# L. A NOTE ON THE KINETICS OF ENZYME ACTION.

By GEORGE EDWARD BRIGGS
and JOHN BURDON SANDERSON HALDANE.

(*From the Botanical and Biochemical Laboratories, Cambridge.*)

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The equation of Michaelis and Menten [1913] has been applied with success by Kuhn [1924] and others to numerous cases of enzyme action. It is therefore desirable to examine its theoretical basis. Consider the irreversible reaction $A \rightarrow B$, unimolecular as regards $A$, and catalysed by an enzyme. Suppose one molecule of $A$ to combine reversibly with one of enzyme, the compound then changing irreversibly into free enzyme and $B$, where $B$ may represent several molecules. We may represent this as:

$$\begin{matrix} A + E & \rightleftharpoons & AE \rightarrow B + E. \\ (a-x)\,(e-p) & & p \quad x \end{matrix}$$

Now let $a$ be the initial concentration of $A$, $e$ the total concentration of enzyme, $x$ the concentration of $B$ produced after time $t$, and $p$ the concentration of enzyme combined with substrate at time $t$. We suppose $e$ and $p$ to be negligibly small compared with $a$ and $x$. Then by the laws of mass action

$$\frac{dp}{dt} = k_1 (a-x)(e-p) - k_2 p - k_3 p,$$

where $k_1$, $k_2$, $k_3$ are the velocity constants of the reactions

$$A + E \rightarrow AE, \quad AE \rightarrow A + E, \quad \text{and} \quad AE \rightarrow B + E,$$

respectively. Now since $p$ is always negligible compared with $x$ and $a - x$, its rate of change must, except during the first instant of the reaction, be negligible compared with theirs.

For during the remainder of the reaction $p$ diminishes from a value not exceeding $e$ to zero, whilst $x$ increases from zero to $a$. Thus the average value of $-\frac{dp}{dt} \div \frac{dx}{dt}$ is less than $\frac{e}{a}$. And provided $\frac{e}{a}$ is small it is clear that if the amount of combined enzyme decreased for a measurable time at a rate comparable with that of its substrate the reaction would come to an end. To take a concrete example Kuhn [1924] calculates that a yeast saccharase molecule at 15° and $p_H$ 4·6 can invert 100 or more molecules of sucrose per second. Even if the enzyme concentration is so unusually large that the inversion of a strong sucrose solution is half completed in 10 minutes, $\frac{a}{e}$ cannot be less than 120,000, and if $-\frac{dp}{dt}$ attained 1 % of the value of $\frac{dx}{dt}$ for 1 second the

reaction would stop owing to all the enzyme being set free. (Actually it may be shown that

$$\frac{dp}{dt} = \frac{-k_3(k_2+k_3)e(a-x)}{k_1\left(a-x+\frac{k_2+k_3}{k_1}\right)^3}.)$$

Hence $$k_1(a-x)(e-p) - k_2p - k_3p = 0.$$

$$\therefore\ p = \frac{k_1e(a-x)}{k_2+k_3+k_1(a-x)}$$

$$= \frac{e(a-x)}{a-x+\frac{k_2+k_3}{k_1}}$$

$$\therefore\ \frac{dx}{dt} = k_3p = \frac{k_3e(a-x)}{a-x+\frac{k_2+k_3}{k_1}}.$$

This is Michaelis and Menten's [1913] equation, $(k_2 + k_3)/k_1$ representing their constant $K_s$. They assume that the reaction

$$A + E \rightleftharpoons AE$$

is always practically in equilibrium, and $K_s$ its equilibrium constant, *i.e.* that $k_3$ is negligible in comparison with $k_2$. Van Slyke and Cullen [1914] on the other hand assumed that the first stage of the reaction was irreversible, *i.e.* $k_2 = 0$, and arrived at the same equation. It is clear, however, that data as to the course of a reaction can give no indication of the ratio of $k_2$ and $k_3$, though when the velocity of the observed reaction, and hence $k_3$, is very large, the upper limit to $k_2$ deducible from the kinetic theory may possibly prove to be of the same order of magnitude.

It may be remarked that with this modification of their theory, Michaelis and Menten's analysis of the effects of the products of the reaction, or other substances which combine with the enzyme, still holds good.

REFERENCES.

Kuhn (1924). Oppenheimer's "Die Fermente und ihre Wirkungen," 185 et seq.
Michaelis and Menten (1913). *Biochem. Z.* **49**, 333.
Van Slyke and Cullen (1914). *J. Biol. Chem.* **19**, 141.