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REVIEW ARTICLE

Commemorating the 1913 Michaelis–Menten paper *Die Kinetik der Invertinwirkung*: three perspectives

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Methods and equations for analysing the kinetics of enzyme-catalysed reactions were developed at the beginning of the 20th century in two centres in particular; in Paris, by Victor Henri, and, in Berlin, by Leonor Michaelis and Maud Menten. Henri made a detailed analysis of the work in this area that had preceded him, and arrived at a correct equation for the initial rate of reaction. However, his approach was open to the important objection that he took no account of the hydrogen-ion concentration (a subject largely undeveloped in his time). In addition, although he wrote down an expression for the initial rate of reaction and described the hyperbolic form of its dependence on the substrate concentration, he did not appreciate the great advantages that would come from analysis in terms of initial rates rather than time courses. Michaelis and Menten not only placed Henri's analysis on a firm experimental foundation, but also defined the experimental protocol that remains standard today. Here, we review this development, and discuss other scientific contributions of these individuals. The three parts have different authors, as indicated, and do not necessarily agree on all details, in particular about the relative importance of the contributions of Michaelis and Menten on the one hand and of Henri on the other. Rather than force the review into an unrealistic consensus, we consider it appropriate to leave the disagreements visible.

Part 1: a critical and passionate biochemist: Leonor Michaelis, pioneer of quantitative enzymology, in Berlin and New York

by Ute Deichmann and Stefan Schuster

Summary

This historical review highlights the life and research of Leonor Michaelis, the German-Jewish-American pioneer in enzyme kinetics and the physical chemistry of proteins. Based on an overview of early research on enzyme kinetics, the outstanding achievements of Michaelis and his research fellow Maud Menten (i.e. their mathematical derivation of the fundamental kinetic rate law and the corresponding affinity constant of the enzyme–substrate bond) are highlighted and discussed. The background and consequences of Michaelis's marginalization in German academia and, finally, his emi-

Abbreviation
MCA, metabolic control analysis.

gration from Germany (already prior to the advent of Nazism) are analysed. An examination of Michaelis's major work in the field of biological redox reactions in New York and a general assessment of Michaelis as a researcher conclude this part of the review.

Introduction

On 3 December 2011, a ceremony was organized by the Department of Biology of Humboldt University of Berlin to mark the change of name of one of its buildings from *Abderhalden Haus* to *Leonor Michaelis Haus* (Fig. 1) This decision was taken after the questionable nature of major parts of Emil Abderhalden's research had come to light (see below). The renaming of the institute after Leonor Michaelis is of dual significance: first, it is a powerful reminder of Leonor Michaelis's seminal and far-reaching research in Berlin around a century ago, which is part of the topic of this special issue; it also draws attention to the lack of acknowledgement he received while in Germany, as a consequence of which he left the country in 1922. Second, as was realized immediately by the young researchers who participated in the renaming ceremony (one of the authors of the present review, UD, participated in the event, with a lecture on Michaelis), it provides Humboldt University's Department of Biology with the role model of an outstanding, internationally renowned researcher whose work has remained fertile to this day, and also of an exceptional human being (Fig. 2).

Fig. 1. Photograph of the Leonor Michaelis Haus in Berlin (taken by UD on 3 December 2011, the day of the rededication ceremony). The building is situated at one of the campus sites of Humboldt University between Reinhardtstraße, Luisenstraße and Hannoversche Straße in Berlin's central district, near the famous Friedrichstraße and the former Checkpoint Charlie.

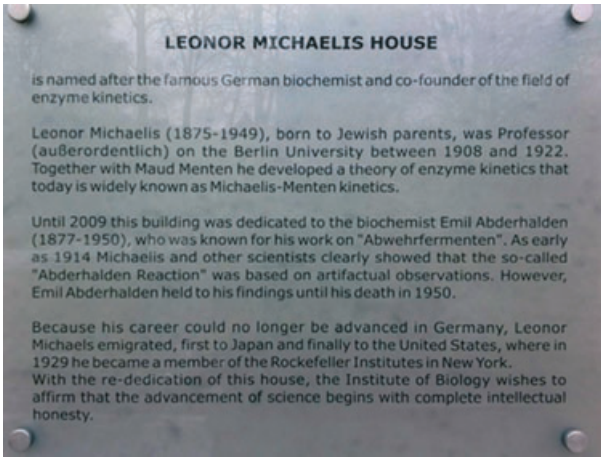

Fig. 2. Photograph (taken by StS) of the plaque commemorating Leonor Michaelis at the building now carrying his name. This plaque in English complemented a similar plaque in German. It should be noted that Michaelis was a German and American biochemist.

