Investigations on the Salivary Phosphatases

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INTRODUCTION

This project began during the summer of 1959 when I (Cooper) received the opportunity to work in the Dental Section of the Oklahoma Medical Research Foundation as recipient of a Sir Alexander Fleming

Scholarship. The purpose was to determine the normal activity and, to some extent, the nature of the phosphatase enzymes found in saliva.

The 104 procedure (Sigma brochure, 1958) was used instead of other methods studied because its accuracy, speed, and simplicity tend to reduce the possibility of technical errors. Also, it is ideally suited to multiple determinations which lessen the chance of error; and, therefore, was employed throughout the investigation.

The modifications which were made were: A. the amount of saliva used in the alkaline phosphatase determination was increased from 0.1 ml. to 1.0 ml., and B. The amount of NaOH was reduced by 0.9 ml. to maintain an equal volume with the original method. The result was a higher value for the active substances. The phosphatase activities found by using the original method were so small that they had to be determined from the extremity of the optical density curve. Since the middle portion of any curve is more accurate than its extremities, the higher activities are more desirable because they are calculated from that portion of the curve. Although these values do not show the actual amount of alkaline phosphatase activity in the saliva, they are more accurate than the smaller amounts; and, therefore, more desirable for the comparative purposes for which they were used.

EXPERIMENTS

A. Activity from Day to Day

The phosphatase activity of saliva from each of four volunteers was determined on saliva collected at 8:30 each morning for 1½ months with the exception of week-ends and holidays. It was collected in quantities of from 5 to 8 ml. over a period of 10 to 15 minutes, sealed, and placed under refrigeration for a short time until the test was set up. In pretesting it was found that saliva sitting open at room temperature for over five minutes had a lower phosphatase activity than that same saliva tested immediately following collection. Saliva sealed and left at room temperature for over five minutes also had a lower activity than that same saliva tested immediately following collection. Saliva unsealed and refrigerated for periods of from five to thirty minutes had a slightly lower activity than that same saliva tested immediately following collection. Saliva sealed and refrigerated for periods not exceeding thirty minutes had the same phosphatase activity as that same saliva tested immediately following collection. Since less than twenty minutes elapsed between collection and testing of saliva, the sealed and refrigerated saliva had the same phosphatase activity that it had upon collection.

B. Activity during one day.

While testing saliva that had been collected during the morning it was suspected that the phosphatase activity might vary during the day. This suspicion was proved correct when the activity of saliva collected at two-hour intervals during the day showed variations. The greatest variation was in saliva collected before and after the mid-day meal. This could possibly account, in part, for the widely divergent values which various investigators state as being the normal value for phosphatase activity. For example: An investigator who conducted daily tests on saliva collected during the morning would find a higher value for phosphatase activity than one who tested saliva collected in the early afternoon since the activity is lower following the mid-day meal.

C. Activity of Frozen Saliva

If it were possible to store saliva by freezing without disturbing the

phosphatase activity, the horizons of the project could be extended. More samples could be collected during the day and it would be possible to keep a stock of saliva on hand since the tests would not have to be made shortly after collection. To determine the stability of phosphatase in frozen saliva, a frozen saliva series was prepared.

First 60 ml. of saliva were collected from one of the volunteers. This saliva was stirred continuously by an automatic stirring device as 3 ml. were pipetted into each of 18 test tubes. These tubes were then sealed and frozen. One after another of the samples were tested over a period

