, the disease is produced by the indirect action of certain aciduric bacteria upon the inert tooth structure, resulting in a decalcification of the enamel and dentin. Clinical experience has shown us that one attack of dental caries does not render one immune to subsequent attacks. Also it has shown us that true, that is, permanent immunity is rare indeed, although some people may go for years without any dental caries, only to be troubled with this affliction unexpectedly later on.

The etiological agents of dental caries are many. They may be grouped into the exciting or instigating, and the predisposing factors.

The predisposing factors are: (1) Diet; (2) General Health; (3) Oral Hygiene; (4) Heredity; (5) Age. Experience has demonstrated to us that, of these, diet has proven to be the leading contributing factor. The exciting factor is now known to be an acid producing organism—Bacillus Acidophilus. This factor is most important and the control of its growth seems to be most essential to the prevention or control of the disease. Bunting, Jay, Fosdick, Friessell, and others have proven conclusively that the activity of the carious process is directly proportional to the growth and presence of this organism.

In recent experimentation, Hoppert, Weber, and Conniff have produced dental caries in rats by feeding them coarse meal diets. Hoppert’s belief is that course particles of food allow circulation of the

* Presented as a faculty seminar, November 17, 1937.
saliva, whereas finely ground particles of meal become so closely packed that they are impervious to the flow of saliva. He feels that acids are not produced so readily because of a lack of moisture under the finely impacted material. However, his experiments are conducted on rats, and therefore conclusions drawn from them are not applicable to human beings without being open to question. Others have controlled the growth of Bacillus Acidophilus by changing the nature of the diet. By the feeding of simple, adequate, and uniform diets low in sugar content, the Bacillus Acidophilus count decreased markedly and stayed at the low level as long as the patient remained on the prescribed diet. From the experiments on diet conducted by the Michigan research group, it has been found that diet controls dental caries by altering the environment of the tooth rather than by affecting the tooth itself.

Bacillus Acidophilus has been found to be constantly present in the mouths of all caries susceptible patients. In the mouths of caries free patients, only sporadic appearances were noted. For some reason, the organisms seemed unable to survive for any appreciable time even when these patients were fed carbohydrate diets, high in sugar contents. Again in some cases, large quantities of Bacillus Acidophilus were implanted in the mouths of these caries free patients and still the organisms failed to live. This interesting finding led to the belief that some immunologic principle must exist which prevented the growth of these organisms.

In a series of experiments conducted at the University of Michigan in the Immunology of Dental Caries, reactions were observed in human beings to subcutaneous injections of Bacillus Acidophilus filtrate which were not unlike the response of the human body to invasion by other organisms. An explanation of these reactions and of the action of antibodies upon Bacillus Acidophilus on the surface of inert tooth enamel is difficult. However, let us briefly explore the experiments and the results obtained.

In dental caries, we are concerned with the elimination of an organism, not merely the neutralization of a toxin as in many diseases. Hence, this is a unique problem. Also, here we are concerned with a pleomorphic organism, for Bacillus Acidophilus assumes different morphologies and even produces different colony growths on the Kulp Tomato Agar media. Some growths are smooth, round, and white colonies; others are rough and still others are mixed in character. Hence, in these experiments vaccines were prepared of the smooth strain, of the rough strain, and of the polyvalent or mixed strain of Bacillus Acidophilus.
The method of preparation of the filtrate was as follows: Forty strains of Bacillus Acidophilus were taken from the mouths of patients with dental caries. The strains were grown separately in 1% glucose meat infusion broth pH 7 at 37.5° C for from 6—10 days. At the end of the incubation period the cultures were pooled and filtered through a Berkefeld candle. Ice cold 95% alcohol (slightly acidified by 0.3% acetic acid) was slowly added to the filtrate in a graduated cylinder. This was shaken thoroughly and the mixture placed in a refrigerator overnight. The resultant precipitate was recovered by centrifugation and taken up in sterile 0.85% salt solution. The precipitate was concentrated at this point to a 50% concentration which was satisfactory for most cases. The solution was once more filtered to insure sterility and 0.5% phenol added as a preservative. The material was placed in vials and stored in the refrigerator until ready for use.

Intradermal injections of 0.1 cc of this filtrate caused definite cutaneous reactions in certain persons. This reaction was characterized by a localized erythema from 10—30 millimeters in diameter which appeared usually within 9—10 hours after inoculation and lasted 3—24 hours. Sterile uninoculated broth produced no skin reaction in the patients or in rabbits. Table 1 shows that persons whose mouths contain Bacillus Acidophilus tend to react locally to intradermal introductions of the unpurified filtrate. Later some of this filtrate was purified by participation with slightly acidified alcohol. The resultant precipitate was dissolved in normal saline solution and refiltered. Results of the skin tests with this purified filtrate show that the relation between the skin reaction and the presence of Bacillus Acidophilus in the mouth was less specific as shown in Table 2.

Later experiments revealed that this purified filtrate which resembled a carbohydrate (having had the nitrogen removed) contained no antigenic factor for the particular bacillus, although it contained the skin reactive substance. In still another experiment 83 caries susceptible patients were skin tested with the purified filtrate. Seventy-five or 90% of them reacted positively while 8 or 9% of the group gave negative reactions. Of 40 caries free patients, 28 or 70% gave negative reactions and 12 or 30% were positive. Although this did not show a 100% correlation, it proved that there is a definite tendency for the negative reaction to occur in the caries free group while in the caries susceptibles the tendency is towards a positive reaction.

