the individual to form detectable antibodies, some differences seem to exist between these 2 phenomena. Relatively large doses of polysaccharide antigens which tend to linger in the system for considerable periods of time(9,10, 11) have usually been used to induce immunologic paralysis, whereas the antigens used thus far to demonstrate actively acquired tolerance were proteins. Where studies have been made, proteins disappear from the system in a relatively short time(12,13,14). In addition, whereas immunological tolerance can be induced at any age, actively acquired tolerance could only be induced in individuals before birth or shortly thereafter. Future studies will probably clarify the relationship of these 2 phenomena.

Actively acquired tolerance seems to bear a superficial resemblance to enhancement of mouse mammary tumor transplants induced by certain tissue factors(15,16,17,18). However, the fact that adult mice have been used in studying cancer enhancement phenomenon would tend to discount such a similarity. Further work needs to be done for an accurate assessment of any similarity.

Summary. 1. Rabbits exposed to staphylococcal toxoid early in life have a reduced capacity to respond to the same toxoid in later life as evidenced by development of low antihemolysin titers. 2. Resistance to dermonecrotic effects of staphylococcal toxin is also reduced by early exposure of rabbits to the toxoid. 3. The Arthus response does not play a role in development of lesions under conditions of these experiments. 4. The possible role of acquired tolerance and similar phenomena in infectious diseases is also discussed.

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Inhibition of Human Salivary and Prostatic Acid Phosphatase and Yeast Enolase by Low Fluoride Concentrations.* (25180)

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The inhibitory effect, *in vitro,* of fluoride on enzymes including enolase and acid phosphatase from several sources is well known. However, little attention has been paid to quantitation of effects which might be observed in the range of possible physiological concentrations of fluoride. The present investigation was designed to study(1) the characteristics of inhibition of acid phosphatases of saliva and prostatic tissue and of enolase by low

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concentrations of fluoride and (2) the possibility that a simple micro analytical method for determination of fluoride might be developed by application of experimental technics involved.

Materials and methods. Fluoride inhibition of acid phosphatase of "unstimulated" saliva samples was investigated both by means of a modification of the procedure of Huggins and Talalay(1) employing phenolphthalein phosphate substrate and that of King-Armstrong (2) using disodium phenyl phosphate substrate. Sodium fluoride was added to the buffer solution in a concentration such that the reaction mixture contained the desired concentration of fluoride ion. Samples utilizing phenolphthalein phosphate as the substrate were incubated for one hour at 37°C. Color intensity of samples containing "0" fluoride concentration was called 100% enzyme activity. Prostatic acid phosphatase solutions were prepared according to the procedure of Abul-Fadl and King(3). Analytical procedures were the same as those for saliva except that due to high concentration of acid phosphatase in extracts of the gland, considerable dilution of enzyme preparations was necessary. The stock solution of enzyme was stored frozen and no change in activity over a 3 month period was noted. The effect of fluoride on crystalline yeast enolase was studied by the method of Warburg and Christian(4) which utilizes the change in optical tranmission at 240 mμ during conversion of β-glycerol phosphate to phosphoenolpyruvic acid by enolase as indicator of enzyme activity.

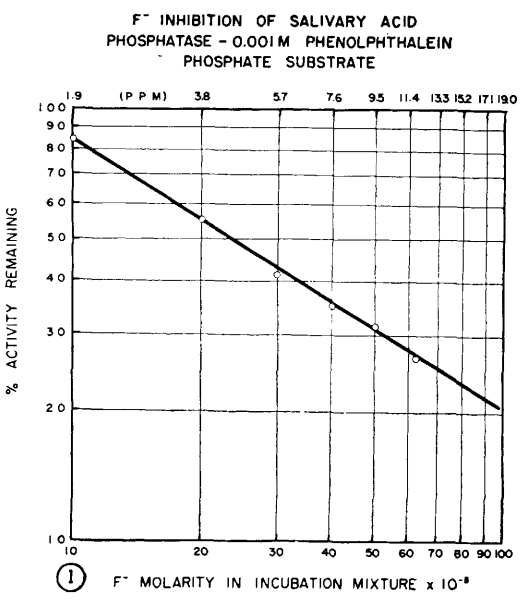
Results. The greatest inhibitory effect/unit of fluoride on salivary acid phosphatase activity occurred over a relatively narrow range of fluoride concentrations and was thoroughly investigated. The results of a typical experiment are illustrated in Fig. 1. When the logarithms of fluoride molarities were plotted on the abscissa and logarithms of activities remaining on the ordinate, a straight line relationship for fluoride concentrations of 10 to 62.5 x 10^{-5} M was obtained. Higher fluoride concentrations gave further reductions in enzyme activity, but the linear relationship described above was no longer followed.