We review Michaelis's life and research, with special emphasis on his work in Berlin. Throughout his life, Michaelis successfully dealt with a variety of different topics, which included experimental embryology, the physical chemistry of proteins, immunology, and biological redox reactions. In addition, he wrote several textbooks on subjects such as embryology [1] and mathematics for biologists and medical students [2]. Here, we focus on his ground-breaking work on the physical chemistry of proteins and enzymes, which culminated in his famous mathematical derivation, together with Maud Menten, of the fundamental kinetic rate law and the corresponding affinity constant of the enzyme–substrate complex. In addition, we review Michaelis's disclosure of the fraudulent nature of Abderhalden's work and its consequences. An examination of Michaelis's major work in the field of biological redox reactions in New York and a general assessment of him as a researcher conclude this part of the review.

Leonor Michaelis in Berlin: his emigration to Japan and the USA

Leonor Michaelis (1875–1949) was born in Berlin, where his father Moritz Michaelis was a merchant. Leonor Michaelis was a member of the orthodox Jewish community in Berlin, Adass Jisroel. Although he left the community in 1915 (Centrum Judaicum Berlin Archive), he did not convert to Christianity. There is very little other information about his family: Michaelis's only

comment was that he was born into an 'environment which was far removed from science' [3]. His autobiographical account, written in the third person shortly before he died in New York, provides hardly any personal background, focussing instead on his research [3].

Michaelis attended a *humanistisches Gymnasium* (a grammar school with a strong emphasis on the classics), the Koellnisches Gymnasium in Berlin, which, unusual for this kind of school at the time, also had a chemistry and physics laboratory. He was talented and interested in many subjects, in particular classical philology, science and mathematics. Despite his great interest in science, he decided to study medicine, his justification being typical of many Jewish students at the time: 'With no one to advise him, and no idea of how pure science could provide a living, he chose the study of medicine as the best approach to science' [3]. Unlike Christian students, who frequently came from academic families, most Jewish students at the time came from the commercial milieu, not the educated middle class. (The social backgrounds of Jewish scientists and the impact of conversion and academic anti-Semitism for their careers are addressed elsewhere [4–6]). In 1893, he entered the Friedrich Wilhelm University of Berlin (renamed the Humboldt University in 1949). His teachers included several outstanding scientists: Emil Fischer in chemistry, Oscar Hertwig in embryology, and Emil du Bois-Reymond in physiology, amongst others. His last semester, including the final examinations in the medical specialities, was in Freiburg im Breisgau. Before going there, he passed his examination as a doctor in Berlin in 1896. Michaelis was also an excellent pianist, and occasionally performed in public.

Following his medical studies, Michaelis accepted the offer of immunologist Paul Ehrlich (1908 Nobel laureate in Physiology or Medicine) to work as his private assistant in the State Institute for Serological Testing, at Berlin Steglitz. There, he used histological staining to investigate general biochemical questions. This was a field pioneered by Ehrlich, who introduced staining with synthetic dyes to the *in vivo* study of oxidation–reduction, and theories of dye structure to biological systems. Among other things, Michaelis introduced a special staining technique with the dye 'Janus green' for what later became known as mitochondria. Ehrlich, who was of the opinion that only men of sufficient wealth should stay permanently in basic research, accepted Michaelis on the condition that, after one year, he would study clinical medicine and become a physician. Thus, from 1900 to 1904, Michaelis studied clinical medicine at municipal hospitals in Berlin and at the Charité (the hospital of the Medical School in Berlin). In 1903, he became a *Privatdozent* and, in 1905, he was appointed an *außerplanmässiger*, or unpaid, professor at the University of Berlin. He never received an academic position in Germany.

