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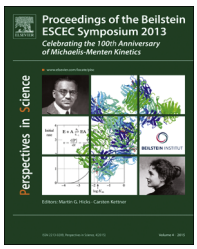

REVIEW

One hundred years of Michaelis-Menten kinetics ☆

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Abstract
The year 2013 marked the centenary of the paper of Leonor Michaelis and Maud Menten (Michaelis and Menten, 1913), and the 110th anniversary of the doctoral thesis of Victor Henri (Henri, 1903). These publications have had an enormous influence on the progress of biochemistry, and are more often cited in the 21st century than they were in the 20th. Henri laid the groundwork for the understanding of enzyme mechanisms, but his experimental design was open to criticism. He reached essentially correct conclusions about the action of invertase, but he took no steps to control the hydrogen-ion concentration, and he took no account of the spontaneous mutarotation of the glucose produced in the reaction. Michaelis and Menten corrected these shortcomings, and in addition they introduced the initial-rate method of analysis, which has proved much simpler to apply than the methods based on time courses that it replaced. In this way they defined the methodology for steady-state experiments that has remained standard for 100 years.

Contents

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Introduction

Many milestones in the history of biochemistry have had anniversaries in 2013, starting with the birth of Claude Bernard in 1813, continuing with Victor Henri's thesis in 1903 (Henri, 1903) the protonic theory of acid-base catalysis in 1923 (Brönsted, 1923), the introduction of flow methods for investigating fast reaction kinetics in the same year (Hartridge and Roughton, 1923), the determination of the structure of DNA in 1953 (Watson and Crick, 1953), the concept of allosteric regulation (Monod et al., 1963) and Cleland's rationalisation of multi-substrate kinetics in 1963 (Cleland, 1963), and the introduction of metabolic control analysis in 1973 (Kacser and Burns, 1973). One other is of special importance for enzymologists, as 100 years ago Leonor Michaelis and Maud Menten placed kinetic studies on a firm experimental and theoretical base (Michaelis and Menten, 1913).

Their paper is the subject of the present chapter. I shall discuss the historical context in which it was written, and will also mention the other contributions made not only by these authors, but also by their distinguished predecessor Victor Henri. Much of the information to be given is based on other recent papers (Cornish-Bowden, 2013; Deichmann et al., 2014; Cornish-Bowden et al., 2014).

The historical context: enzyme catalysis before 1913

Michaelis and Menten (1913) did not, of course, find enzyme kinetics in a virgin state, and they built on the work of Adolphe Wurtz (1880), O'Sullivan and Thompson (1890), Adrian Brown (1902), and, most important of all, Victor Henri (1902, 1903).

Wurtz introduced the idea of an enzyme–substrate complex, Brown used it to explain enzyme saturation, and Henri was the first to write the equation commonly called the Michaelis-Menten equation[1]. This obviously raises the question of why Michaelis and Menten tend to be the ones mainly remembered today. Their contribution was indeed important, but not to the point where their predecessors should be forgotten.

Much of the early work was done with invertase, an extracellular enzyme from yeast that catalyses the hydrolysis ("inversion") of sucrose to "invert sugar", a mixture of glucose and fructose. It plays little part in modern academic research, but it still has considerable industrial importance because it provides a simple and convenient method of producing chocolates with liquid centres, using the fact that invert sugar is more soluble in water than sucrose: a solid filling of sucrose to which a little invertase is added at the last moment becomes liquid after the chocolate coating has hardened. This was not of course the reason why it was so much studied at the end of the 19th century and the beginning of the 20th. Its importance then was that it catalysed one of the only reactions that could be assayed very easily. Although primitive colorimeters had been available since 1827 (Warner, 2006) their operation would have been too time-consuming for following reactions even if the enzyme-catalysed reactions now studied spectrophotometrically had been known, and nothing resembling a pH-stat existed. The inversion of sucrose, however, was easy to follow in a polarimeter, as sucrose is dextrorotatory whereas invert sugar is laevorotatory.

