the total amount of easily soluble proteins per g had decreased to some extent. Thus it appeared that protein II and the components of the level part of the diagram had only increased in relative proportion to the other components, and that their absolute amounts in the total muscle had remained constant.

Rigor mortis also caused a distinct change in the diagram as compared to the fresh contralateral muscle. The percentage of component V had increased 2 to 3 fold, while the percentages of components VIII and IX had decreased. The protein composition of muscles in rigor mortis thus differs essentially from that of normal as well as from that of atrophic muscles.

As an introduction to the study of patients suffering from muscle diseases, twelve normal human muscles were investigated. The electrophoretic diagrams closely resembled the diagrams obtained with rabbit's skeletal muscles. The latter diagrams, however, differed very much from the diagrams obtained from rabbit's heart, uterus, diaphragm and stomach, while the diagrams of these latter muscles were also mutually different.

This work forms part of the investigations on the chemistry of muscle diseases by H. G. K. WESTENBRINK and collaborators. It has been communicated in the Meeting of the Netherlands Society for Biochemistry on December 1st, 1951. Full details will be published. The support of the Netherlands Organisation for Pure Scientific Research (Z.W.O.) is gratefully acknowledged.

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# QUATERNARY AMMONIUM SALTS AS INHIBITORS OF ACETYLCHOLINE ESTERASE

## II. pH DEPENDENCE OF THE INHIBITORY EFFECTS OF QUATERNARY AMMONIUM SALTS, AND THE DISSOCIATION CONSTANT OF THE ANIONIC SITE

by

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Quaternary ammonium salts, in which one part or the other of the ester grouping R–O–C(=O)–R′ is missing, were compared as inhibitors for ACh esterase. Whereas the inhibitory effect decreases in the series prostigmine > acetophenone trimethylammonium iodide > choline chloride > tetraethylammonium bromide, all members of the series show a very similar relative decrease of activity with decreasing pH. Near pH 5 the activity of all quaternary ammonium salts approaches zero. On the other hand, over the whole range of pH 7–10.5 their inhibitory efficiency remains practically constant. The progressive inactivation with decreasing pH can not be ascribed to the inactivation of the nucleophilic group $G_1$ in the esteratic part of the active surface[1], as claimed by WILSON[2], since *e.g.* the tetraethylammonium ion has no "specific" chemical affinity to such a group (its alkyl chains are attached to the active surface by unspecific VAN DER WAALS forces). The dependence of inhibitory effects on pH changes must be due to the *combination of the anionic site with the hydrogen ion*, which inactivates both $G_1$ in the esteratic site and the negative charge of the anionic site. The dissociation constant $K_{EH_2^+}$, determined previously as characteristic for $G_1$, thus appears now to be incorrect and requires redetermination.

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

by

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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 $\mu M$ of *dl iso*citrate were then added and the solution placed in a spectrophotometer. The formation of *cis*-aconitate was followed at 240 m$\mu$ 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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