Also in 1905, Michaelis accepted the post of bacteriologist at the hospital Am Urban, Berlin (today Vivantes Klinikum Am Urban, named after a river port), where, in addition to his salaried position, he worked in a very small laboratory that he built with his friend, the biochemist Peter Rona. Here, Michaelis carried out research on physical chemical problems of biochemistry, in particular proteins. It was in this laboratory that he conducted the research on enzyme kinetics that culminated in the mathematical derivation of the fundamental enzyme kinetic rate law and the affinity constant of the enzyme–substrate complex (see below).

Michaelis's chances of receiving an academic position deteriorated after he showed that the work of respected Professor Emil Abderhalden (his claim for the existence of specific defence enzymes, see below) did not stand up to scrutiny. In 1922, he was offered a post as visiting professor at the Aichi Prefectural Medical College in Japan. Being very discontented with his work situation in Germany, he immediately accepted this offer, despite his being closely attached to Berlin and its culture (personal communication to UD from Michaelis's granddaughter, Sylvia Cohn, 16 September 1996).

Since Michaelis had become an internationally renowned biochemist in about 1910, the fact that he never received a university professor's position in biochemistry (called physiological chemistry at the time) in Germany, and was not offered a position at a Kaiser Wilhelm Institute, requires explanation. It was in part due to the fact that there were only very few departments of physiological chemistry until the 1930s, in part due to academic anti-Semitism, and in a major part due to the nature of Michaelis's work. Several other outstanding Jewish biochemists also failed to become professors, even before Hitler came to power. Among them was Otto Meyerhof, who wrote in 1921, shortly before he received the Nobel Prize for Physiology or Medicine in 1922, to his colleague in the USA, Jacques Loeb, about the prejudicial treatment he had received from the faculty at the university in Kiel, which did not consider him for a professorship in physiological chemistry, because:

> I am a democrat and a Jew… in particular Michaelis and myself who, in inferior positions, are suffering the most from anti-Semitism and the faculty's conceitedness (*Fakultätsdünkel*).
>
> (Meyerhof to Loeb, 10 October 1921, Jacques Loeb Papers; translation by UD)

After he received the Nobel Prize, Meyerhof received an offer from Yale University, and another to head a department of physiology from the Kaiser Wilhelm Society (which he accepted). Michaelis did not receive such an offer and finally left Germany. In addition, the nature of his work (i.e. his focus on basic research and exact scientific experimentation and the preference of the modern physical chemical concepts of ions and pH over the then widespread concepts based on the so-called colloidal theory) contributed decisively to his failure to secure an academic position in Germany and also in other European countries [7]. His pioneering work in physical chemistry of proteins was not recognized in medical biochemistry in Germany. The colloidal theory, which, around 1900, became fashionable in the chemistry of cell constituents and processes, treated antibodies, enzymes, other proteins and DNA not as macromolecular entities, but as colloidal aggregates of a changing composition. Colloidal chemists considered structural organic chemistry unnecessary for the understanding of biological phenomena, such as the behaviour of proteins. Further details are provided elsewhere [8].

Explaining Michaelis's lack of recognition solely by saying that 'academic positions were scarce in post-World War I Germany' [9] is not sufficient, and the statement that he 'was honoured with an unpaid professorship' [9] certainly misses the point. Although it is true that there was a lack of academic positions, especially in biochemistry (physiological chemistry at the time) [10], openings did exist, although two of the best German biochemists, Meyerhof and Michaelis, both Jewish and liberal, never received a call from a university. In addition, it is important to ask why there were only so few university positions in biochemistry. This number increased drastically after Jewish scientists had been expelled in 1933. The fact that many (medical) biochemists were Jewish was most probably one of the reasons for the slow institutionalization of biochemistry at universities; non-Jews considered the large number of scientifically influential Jews an impediment to joining together [8].

The offer of a professorship in Japan was something quite extraordinary. Michaelis was the first foreign researcher to be invited to Japan. He was asked to be the founding director of the Department of Biochemistry at the Aichi Prefectural Medical College, which wished to become elevated from a college to a university department (it became later the Medical Department of Nagoya Imperial University). His colleagues in Nagoya appreciated him highly; he enjoyed his stay there and even learned Japanese [11]. The original contract for one year was extended to three years. As Nagatsu [11] has described, Michaelis lectured in many places throughout Japan, and had a major influence on the development of biochemistry there, and he created a thriving school of biochemistry in Nagoya. This was later chaired by Kunio Yagi, who was stimulated by Michaelis's work to crystallize the enzyme–substrate ('Michaelis') complex of D-amino acid oxidase [12]. In his own work in Nagoya, Michaelis profited from his expertise in the chemistry of ions and electrodes [13], and studied the permeability of membranes [14].

