Spectrophotometric methods for measuring succinate oxidase and dehydrogenase are described. Fluoride and phosphate separately, but more strongly when acting together, are competitive inhibitors of succinate dehydrogenase. The limiting assumptions of the Michaelis-Menten equation are not applicable to this enzyme; the Briggs-Haldane equation should be used. A method of determining the rate constants for association of substrate and enzyme and dissociation of the enzyme-substrate complex is given. (The SC® indicates that this paper has been cited in over 570 publications.)

Fluoride and Succinate Dehydrogenase Kinetics

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This is the last of a series of papers on the succinate oxidase system, starting in 1949, that one of us (ECS) published in the Biochemical Journal while working under the direction of David Keilin. By 1950 his interests had turned to the mechanism of oxidative phosphorylation and when a postdoctoral visitor (WDB) to the institute found that phosphate is necessary for maximal inhibition of succinate oxidase1 by fluoride, we joined forces, in the hope that a further investigation of this requirement might provide a clue towards the mechanism of oxidative phosphorylation.

It did not. However, it did resolve conflicting conclusions in the literature concerning the locus of action of fluoride on respiratory systems. We were able to show that either fluoride or phosphate alone acts as a classical competitive inhibitor of succinate dehydrogenase. The two together are, however, much more effective, suggesting the formation of fluoromethylene as the active site of the enzyme. Using the classical procedures of enzyme kinetics, we measured the various inhibitory constants. However, there remained a problem. We confirmed a previous finding by Massart that the degree of inhibition is greater when the complete succinate oxidase system is measured than when its component, succinate dehydrogenase, is determined with methylene blue as acceptor.

The degree of inhibition is determined by the ratio $k_2/k_3$, where $k_2$ is the dissociation constant of the inhibitor-enzyme complex, and $k_3$ is the Michaelis constant of the enzyme. It was widely believed at that time that $K_M$ is identical with the dissociation constant of the enzyme-substrate complex, which would be independent of the hydrogen acceptor. However, already in 1925, Briggs and Haldane had shown that a more general expression for $K_M$ is $(k_2 + k_3)k_3$, where the reaction constants are defined by:

$$E + S \rightarrow ES \rightarrow E + P.$$  

This reduces to $k_2/k_3 = K_D$, when $k_2 > k_3$. It occurred to us that, if the Briggs-Haldane equation were applicable to succinate dehydrogenase, then, since $k_3$ and therefore $K_M$ would be dependent on the hydrogen acceptor and its concentration, the corresponding dependence of the degree of inhibition by a competitive inhibitor would be explained. This idea was confirmed by the demonstration of a linear relationship between $V$ (the velocity at infinite substrate concentration) and the observed velocity at different values of $V$, obtained by varying the nature of the acceptor and its concentration. By extrapolation of this straight line to $V=0$, $k_3$ could be calculated. This demonstration of the validity of the Briggs-Haldane equation we considered to be the most important part of our paper.

The paper and a more general exposition during a symposium on enzymes organized by the Faraday Society aroused considerably more interest than we had expected, and we had the satisfaction of seeing the method for determining $k_3$ included in M. Dixon and E.C. Webb's book.1 However, this is now all old hat and cannot be the reason for the high citation score. Perhaps, the interest in fluoride toxicology and pharmacology is a partial explanation. However, consultation of some recent citations has probably revealed the main reason. We had completely forgotten that, in this paper, we had introduced convenient spectrophotometric methods for measuring succinate dehydrogenase and succinate oxidase. It appears that these methods are still widely used for measuring the activity of this enzyme as a mitochondrial marker (see, for example, reference 5).