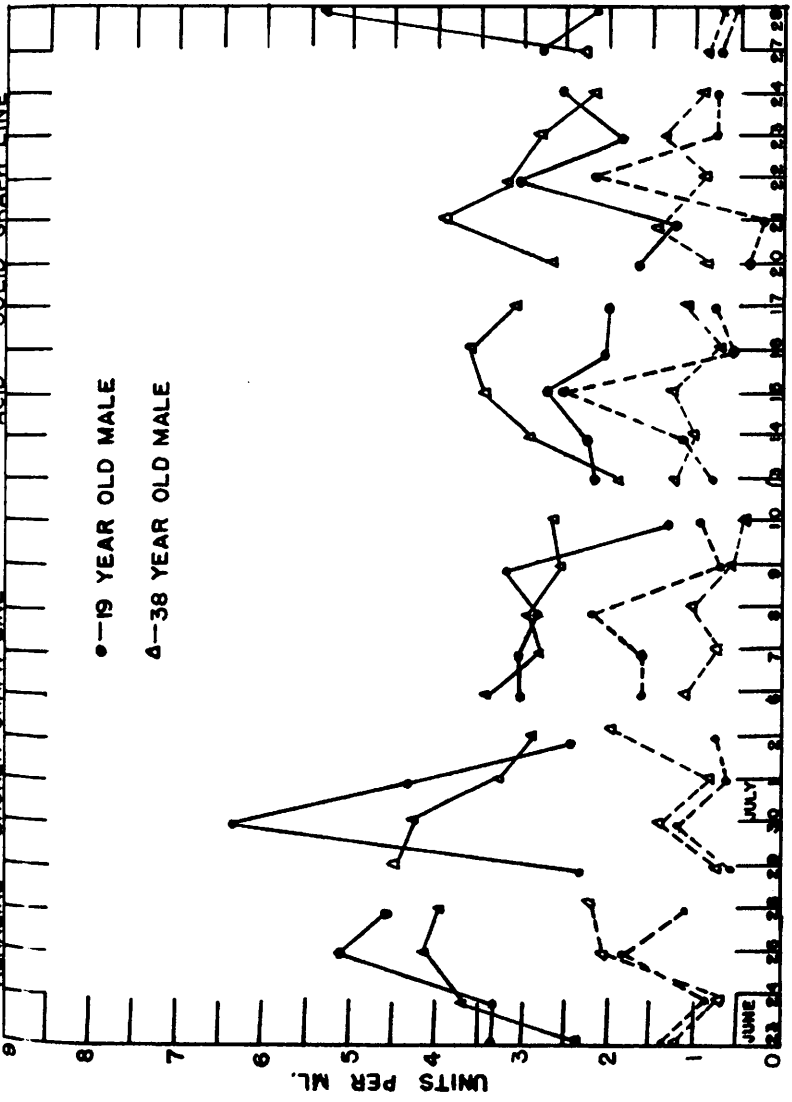

Figure 1.

of six weeks. In order that each sample might be tested as nearly like every other sample as possible, a standard procedure was followed.

The tube of frozen saliva was removed from the freezer and allowed to thaw at room temperature while still sealed . Then the seal was removed, the saliva was gently mixed using a stirring rod with every effort to keep from damaging the enzyme, and saliva tested for phosphatase activity.

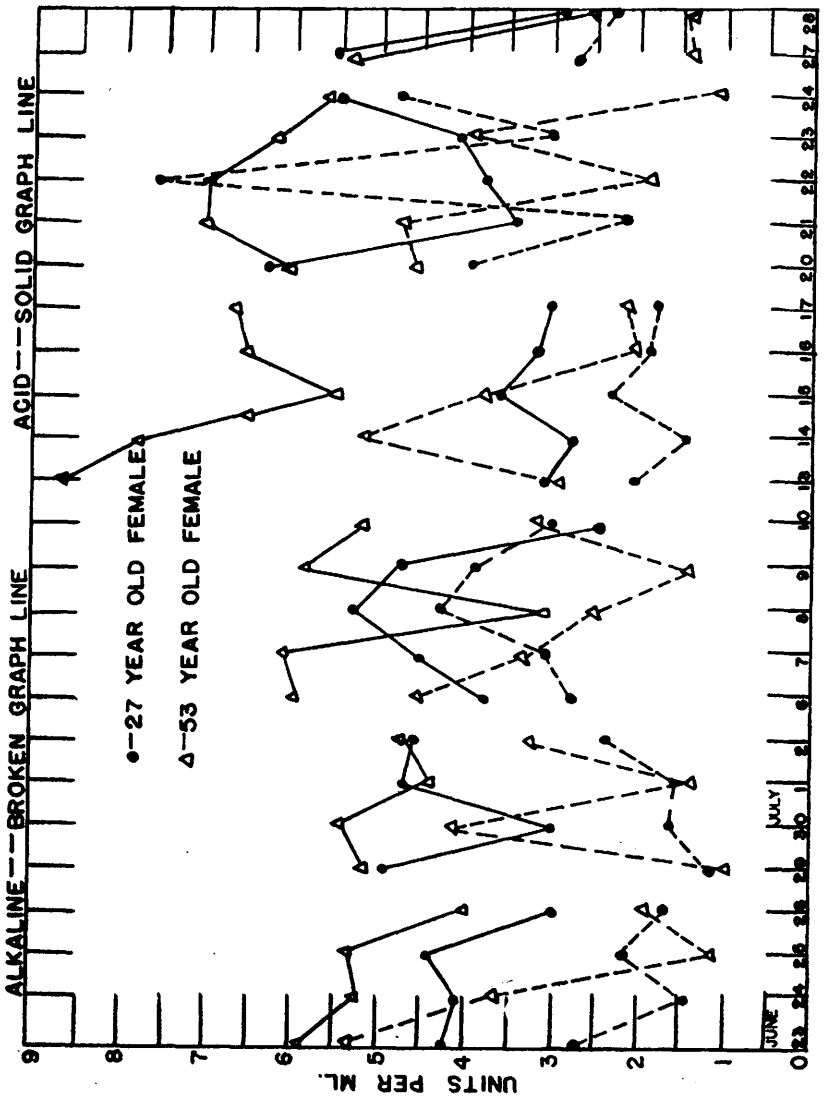

Figure 2.