On a US lecture tour, which was organized by Jacques Loeb (who died, however, in 1924, shortly before Michaelis arrived), Michaelis sought out future positions in the USA. As a result, after he completed his stay in Japan in 1926, he was appointed lecturer at Johns Hopkins University, Baltimore, again with a contract of 3 years. Michaelis liked his stay in Baltimore as much as he had Nagoya. Undoubtedly, because he had been strongly integrated into the cultural life of Berlin, he would not have left Berlin had his work situation there been more rewarding. Quite unlike many other German immigrants in the USA, he had no problems with the American academic environment [10]. Thus, when he was asked by the President of Johns Hopkins University to find out whether Einstein, a friend of his, would accept a professorship in Baltimore in principle, he wrote to Einstein (on 25 January 1927):

> The scientific life and the personal acquaintance with colleagues are as agreeable as at all imaginable.
>
> (Michaelis to Einstein, 25 January 1927, Einstein Papers Project; translation by UD)

Einstein, however, preferred to stay in Berlin, and did not leave Germany until immediately after the Nazis came to power in 1933.

Michaelis's high scientific status and his reputation as a teacher at Johns Hopkins was such that other universities were interested in offering him posts, including the Rockefeller Institute for Medical Research in New York City, one of the leading institutions of experimental medicine at the time. Expert opinions on Michaelis, solicited from faculty at Johns Hopkins by Rockefeller Institute's director Simon Flexner[1], were unanimously favourable, stressing his great knowledge, readiness to cooperate and give advice to young coworkers, as well as his personal modesty:

> He is extremely modest and unassuming and I think one would be justified in saying that he has a brilliant

[1]Flexner had been an early collaborator of Maud Menten (as described in Part 2).

> mind. He has gone to no end of pains and trouble to assist the younger men in the clinic with their work and it is no exaggeration to say that many of these men not only regard him very highly but have become very fond of him. He would be perfectly happy, I am sure, if he had laboratory facilities for investigation, with a few younger men working with him. He apparently cares nothing for titles or position, and requires only sufficient income to live on comfortably.
>
> (Garfield Loncope to Simon Flexner, January 26 1929, Leonor Michaelis Papers)

Flexner offered Michaelis the position, which he accepted. In 1929, Michaelis was appointed a permanent member of the Rockefeller Institute and remained there for the rest of his life, continuing research also after he became member emeritus in 1941. In 1949, he died in New York, at the age of 74 years.

Michaelis's research on enzyme kinetics: the Michaelis–Menten constant

Shortly after physical chemists had developed a theory of matter in solution based on the new concept of ions, Michaelis and, independently, Søren Sørensen in Denmark [15] were the first to recognize the importance of the ion theory for the explanation of biological phenomena. They both conducted research on the influence of the hydrogen ion concentration on the properties of proteins and enzymes and expanded the theoretical basis of the concept. Michaelis formulated the theory of buffers (which he called 'hydrogen ion regulators') and proposed quantitative theories for the dissociation of amphoteric electrolytes and the isoelectric points of proteins; the latter in part in cooperation with Heinrich Davidsohn [16]. He introduced the method of electrophoresis under constant pH and, with this, determined the isoelectric points for a number of proteins, such as haemoglobin and serum globulin.

Michaelis's mathematical derivation, together with the Canadian researcher Maud Menten, who had just completed her MD, of the affinity constant of the enzyme–substrate complex in 1913, as scrutinized below, marked a turning point in existing work in enzyme kinetics [17]. Michaelis and Menten implemented methodological novelties and formulated their results with the greatest clarity and precision and in a generally applicable way, that transcended the experimental system that they used. By contrast to others, including Maud Menten, who turned to different fields of research after her return to the USA (see Part 2), Michaelis continued to develop the field.