Jay also reports that he was able to desensitize caries susceptible...
patients with intradermal injections of the filtrate. The change in the reaction to the filtrate was accompanied by the appearance of Bacillus Acidophilus agglutinins in the blood stream. This led to a suggestion that the caries free might also have agglutinins which inhibit the growth of the organisms in their mouths. From table 4 we see that this is true. However, here again there is not a 100% correlation with susceptibility to caries but a definite relationship is seen to exist between the agglutinin titer and susceptibility to dental caries. For 30 caries free patients, the average titer was found to be 1—211 as compared to 1—20 for 31 patients with active caries. Of these, two children, both caries susceptibles with low agglutinin titers, had the titer raised by use of a polyvalent vaccine, but they developed such severe abscesses after the injections of the vaccine that it was necessary to stop the experiments until the irritating property of the vaccine had been eradicated.

Next, a series of rats were divided into three groups for the purpose of testing the vaccines made from the rough strain, smooth strain, and the mixed strain of Bacillus Acidophilus. They were fed on the Hoppert diet and 4.5 cc of the vaccines were given over a period of 10 weeks. At the end of this time, the rats were killed and examined for caries. Those that had received the rough strain vaccine had no caries, whereas the remaining six had a large amount of caries. At first glance it seems that the rough strain vaccine had protected the rats from caries. But when serums from the rats were examined, they were all found to be devoid of agglutinins. This proved that the vaccines were not antigenic for the rats, but the failure to develop caries was due to some other circumstances than the vaccine. From this experiment, however, it was observed that the animals injected with the smooth strain developed no abscesses while those injected with the mixed strain developed moderately severe abscesses. Very severe abscess formation was noted following the use of the vaccine of the rough strain. Apparently Bacillus Acidophilus in the rough phase contained the irritant that provoked abscess formation in the children in the previous experiment.

This same experiment was repeated on a large group of rats. From Table 5, it can be seen that neither vaccine was antigenic for the rat since no agglutinins were produced. Therefore, the vaccines had produced no protection against dental caries. Here also it is seen that abscess formation followed the administration of rough and mixed strain vaccines. In another experiment, rabbits were used and within 48 hours following the injection of dead rough
strain Bacillus Acidophilus cultures, abscesses appeared. In each case the abscess was sterile. It was found that agglutinins appeared in the blood of these rabbits. Therefore, because of the apparent lack of antigenicity of Bacillus Acidophilus in the rat, no further experiments were conducted on rats.

Still more recently, for another experiment ten children who were highly susceptible to dental caries and who had high Bacillus Acidophilus counts were selected. Preliminary mouth cultures were made. This time, autogenous vaccines were used since through the use of autogenous vaccines, the dangers of abscess formation were thought to be minimized. Each subject was examined for agglutinins before and after vaccination. Seven of the vaccines were smooth. Only three contained the rough strain. The vaccine was administered in 0.5 cc doses (about 200,000,000 organisms per cc) one week apart. After the fourth dose, two children who had been given the rough vaccine had severe abscess formations. Therefore, at this time all of the children were bled and their serums examined for agglutinins. The titers of the children with the abscesses increased from 0 to 1—640 and from 1—30 to 640 respectively, whereas the children who had no abscess formation showed no appreciable change in the agglutinin titer. Also ten days after the last injection, there was no change in the Bacillus Acidophilus count in their mouths. However, the time was entirely too short to expect a change in the bacteriological picture. Additional sustaining doses of the vaccine would have been necessary to keep the elevated titer of agglutinins in the blood stream. These were not given because of the extreme discomfort that the abscesses caused in the children. And before any determination of the practicability of this form of immunization against dental caries can be made, the irritating factors of the rough strain vaccine must be removed.

It has been thought that phenol, which is used in the preparation of the filtrate, may have been responsible for the abscess formation. Again it may have been the acidified alcohol; or the bodies of the dead Bacillus Acidophilus of the rough strain may contain some protein which may cause the abscess. So far, all we can say is that the cause of the abscesses is unknown. From the above experiments, it is seen that there is a possibility of immunization to dental caries being effected through the use of vaccines. However, the surface of this problem is only scratched and it will be left to future research workers to remove the irritating factors from the vaccines in order to carry on further studies in immunology.
Summary

1. Dental caries has a complex etiology with the following predisposing factors: (1) Age; (2) Oral Hygiene; (3) Diet; (4) General Health; (5) Heredity. The exciting factor is an aciduric organism—Bacillus Acidophilus.

2. Correlation exists between the skin reaction to polyvalent filtrate injections and dental caries susceptibility.

3. Use of vaccines of the rough and mixed phases of Bacillus Acidophilus is followed by abscess formation.

4. Smooth phase vaccine is not followed by abscess formation.

5. Increases in the agglutinin titer were noted in the patients following use of vaccines of the rough phase.

6. No increase in the agglutinin titer was noted following use of vaccine of the smooth strain of Bacillus Acidophilus.

7. Causative agent of the abscess formation is not known.

Bibliography


Discussion—By Ruth E. Moore, Ph.D.

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In this paper on the immunology of dental caries, a number of facts should be mentioned which are of importance from an immunological as well as bacteriological standpoint; namely, etiological