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Task Order #3

OFFICE OF NAVAL RESEARCH

(16) NR-103-005

(6) Semi-Annual Progress Report

1 January 1951 to 30 June 1951,

~~by~~

(10) Vincent F. Lisanti

(11) 1951

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1. General and Specific Objectives of the Problem.

The relationship between substances occurring in saliva and oral health or disease is the basic concern of this investigation. A search is being made for the source of hyaluronidase in saliva and other substances that have a bearing on susceptibility of disease. Efforts are being made to identify the substances and their source and to establish their relations to oral disease.

2. Progress: Methods, Results, Conclusions.

The hyaluronidase concentration in saliva was evaluated by comparison with Wyeth bull testis hyaluronidase. This standard was reacted in a concentration of 6.4 trubidimetric reduction units in 4 cc. with buffered hyaluronic acid solution which was adjusted to amount to have an initial viscosity of 1.8 times the buffer solution. The reduction in viscosity in forty-five minutes by the standard enzyme was used as the base (1.0). The fraction of reduction achieved by the unknown within the same time was the unit selected. The range in values found for saliva was from 0 to 0.99.

The hyaluronidase content of saliva has been determined for one hundred and fifty dental students. Nearly 95 per cent had hyaluronidase activity to a measurable degree. Nine subjects from the group possessed no or questionable activity, where the units of enzyme activity was .06 or less.

The study began in January 1951 at which time there happened to be an epidemic of upper respiratory infections. Several students were observed to have hyaluronidase activity greater than 0.8, which according to previous studies had been indicative of rampant dental caries or advanced periodontal disease. When the subjects were examined, the expected incidence of dental and oral disease was not found, but it was noted that these persons had symptoms of upper respiratory disturbances. This caused us to collect information concerning the presence or absence of upper respiratory infection. By the time the study was completed, a

total of sixty subjects had had colds or described symptoms of upper respiratory involvement while ninety-five never reported such symptoms. Hyaluronidase levels could be computed on thirty subjects both before and during upper respiratory infection. The mean level before the cold was 0.13 and during the cold was 0.42. The difference between means was found to be significant beyond the 1 per cent level. The same finding was obtained when ninety-five subjects without colds were compared with the sixty subjects with colds. Without colds the mean was 0.18 ± 0.014 and with colds the mean level was 0.45 ± 0.22. Here again the difference was significant at the 1 per cent level or better. Recovery from the symptoms and clinical signs of respiratory disturbance resulted in a lowering of the enzyme activity.

This finding appeared to be important from the medical viewpoint and was called to the attention of Drs. B. Walker, M. Derow, F. Lionetti of the Boston University Graduate School of Sciences. These men have worked with hyaluronidase and quickly foresaw possibilities from this lead. They have requested research funds from the Armed Forces for study of the relation of respiratory infection and salivary hyaluronidase. If funds are provided for this study, it will be done in cooperation with our laboratory.

In the original plan for this work it was postulated that the enzyme could come either from salivary glands or from salivary bacteria. In order to test the salivary gland hypothesis, an attempt was made to cannulate the parotid duct on two persons. Cannulation proved to be quite uncomfortable and caused swelling of the duct so that it became impractical to evaluate gland sources in this manner. A different approach was tried. Saliva was collected repeatedly once an hour on twenty-five patients for five hours. In comparison with the original before-breakfast sample the salivas on the average lost 90 to 100 per cent of their activity with repeated stimulation. This suggested that the enzyme did not

have a glandular source. On the other hand fifteen patients with periodontal lesions lost merely 50 per cent or less of their salivary enzyme as the collection continued, suggesting that the enzyme was being replaced from the periodontal lesions. Next bacterial sources were examined. The total bacterial count in saliva was obtained for fifty persons. A broth culture of saliva was made, incubated for forty-eight hours, plated and counted for total microorganisms. From the morphological appearance on the plate the organisms counted were classified into various groups. These results were plotted against the hyaluronidase content for the same saliva. The data appeared to be completely unrelated. Apparently the predominating organisms were not the chief source of the enzyme. The same question was approached by still a different method as indicated on Page 2 of the last Semi-Annual Progress Report. Saliva samples for testing were collected in sterile tubes and small samples were plated on several specific media at dilutions ranging from 10^{-3} to 10^{-6}. The media used were specific for streptococci, staphylococci, pneumococci, lactobacilli, and yeast. The counts derived from these plates were found to be uncorrelated with the enzyme activity readings obtained from the original saliva.