Early research on enzyme kinetics

First studies on invertase

Scientific research on the kinetics of enzyme reactions traces back to the end of the 19th century. The idea to use invertase, which hydrolyses sucrose into glucose and fructose, as a model enzyme for studying enzyme kinetics dates back at least to 1890 [18]. At the beginning of the 20th century, several researchers were working on enzyme kinetics both experimentally and theoretically (most of them using invertase), mainly in France, England, Germany and the USA. Among them were Émile Duclaux at the Institut Pasteur in Paris, Victor Henri at the Sorbonne in Paris, Leonor Michaelis in Berlin, Max Bodenstein in Leipzig, Donald D. Van Slyke in New York, Adrian J. Brown in Birmingham and, with a slightly different topic and independently of the others, Archibald Vivian Hill in Cambridge. Before highlighting the achievements of Leonor Michaelis in that field, we first outline the preceding results that Michaelis was able to build on.

O'Sullivan and Tompson, in 1890, assumed that the time course of invertase action could be described by a simple mass action kinetics (called Harcourt's law at that time), which means that the reaction velocity, v, is proportional to the substrate concentration, S [18]. However, they expressed some doubts because they had noted some deviations. These doubts were expressed more clearly by Duclaux in 1899 [19], Henri in 1901 [20] and Brown in 1902 [21].

Victor Henri and Max Bodenstein

The important contributions of these individuals (thoroughly recognized by Michaelis and Menten) are covered in detail in Part 3, and so we discuss them only briefly here. Henri [22,23] took into account the formation of an enzyme–substrate complex, and an enzyme–product complex and the conservation sum of these complexes and the free enzyme, arriving at:

$$v = \frac{K\Phi S}{1 + mS + nP} \tag{1}$$

where we have deviated from Henri's notation in using the symbols S and P for the concentrations of substrate and product, respectively [22].[2] When Max Bodenstein examined Henri's results on invertase, he suggested that the action of the enzyme is inhibited by sucrose and invert sugar, so that the rate constant is

[2]Apart from these changes in notation, this is the same as Eqn (29), below.

divided by ($mS + nP$), a suggestion that led to an incomplete version of Eqn (1) lacking the constant 1 in the denominator [23] (see Part 3).

Leonor Michaelis

Michaelis's work on enzyme kinetics dates from at least 1907. He had analysed in detail the time course of the catalytic cleavage of polypeptides [24]. Today, his early results can be explained as outlined below. We know that, at large substrate concentrations, the reaction rate is nearly constant, $v = V_{max}$, because the enzyme is saturated. This leads to the differential equation

$$\frac{dS}{dt} = -V_{max} \tag{2}$$

Integration gives:

$$t = \frac{S(0) - S(t)}{V_{max}} \tag{3}$$

By contrast, at low substrate concentrations, the reaction rate follows mass-action kinetics, $v = kS$. This leads to the differential equation:

$$\frac{dS}{dt} = -kS \tag{4}$$

Integration gives:

$$t = \frac{1}{k}\ln\frac{S(0)}{S(t)} \tag{5}$$

Abderhalden and Michaelis, in 1907, noticed that the measured time course is somewhere in between the curves determined by Eqns (3) and (5) [24]. They therefore proposed to take a linear combination of the two. Thus, they came very near to the correct formula but did so via a detour because they considered the integrated form of the rate equation. It is of historical interest that Michaelis had published this paper [24] together with Emil Abderhalden, who was then a young protein researcher working under Emil Fischer at Berlin University but whose work Michaelis criticized for good reasons later (see the section below on the controversy of Michaelis and Abderhalden in 1914).

The famous paper *Die Kinetik der Invertinwirkung*

Michaelis's most famous paper (with Maud Menten in 1913) [25] was published in *Biochemische Zeitschrift* (the predecessor of the *FEBS Journal*) in 1913. They gave a detailed derivation of the equation:

$$v = \frac{V_{max}S}{K_m + S} \tag{6}$$

which is nowadays called the Michaelis–Menten equation. Using invertase as their model enzyme, they took into account (similar to Henri, following Emil Fischer's 1894 lock-and-key suggestion for the interaction of an enzyme and its substrate [26]) that an enzyme–substrate complex is formed. Moreover, they used the law of mass action for the formation of that complex and reasoned that only a minor fraction of the substrate is bound to enzyme, such that its free concentration is nearly equal to its total concentration.