In his thesis (Henri, 1903), therefore, Henri's main focus was on understanding the kinetics of the invertase-catalysed reaction, but he also considered the reactions catalysed by emulsin and amylase, primarily to confirm that his conclusions with respect to invertase had some generality. His major objective was to show that enzyme-catalysed reactions followed the laws of physical chemistry—something that seems obvious today, but was still controversial at the beginning of the 20th century. Although Buchner's experiments (Buchner, 1897) are now considered to have sounded the death-knell of vitalism (Friedmann, 1997), their effect on biochemical thinking was not instantaneous, and Henri was working at a time when vitalistic ideas were far from dead. Moreover, at that time almost nothing was known about the molecular nature of enzymes. This was the heyday of colloids, and there were doubts as to whether enzymes could be regarded as molecules at all, and, even after the crystallisation of urease (Sumner, 1926) and pepsin (Northrop, 1930), the protein nature of enzymes continued to be controversial.

Like all of his predecessors, Henri tried to analyse the time course of the reaction. This approach was known to work very well with simple chemical reactions, and even some catalysed reactions, such as the effect of hydrogen iodide on the reaction between potassium persulphate and phosphorous acid, but the time was not yet ripe for it to be applied with success to enzyme-catalysed reactions. Henri derived an equation equivalent to the Henri-Michaelis-Menten equation

$$\text{Initial rate} = \frac{K_3 a}{1 + ma} \quad (1)$$

[1] In this chapter I shall call it the Henri-Michaelis-Menten equation, for reasons that will become apparent.

in which a is the initial amount of the substrate sucrose, K_3 is a constant proportional to the enzyme concentration, and m is another constant. He obtained this as a special case at time zero of a more general equation

$$\frac{dx}{dt} = \frac{K_3\,(a-x)}{1+m\,(a-x)+nx} \qquad (2)$$

in which x is the amount of product at time t, and n is a constant. Henri noted that Eq. (1) predicted a hyperbolic dependence of initial rate on the amount of sucrose and added that that was what he observed experimentally, but he did not illustrate the curve[2], and he did not take what today seems the obvious next step of analysing his data in terms of the initial-rate equation. Instead he preferred to use the integrated form of Eq. (2).

Michaelis and Menten's contribution

As we have seen, Henri derived the equation for the initial rate of an enzyme-catalysed reaction, but he did not use it, and Michaelis and Menten (1913) were the first to recognise the advantages that would result from analysis in terms of it:

1. Complications due to the progress of the reaction vanish: inhibition by accumulated products, loss of catalytic activity, and, in the special case of the polarimetric methods used for studying invertase, spontaneous mutarotation of the products.
2. The reverse reaction can be ignored, because it cannot occur until some products have had time to accumulate.
3. Initial-rate equations are easier to derive and use than integrated equations for the progress of reaction.
4. There is no drift in the pH, temperature or other conditions at zero time.

The introduction of initial-rate methods was thus the most important contribution of Michaelis and Menten to the theory and practice of methods of studying enzyme-catalysed reactions, and the principal reason why they are still remembered today. However, it was not their only contribution, and in addition they had two criticisms of Henri's experiments (though without contesting his conclusions).

1. The α-D-glucose released in the reaction catalysed by invertase is not stable in aqueous solution: it changes spontaneously into an equilibrium mixture of α-D-glucose and β-D-glucose in which the latter predominates, and although this is also dextrorotatory it is much less so. The result is that the optical rotation of the products changes spontaneously, a process known as mutarotation.[3] Henri took no account of this in his experiments with invertase[4].
2. Henri took no steps to control the pH of his reaction mixtures. Although the pH scale was not proposed until several years later (Sørensen, 1909), the concept of "acidity" was certainly known to Henri, and had already been used in experiments on invertase (O'Sullivan and Thompson, 1890), and the use of indicators for estimating the hydrogen-ion concentration was described shortly after Henri's thesis was written (Friedenthal, 1904).

