Our observations shed new light on the different pH-activity curves of ACh esterase and other "unspecific" esterases and on the apparent disagreement in the pH dependence of ACh as substrate and tetraethyl pyrophosphate as inhibitor.

A detailed report of this work will appear in this Journal*.

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A FURTHER STUDY OF THE INHIBITION OF ACONITASE BY 'INHIBITOR FRACTION' ISOLATED FROM TISSUES POISONED WITH FLUOROACETATE

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Fluoroacetate has been shown to inhibit the oxidation of citric acid in animal tissues *in vitro*[1,2] and *in vivo*[3]. This poison is metabolized to a fluorotricarboxylic acid, via a similar pathway as that of acetate. As shown recently by LOTSPEICH, PETERS, AND WILSON[4] this fluorotricarboxylic acid, obtained biosynthetically, inhibits the enzyme aconitase. This preliminary communication* reports a further study of the reaction which indicates that the inhibition is competitive.

The first type of experiment consisted of measuring the initial velocity of the reaction citrate $\longrightarrow$ *iso*citrate at various citrate concentrations with and without inhibitor. Substrate and inhibitor were added simultaneously to the enzyme solution. The reciprocal of the initial velocity was plotted against the reprocal of the substrate concentration[5]. Both the inhibitor and control gave the same initial velocity at infinite substrate concentration (by interpolating the straight lines) which is characteristic of competitive inhibition.

In the second type of experiment 9 units of inhibitor (see[6]) were preincubated for 20 min with a crude muscle extract containing aconitase. 5.6 μM of *dl iso*citrate were then added and the solution placed in a spectrophotometer. The formation of *cis*-aconitate was followed at 240 mμ according to the method of RACKER[7]. Following the addition of *iso*citrate an initial inhibition of about 70% was observed at 30 sec. This decreased in the next minute to 39% inhibition, which remained constant at this level throughout the remainder of the experiment. This is consistent with the hypothesis that inhibitor once attached to the enzyme can subsequently be readily displaced by added substrate.

The previous paper[6] demonstrated an inhibition of four of the possible six reactions of the enzyme aconitase. The other two reactions *iso*citrate $\longrightarrow$ *cis*-aconitate and citrate $\longrightarrow$ *cis*-aconitate have since been tested and show a similar inhibition using the fluorotricarboxylic acid.

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