Michaelis and Menten recognized the formal equivalence of Eqn (6) with the dissociation equation of a weak acid (called *Restdissoziationskurve* in their 1913 paper):

$$\rho = \frac{H^+}{K + H^+} \tag{7}$$

where $\rho = HAc/Ac_T$ is the fraction of nondissociated acid. This is a special case of the hyperbolic binding curve:

$$\rho = \frac{A}{K + A} \tag{8}$$

of the binding reaction $A + B \rightleftharpoons AB$, with ρ being the fraction of B bound. This equation results from the law of mass action $A \times B/AB = K$ and the conservation sum $B + AB = B_T$. In the special case of enzymes, A and B are the substrate and enzyme, respectively. Michaelis and Menten put their understanding of acid dissociation curves to use in their method of estimating the kinetic parameters of Eqn (6), which is illustrated in Fig. 3.

Returning to Eqn (1), we see that, in the special case $P = 0$, Eqn (6) is obtained. The term nP in Eqn (1) describes the partial saturation of the enzyme by product. Note that this may be relevant even in an irreversible overall reaction if the dissociation step of the product is reversible. On the other hand, Henri's derivation of the equation was very brief [22] and he did not analyse the problem in as much detail and depth as Michaelis and Menten.

Michaelis and Menten were aware of the phenomenon of product inhibition. In section 2 of their paper, they analysed the influence of the reaction products and also of other substances. In section 3, they derived a more complex equation than that of Henri, by considering that invertase (similar to many other cleaving enzymes) gives rise to two products: glucose and fructose. Written in modern notation, this reads as follows:

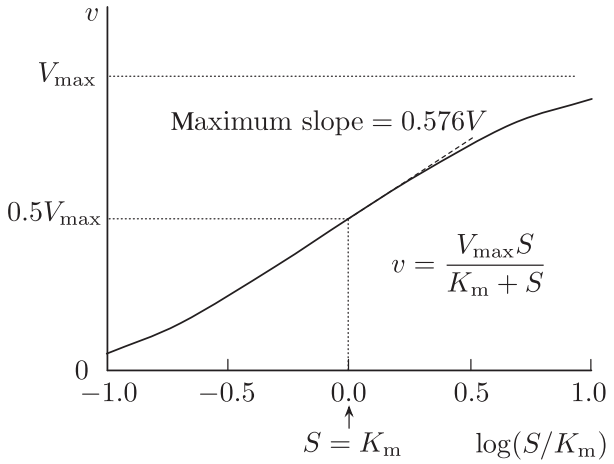

Fig. 3. Plot of v against log (S/K_m) as defined by Eqn (6). In plotting their data in this way, Michaelis and Menten were influenced by their deep knowledge of the effect of pH on the dissociation of a weak acid (Eqn 7). By contrast to what is widely believed, they did not plot v against S (Fig. 6, below) and were thus able to avoid the difficulty of estimating the location of the asymptote $v = V_{max}$ because, over a range of two logarithmic units (or less), the line is sufficiently straight to make it easy to estimate the maximum slope, which is $0.576V_{max}$ (i.e. 0.25 V_{max}·ln 10).

$$v = \frac{V_{max}S/K_s}{1 + S/K_s + P(1/K_{P1} + 1/K_{P2})} \quad (9)$$

where K_{P1} and K_{P2} denote the dissociation constants of the first and the second product, respectively, and P is the concentration of either product (assuming that they both start at zero, so that they are equal).

Michaelis and Menten cited and discussed Henri's equation [25]. They not only clearly stressed its usefulness, but also made it clear that it should be improved in two directions:

- Henri did not study the role of pH or even 'acidity'. He could not use pH because its effects on enzymes were only demonstrated after 1902, in particular by Sørensen in 1909 [15] and by Michaelis himself (e.g. with Davidsohn in 1911) [16]. Although the term pH did not exist in 1902, the concept of acidity certainly did, and had been used (clumsily) by O'Sullivan and Tompson [18].
- Henri did not consider the mutarotation (at that time called 'multirotation') of glucose. Mutarotation is the spontaneous (non-enzymatic) conversion of the ring-shaped α-D-glucose into an equilibrium mixture with its stereoisomer β-D-glucose.

Because mutarotation only concerns invertase and a few other enzymes, it is of minor importance for enzyme kinetics in general. Considering the pH is more generally important, notably for all enzymatic reactions producing or consuming protons because the activity of enzymes depends on pH.