For these various reasons Michaelis and Menten thought it essential to re-examine the invertase reaction, and in doing so they defined the methodology for experimental study of enzyme catalysis that has remained standard for 100 years. Contrary to what seems to be widely believed, they did not estimate their kinetic parameters from the rectangular hyperbola obtained by plotting the initial rate against the substrate concentration (i.e., the curve described but not illustrated by Henri (1903)), but instead plotted the rate against the logarithm of the substrate concentration. This may seem a trivial point, but it enabled them to avoid the difficulties inherent in locating the asymptote of a curve when the observations do not approach it closely. It is probably at least in part for this reason that when Johnson and Goody (2011) applied modern methods of data analysis to Michaelis and Menten's data they found that they could reproduce the conclusions. It seems quite unlikely that this would have been the case if the original analysis had been made in terms of the hyperbola, as it was not until much later that satisfactory methods for direct fitting of the hyperbola were introduced (Cleland, 1967; Johansen and Lumry, 1961; Wilkinson, 1961).[5]

Although the plot of initial rate against the logarithm of the substrate concentration has not survived as a method of parameter estimation, it remains indispensable for one purpose, as it provides the only satisfactory way of displaying data for enzymes with very different kinetic properties in a single graph with a single scale. For example, the four isoenzymes of hexokinase found in rat liver differ in affinity for glucose by more than 300-fold, but they can easily be compared with a semi-logarithmic plot (Cárdenas, 1995).

Landmarks in the development of steady-state enzyme kinetics

The main advances in steady-state enzyme kinetics, both theoretical and experimental, are presented in many textbooks, so here I shall simply note some of the principal landmarks.

[2]He had no figures in his thesis.

[3]Other terms used in the older literature are birotation (Henri, 1903) and multirotation (Michaelis and Menten, 1913).

[4]Curiously, he did take account of it in his experiments on emulsin, which were done later. So it is possible that he did allow for mutarotation in his experiments on invertase but failed to mention it, or maybe he did not become conscious of the problem until after the studies of invertase were completed.

[5]This is not entirely fair, as Lineweaver and Burk sought advice from the distinguished statistician Deming and described a valid method much earlier, in a paper with Deming (Lineweaver et al., 1934) published in the same year as their more famous paper (Lineweaver and Burk, 1934) (and referred to in it), but the influence on the progress of biochemistry of this earlier paper was essentially non-existent. The many authors who refer to the later one (nearly 12000 citations listed in the Web of Science) and claim to have used the "method of Lineweaver and Burk" show little sign of having read what they actually did.

The steady-state hypothesis

Michaelis and Menten, like Henri before them, regarded formation of the enzyme-substrate complex as an equilibrium process, but almost at the same time Van Slyke and Cullen (1914) treated it as irreversible. A decade later Briggs and Haldane (1925) pointed out that both interpretations were special cases of the steady-state hypothesis, and this is now regarded as the correct starting point for deriving the Henri–Michaelis–Menten equation.

Two-substrate reactions

Haldane gave a brief account of the kinetics of reactions with two substrates in his influential book *Enzymes* (Haldane, 1930), but these were not thoroughly analysed until the 1950s (Alberty, 1958; Dalziel, 1957; Segal et al., 1952). A major difficulty, however, came from the obvious fact that the relevant equations are more complicated, and hence more difficult to derive and analyse, than those for one-substrate reactions, but this was greatly eased by the graphical method of King and Altman (1956). Later Cleland (1963) organised and systematised all of this work, and circulated computer programs to facilitate the statistical analysis (Cleland, 1967), with a great influence on the subsequent development of the subject.