Discussions and Conclusions

The wide variations (Figs. 1 and 2) in the phosphatase activity from day to day suggest that no predetermined value for phosphatase activity can be stated but rather a maximum and minimum range for normal saliva should be used clinically. It is believed that for genetic reasons the efficiencies of the different enzymes and enzyme systems vary from individual to individual (Williams, 1956). Also the theory that phosphatase activity is contributed to partly by exogenous salivary constituents such as cellular debris, food residues, and oral bacteria could account for the variations (Fitzgerald, 1952).

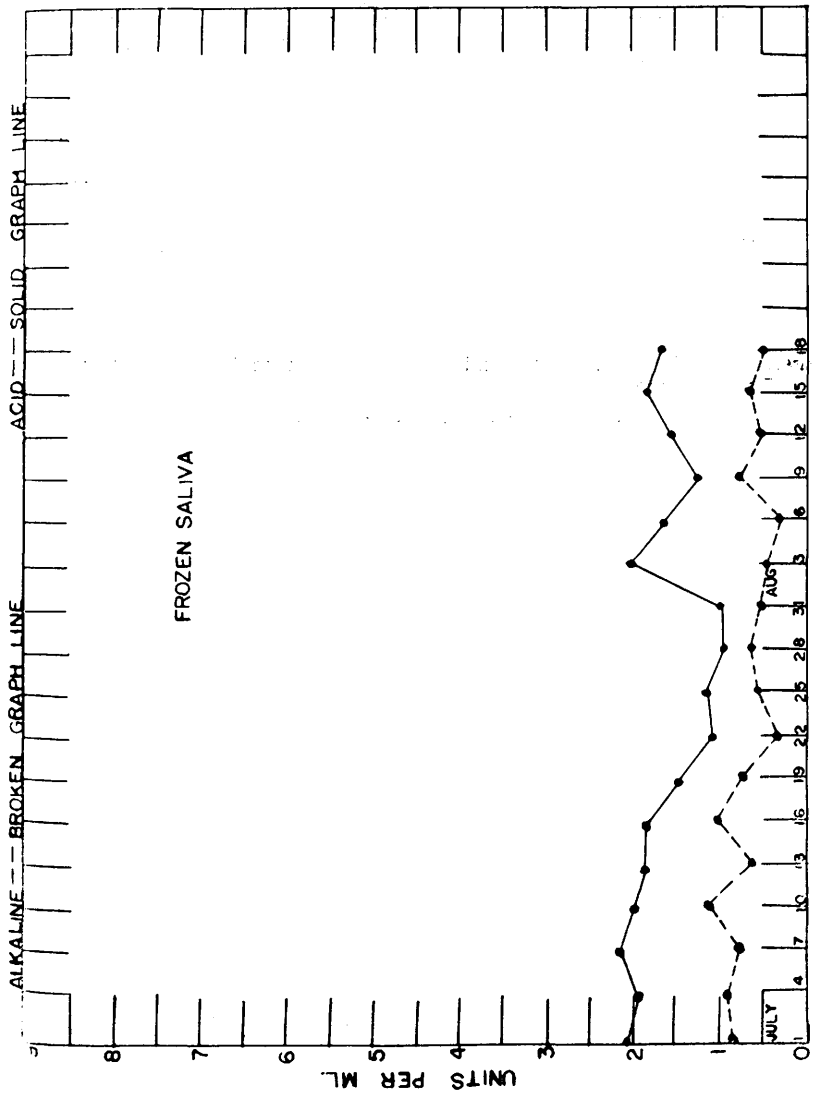

Figure 3.

The decrease in the phosphatase activity after a meal is comparable to the decrease after the chewing of wax for several minutes. Since the saliva tested was collected immediately following the meal, bacterial action had not begun. The chewing and drinking actions could possibly have provided a cleaning effect just as the chewing of wax for the same period would have done.

The phosphatase activity of frozen saliva showed a slight decrease (Fig. 3). The variation in the 18 samples were 1.5 units in acid phosphatase and 1.0 unit in alkaline phosphatase. The upward and downward shifts in activity can be accredited to the fact that no way was found to distribute saliva into several uniform quantities without destroying some phosphatase activity. Although all the samples were not alike, the activity did show a gradual decrease due to aging.

SUMMARY

The project has been a study of the variations in the phosphatase activity of saliva and the factors contributing to these variations. No value for normal phosphatase activity can be stated because it is influenced to such a degree by external and internal factors.

Saliva which is sealed and refrigerated will retain its original phosphatase activity for periods up to 30 minutes. Freezing alone does not preserve the activity of the salivary phosphatases.

LITERATURE CITED

Fitzgerald, R. J. 1952. The acid phosphatase in oral microorganisms. J. Dental Research 31: 189-194.

Williams, Roger John. 1956. Biochemical Individuality. John Wiley and Sons, N. Y.