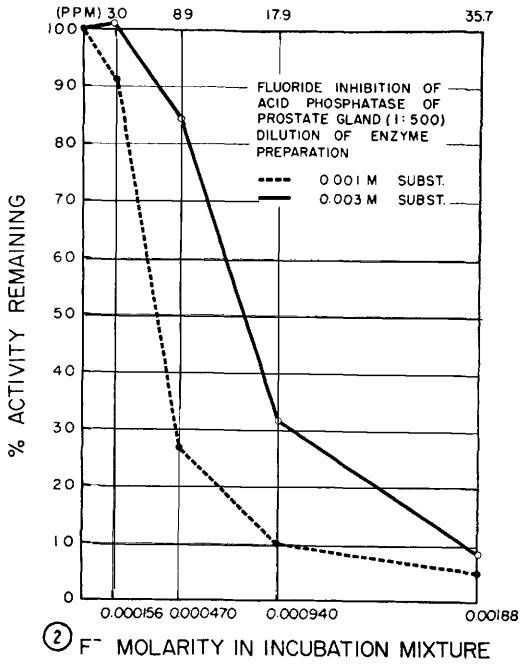
A phenomenon observed with many salivary samples was a slight activation of acid phosphatase activity in the presence of the smaller concentrations of fluoride investigated. For example, in the presence of 7.8 x 10^{-5} M fluoride one sample gave 2% more activity than when no fluoride was added. This observation, and similar data from other samples, was not due to an analytical error, but was observed upon repeated analysis of the same sample. This finding may have been due to a stabilizing effect of small quantities of fluoride on the enzyme rather than an activation. Stabilizing effects of ions against denaturation by factors including heat are known, such as protection of activity of trypsin by calcium(5).

Results obtained by varying substrate concentration of the incubation mixture while keeping the enzyme concentration and fluoride molarity constant, indicated that it was possible to overcome partially the inhibitory effect of the fluoride by increasing the substrate molarity. These experiments were performed with various concentrations of fluoride between 2.0 and 6.25 x 10^{-4} M.

The King-Armstrong phosphatase method using disodium phenyl phosphate as substrate gave similar results. The principal difference between data obtained by this procedure and those of the Huggins and Talalay method was that a higher concentration of fluoride was required to cause inhibition. For example, when disodium phenyl phosphate was the substrate, the acid phosphatase activity was decreased by 53% in presence of 5.0 x 10^{-3} M fluoride. The activity of the same sample was decreased by 61% in the presence of 2.35 x 10^{-4} M fluoride when the substrate was phenolphthalein phosphate. The difference in degree of inhibition of activity was probably due to higher concentration of disodium phenyl phosphate (0.005 M) than that of phenolphthalein phosphate (0.001 M).

Investigations with human prostatic acid phosphatase were largely directed towards use of this enzyme as a simple analytical method for determination of fluoride. This enzyme source was selected because it furnished a stable source of a large quantity of homogenous activity. Whereas 100 ml of saliva





would liberate 5-10 mg of phenolphthalein/hour at 37°C from 0.001 M phenolphthalein phosphate solution, one g of wet prostate tissue released about 1000 mg under the same conditions. All findings with prostatic acid phosphatase discussed subsequently are from