Specificity

The last major advance in understanding steady-state kinetics was made by Fersht (1974), who introduced for the first time a meaningful definition of specificity. Everyone had always agreed, of course, that this was a vitally important property of enzymes, but there was little agreement of which of the parameters of the Henri–Michaelis–Menten equation is the most appropriate measure of it. The equation can be written with modern symbols as follows:

$$v = \frac{k_{cat} e_0 a}{K_m + a} \tag{3}$$

in which k_{cat} is the catalytic constant, e_0 is the enzyme concentration, a is the substrate concentration and K_m is the Michaelis constant. Higher values of k_{cat}, lower values of K_m, and higher values of k_{cat}/K_m, were all regarded at one time or another as indications of greater specificity. For a substrate A with parameters k^A_{cat} and K^A_m the rate v_A measured in the presence of a competing substrate B with parameters k^B_{cat} and K^B_m is as follows:

$$v_A = \frac{k^A_{cat} e_0 a}{K^A_m \left(1 + b/K^B_m\right) + a} \tag{4}$$

with an analogous expression for the rate v_B of the competing reaction. Division of one expression by the other gives

$$\frac{v_A}{v_B} = \frac{k^A_{cat}/K^A_m}{k^B_{cat}/K^B_m} \cdot \frac{a}{b} \tag{5}$$

Fersht pointed out that k_{cat}/K_m, is thus the parameter that measures the capacity of an enzyme to discriminate between substrates that are available simultaneously, and so it provides the only meaningful physiological definition of specificity. For that reason the International Union of Biochemistry and Molecular Biology has recommended the name *specificity constant* for this ratio (International Union of Biochemistry, 1982).

Fast reactions

Before 1923 no method was known for studying reactions that occurred in the millisecond range or faster, but this became possible surprisingly early, with the introduction of the *continuous-flow method* (Hartridge and Roughton, 1923). In its original form this was only suitable for proteins available in very large quantities, such as haemoglobin, but it formed the starting point for developing other flow methods, including the *stopped flow method* (Gibson and Milnes, 1964), which has become the method of choice for the study of fast reactions.

Integrated rate equations

As already mentioned, Henri and his predecessors tried to analyse invertase kinetics in terms of integrated rate equations that could, at least in principle, describe the whole time course of a reaction. This approach was largely discarded after Michaelis and Menten showed that it was much simpler to analyse enzyme kinetics in terms of initial rates. Some biochemists, such as Niemann (Jennings and Niemann, 1955), and other authors more recently (Boeker, 1984, 1985; Cornish-Bowden, 1975; Goudar et al., 1999; Schiller et al., 1996), have attempted to revive interest in it, but it has remained little used.

Other work of the main participants

Henri, Michaelis and Menten are all known primarily to biochemists for the work described above, the development of the Henri–Michaelis–Menten equation. However, all three of them made other important contributions. This section will briefly describe their lives and other work, but more detail can be found elsewhere (Deichmann et al., 2014; Cornish-Bowden et al., 2014).

Victor Henri (1872–1940)

Victor Henri was born in Marseilles, but his family background was wholly Russian.[6] His parents were not married, and came to France to ensure that he would be born there, and thus benefit from the much greater rights of illegitimate children in France, compared with Russia. He was then adopted by his father and his wife, the sister of his natural mother, and after several years in France he received most of his education in St Petersburg. Henri's mothers came from the scientifically very distinguished Lyapunov family, of whom the best-known was their cousin, the mathematician Alexander Lyapunov. His niece married Peter Kapitsa, who discovered superfluidity and developed low-temperature physics, work for which he received the Nobel Prize for Physics in 1978.

[6] A misleading account that described him as being orphaned when very young was circulated for 130 years, with the aim of preventing the scandalous truth from becoming known.

Henri himself pursued an extremely varied career. Before his studies of enzymes he worked in experimental psychology, and was the first collaborator of Alfred Binet, the pioneer of intelligence testing. He received his second doctorate in 1903 on the basis of his thesis on diastases (Henri, 1903), but he appears to have done no further work on invertase. He was Professor of Physiology in Paris, and afterwards was responsible for the organisation of the chemical industry of Russia for defence. After the First World War he spent ten years at the University of Zürich, after which he was to be incharge of a planned great institute of petrochemistry at Berre L'Étang (near Marseilles). However, he moved to Science Faculty in Liège before this was finished. His later work was mainly in physical chemistry, with a particular interest in the use of absorption spectra as a source of information about molecular structures. He died in Bordeaux in 1940.