Apparently if organisms were the sources of the enzyme, it was not the predominating organism which had to be investigated. Staphylococci were studied because reports in the literature indicated that these organisms can produce hyaluronidase. Saliva samples obtained before breakfast were incubated with brain-heart infusion broth for forty-eight hours, centrifuged, passed through a Berkfelt V. filter, and tested for hyaluronidase activity. Broth cultures which exhibited clear or marked activity were plated for forty-eight hours and morphologically different colonies were transferred to brain-heart infusion broth and incubated for another twenty-four hours. Hyaluronidase-producing microorganisms were recovered in twenty-three out of twenty-five samples tested.

Staphylococcus of the aureus type (grey and yellow) were isolated from whole saliva in twenty-five of the one hundred and fifty cases studies. A streptococci tentatively identified as an alpha, was also found to be an enzyme producer. This organism has been isolated from stimulated and unstimulated whole saliva, from carious dentin and from deep and shallow periodontal pockets. High enzyme activity was also found to be present in infected periodontal tissue. Four and a half grams of infected periodontal tissue were obtained by surgical removal of gingival tissue from twenty-four patients, digested with pepsin, precipated with ammonium sulfate, and dialyzed. The activity per gram of dry substance was four to five times as great as that of saliva. The enzymes obtained from streptococci and staphylococci were compared with commercial bull testis hyaluronidase. All three enzymes were inactivated by heat treatment for thirty minutes, were sometimes inhibited by saliva, were inactivated by serum and by nitrated hyaluronic acid, and were not inhibited by serum to which snake venom had been added. It appears that hyaluronidase produced by oral microorganisms is quite similar to testicular hyaluronidase. The activity of the enzyme in whole saliva and partially purified saliva is not inhibited by the serum inhibitor or by nitrated hyaluronic acid. Some salivas inhibit the activity of the enzyme and others do not, indicating possibly that some salivas have a defensive action against the hyaluronidase or the microorganisms which produce it.

3. Bibliography

No papers have been submitted for publication or appeared in print during the six months period covered by the report.

4. Other Information Desired.

A. Change in direction or emphasis of project title.

Work is still progressing within the framework of the outline set forth in the proposal for this investigation.

B. Personnel changes

Mrs. I. R. Mahler has resigned as research assistant in order to follow her husband, Dr. Mahler, who joined the Army as a 1st Lieutenant on July 7. There is some possibility that Mrs. Mahler will return to Boston for work on this Task Order #3 in case her husband is sent overseas. We will know soon whether to seek a new research assistant or not.

C. Number of graduate students on contract.

None

D. Research support received or withdrawn from other sources.

At the present time there is no research support from other sources.

E. Difficulties encountered.

One of the major difficulties encountered during the past six months actually proved to be an advantage some few weeks after the problem was encountered. The epidemic of colds which occurred simultaneously with our attempt to collect saliva samples caused problems both from the illnesses which kept the dental students from attending school for several days at a time and from the effect on the concentration of hyaluronidase in saliva. From the standpoint of the dental effect our work was held up considerably by this epidemic, but it caused us to discover a new lead which has many potential benefits to some other research group and to the military services. The rise in hyaluronidase content in saliva should be useful in screening large groups of persons for respiratory infection and in prognosticating the severity complications and sequellae of such infections.

Submitted by

Vincent F. Lisanti