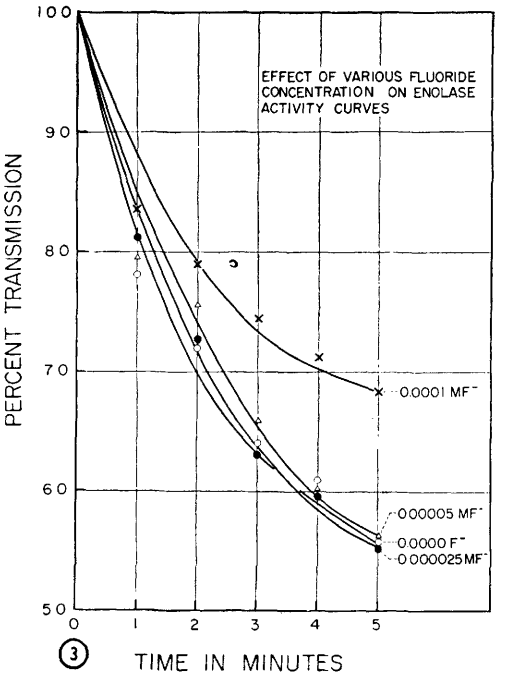


FIG. 1. F⁻ inhibition of salivary acid phosphatase —0.001 M phenolphthalein phosphate substrate.

FIG. 2. Fluoride inhibition of acid phosphatase of prostate gland (1:500) dilution of enzyme preparation.

FIG. 3. Effect of various fluoride concentration of enolase activity curves.

experiments in which phenolphthalein phosphate was the substrate.

Experiments with varying enzyme concentrations (0.0001 to 0.0004 g of wet prostate gland/ml of enzyme solution) were conducted to study inhibition of acid phosphatase activity when substrate concentration and fluoride concentrations were constant. The *percent* inhibition was the same for each enzyme concentration tested, although total amount of substance acted upon varied considerably. In Fig. 2 typical data obtained for enzyme inhibition from 2 concentrations of substrate when enzyme concentration was constant, are presented. These data clearly indicate that inhibition of acid phosphatase from the prostate gland by fluoride may also be partially overcome by increasing the substrate concentration. The activating effect of small concentrations of fluoride under certain conditions is again illustrated.

The range of greatest inhibitory effect/unit of fluoride on activity of acid phosphatase of

the prostate gland was thoroughly investigated. When the data were plotted on log-log scale in the same manner as results of salivary acid phosphatase studies, a straight line relationship similar to that of Fig. 1 was obtained. Application of the straight line relationship to possible analysis of fluoride was considered. The percent inhibition of the acid phosphatase from a single prostate gland was constant with a given fluoride concentration. Thus once the percent inhibition of activity by various fluoride concentrations was well established, it was possible to determine the fluoride content of pure aqueous solutions. A 3 ppm solution of fluoride analyzed in duplicate tubes on 6 different days gave 8-11% inhibition of activity. Results of analysis based on a single assay in duplicate were 3 $\pm$ 0.4 ppm of fluoride. The error of determination could readily be reduced by using a series of replicate tubes for a single assay. Higher concentrations of fluoride gave a smaller analytical error on a percentage basis. Attempts were made to apply this procedure to direct analysis of fluoride in blood serum. This method suffered from the disadvantage that proteins in serum stabilized the enzyme preparation and resulted in elevated activities. If the method is applied to tissue samples, separation of proteins and fluoride must be accomplished in a preliminary procedure.

In Fig. 3, curves show effects of varying concentrations of fluoride on activity of yeast enolase as measured by conversion of beta glycerol phosphate to phosphoenolpyruvic acid. The "0", 0.5, and 1 parts/million fluoride curves are essentially the same and indicate that at these concentrations fluoride did not appear to greatly influence conversion of substrate by the enzyme. At 1.96 ppm there is marked inhibition of enzymatic activity. This is reflected by 30% reduction in amount of substrate acted on in 5 minutes.

The concentration of fluoride (2-3 ppm) required to cause significant inhibition was much above the 0.2 ppm found in body fluids (6). It is inconceivable in view of the rapid excretion of fluoride, the sequestration of fluoride by calcified tissues, and the total volume of body water, that concentrations of fluoride would reach a level which would inhibit the activity of these enzymes, with the possible exception of a transitory effect on salivary acid phosphatase, by addition of 1.0 to 1.2 ppm of fluoride to drinking water.

Summary. A study was made of the effects of low concentrations of fluoride on activity of salivary and human prostate acid phosphatase and yeast enolase. Concentrations of fluoride or concentrations that could be derived from fluoridation of drinking water did not significantly alter enzymatic activity. The straight line relationship between the logarithms of low fluoride concentrations and logarithms of percent of enzyme activity remaining suggested that it is possible to analyze fluoride content of pure aqueous solutions by degree of inhibition of activity of acid phosphatase of human prostate tissue.

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