Leonor Michaelis (1875–1949)

Leonor Michaelis was born into a Jewish commercial family in Berlin. He started his career as assistant to Paul Ehrlich, and at whose insistence he qualified as a physician. Subsequently he undertook his own research, but in very unsatisfactory conditions: as an unpaid professor at the University of Berlin, he lived on his earnings as a doctor in a city hospital, and carried out his research in a small laboratory in the hospital that he and his friend Peter Rona had built themselves. Nonetheless, he was highly productive, and in the five years preceding the First World War he had nearly 100 publications, some of them still cited today. His major motivation, like Henri's, was to put studies of enzymes on a firm foundation of physical chemistry, with a particular interest in hydrogen-ion concentration (Michaelis and Davidsohn, 1911). He was the first to distinguish between different kinds of inhibition, in the context of the different effects of glucose and fructose on the reactions catalysed by maltase (Michaelis and Rona, 1914) and invertase (Michaelis and Pechstein, 1914). His division of these into competitive inhibition, characterised by its effect on K_m, and non-competitive inhibition, characterised by its effect on k_{cat}, remains widely used. That is unfortunate, however, as it is now understood that competitive inhibition is better described as an effect on k_{cat}/K_m with no effect on k_{cat}, and that the other extreme is uncompetitive (not non-competitive) inhibition, with an effect on k_{cat} but not on k_{cat}/K_m (Cornish-Bowden, 2012). When both effects occur simultaneously we have mixed inhibition, and classical non-competitive inhibition is the special case of mixed inhibition in which the two effects are equal.[7]

Michaelis's lack of possibilities for promotion to a real academic position in Germany, coupled with the problems created by a scientific dispute that he had with Emil Abderhalden, one of the leading figures in physiology at that time (Deichmann et al., 2014), led him to accept an invitation to go to Japan as Professor of Biochemistry at Nagoya. He spent four years there, and had a major influence on the development of biochemistry in Japan (Nagatsu, 2013). Afterwards he moved to the USA, first to Johns Hopkins University in Baltimore, and then to the Rockefeller Institute in New York, where he worked for the remainder of his life. In that period he was primarily interested in biological redox reactions, and advocated the view that radical semiquinones were intermediates in these reactions, a view that is today well accepted, but was highly controversial when Michaelis proposed it.

[7]Some authors follow Cleland (1963) in using the term "non-competitive inhibition" for any variety of mixed inhibition, without requiring the two inhibition constants to be equal.

Maud Leonora Menten (1879–1960)

Maud Leonora Menten was born in Ontario, but grew up in British Columbia. She studied at the University of Toronto, and was one of the first Canadian women to be qualified to practise medicine. She had already published work on the distribution of chloride and potassium ions (Macallum and Menten, 1906; Menten, 1912) and co-authored a book on animal tumours (Flexner et al., 1910) before she went to Berlin to work with Michaelis. She was probably motivated to do that by a desire to learn how to measure and control the hydrogen-ion concentration, knowledge that she applied soon after her return to the USA (Menten and Crile, 1915).

As Menten's primary interest was in experimental pathology, and her research at the University of Pittsburgh was mainly in this area, she rather faded from the view of biochemists. Among her various important contributions one can mention her development of a method of histochemical detection of alkaline phosphatase in the kidney (Menten et al., 1944) that was considered by a major textbook of the 1950s (Pearse, 1953) to have revolutionised the field, and the use of sedimentation and electrophoresis for detecting haemoglobin variants (Andersch et al., 1944) that anticipated Pauling's much better known work (Pauling et al., 1949) on sickle-cell disease by several years.

Impact of the early work today

As may be seen from Figure 1 the papers at the origin of steady-state enzyme kinetics have been well cited since about 1950, but what is especially striking is the large increase during the 21st century. This is especially noticeable for Michaelis and Menten (1913), but the same trend can be seen for Henri (1902, 1903) (taking the 1902 paper and the thesis together): in both cases the year with the highest number of citations is 2013. The occurrence of the centenary of Michaelis and Menten (1913) in 2013 is of course partly responsible for this, but only partly, as the steep increase started at the beginning of the century.

How can we explain this? Various topics in biochemistry have grown substantially since the beginning of the century, such as systems biology and studies of single molecules, and others, such as drug development—which in the 20th century was even more obsessed with structure than it still is today—pay much more attention to kinetics than they did. However, even taking all of these together they do not account for all of the growth in citations to the early papers, and so there seems to be a general revival of interest in enzymes and their properties.

For several reasons, in fact, the Henri–Michaelis–Menten equation remains crucial for understanding biochemistry:

1. It is the starting point for teaching any aspect of enzymology.
2. It provides the basis for studying fast reactions.

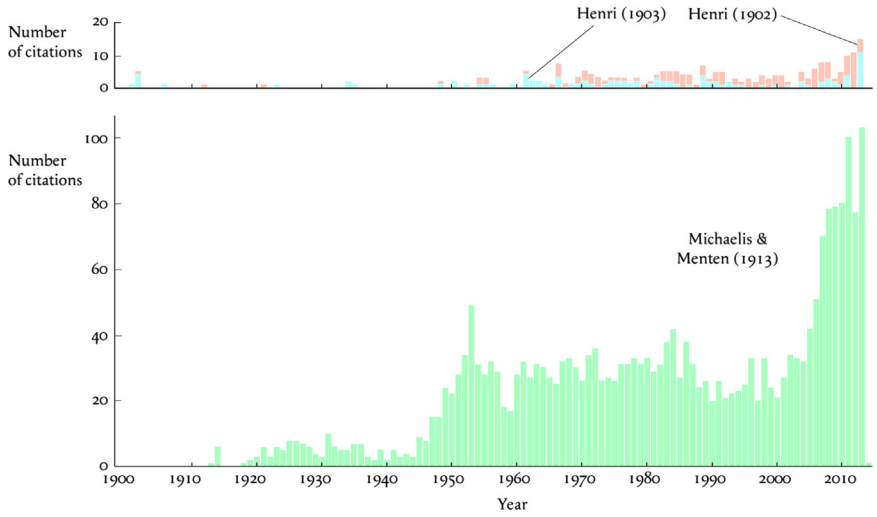

Figure 1 Citations to Henri (1902, 1903) and Michaelis and Menten (1913). The data were obtained from the *Web of Science* on 18 December, 2013, so the values for 2013 are not complete and probably need to be increased by about 10%.

3. It is the basis for studying mechanisms of enzyme catalysis.
4. It provides a point of reference for understanding enzyme regulation, including non-classical kinetics, as seen in allosteric and cooperative interactions.
5. It is essential for adequate progress in drug development, which is increasingly understood to be more than just a matter of structure.
6. It is the basis of enzyme engineering, and technological uses of enzymes in general.
7. It is necessary for understanding the properties of single molecules.

So far as non-classical kinetics are concerned, we may wonder why it took so long for deviations from Henri–Michaelis–Menten kinetics to be recognised, about 30 years from the introduction of the steady-state hypothesis (Briggs and Haldane, 1925) to the discovery of feedback inhibition in threonine deaminase (Umbarger, 1956) and aspartate transcarbamoylase (Yates and Pardee, 1956). The point, however, is that deviations from classical behaviour could not be recognised until the classical behaviour itself was well defined, and that required time. At the beginning of the century almost nothing was known about the chemical nature of enzymes, very few enzymes had been characterised, and very little was known about metabolic pathways. It is not surprising, therefore, that it was not until the 1950s that deviations from classical kinetics came to be recognised, and associated with metabolic regulation (Cárdenas, 2013).

Conflict of interest

The author declares that there is no conflict of interest.

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