As pointed out by many individuals [27], a very important achievement of Michaelis and Menten was to give a detailed derivation and interpretation of the constant K_m in Eqn (1). Although Henri correctly assumed that the formation of the enzyme–substrate and enzyme–product complexes proceeds according to the law of mass action, he did not recognize (or did not write about) the physico-chemical meaning of the constants K, Φ, m and n. Michaelis and Menten recognized that K_m can be considered as the dissociation constant of the enzyme–substrate complex.

Another important achievement that they made was the introduction of initial-rate measurements [17]. Because the term corresponding to the reaction product in Eqn (1) makes fitting of experimental data difficult, it represents a clever approach for measuring the initial reaction rate, when P is still zero. This was one motivation for deriving Eqn (6). Another advantage of that approach is to focus on the essential properties and to neglect all irrelevant details. As is always the case in mathematical modelling, it is important to make the description as complex as necessary and as simple as possible, and this goal was excellently achieved by Michaelis and Menten. This is also true with respect to notation; they used the symbol S in Eqn (2) for the time-dependent substrate concentration, whereas Henri used ($a-x$), where a represents the substrate concentration at time zero and x denotes what is now referred to as the 'extent of reaction' in physical chemistry.

Michaelis and Menten not only reduced the kinetic equation to a minimalist form describing the essential characteristics, but also extended the approach in many respects. For example, they derived the more general Eqn (9) and studied the impact of effectors on enzymes.

Notice that Henri's equation, Eqn (1), written as follows in present-day notation:

$$v = \frac{V_{max}S/K_s}{1 + S/K_S + P/K_P} \quad (10)$$

is the equation for competitive inhibition by a product, and becomes the ordinary equation for inhibition by any competitive inhibitor I if P/K_P is replaced by I/K_I. Henri essentially suggested this equation, even though with an unnecessary restriction on the coefficients in the denominator (see above). Michaelis and Menten made the excellent suggestion to measure the kinetics in the case of $P = 0$ (initial rate measurements), which simplifies Eqn (10) to Eqn (6). However, in many

situations *in vivo*, Eqn (10) is more appropriate, a consideration that is often overlooked in biochemistry, including large-scale kinetic models. Of course, Michaelis and Menten realized the importance of considering the influence of product(s) and, accordingly, derived Eqn (9).

Michaelis and Menten also considered inhibition by two products, Eqn (9), and derived an integrated equation that describes the whole time course, accounting for the effect of the increasing product concentrations, as discussed by Johnson and Goody [28].

Archibald Vivian Hill

In this context, the work of Archibald Vivian Hill in Cambridge is also worth mentioning. Three years before Michaelis and Menten's famous paper, he published a four-page paper in a Supplement of the *Journal of Physiology*, which included the proceedings of a meeting of the Physiological Society [29]. There, he proposed an equation of the following form as a way of representing a binding function approximately:

$$f = \frac{Kx^h}{1 + Kx^h} \tag{11}$$

in which f, x, and K denote the fractional saturation, oxygen tension, and an equilibrium constant, respectively, and h is a constant that is not required to be an integer.[3] This equation is now widely known as the Hill equation and is used to describe many cooperative phenomena [27,31,32]. It is more complex than the Michaelis–Menten equation because it allows higher exponents and, thus, cooperative behaviour (sigmoidal curves). Hill himself did not extend his results to enzyme catalysis; this was carried out later by others, using the term 'Hill equation'. Therefore, Hill kinetics were in fact formulated later than Michaelis–Menten kinetics. Strictly speaking, what Hill himself had done was to extend the dissociation equation, Eqn (7).

The quasi-steady-state approximation

To make the story of development of enzyme kinetics more complete, we also mention here the paper by Van Slyke and Cullen in 1914 [33]. Based on Henri's work, they derived a basic enzyme kinetic rate law similar to Eqn (6), apparently independently of Michaelis and Menten (but citing the earlier paper by Abderhalden and Michaelis [24]). Perhaps Van Slyke benefitted from his one-year visit in Emil Fischer's laboratory in 1911. In Van Slyke and Cullen's paper, the binding step was assumed to be irreversible, which is, however, rarely the case for enzymes because the substrate is usually bound by weak interactions such as electrostatic or hydrophobic forces. They could not therefore use the rapid-equilibrium approximation and used, implicitly, an approach that was later outlined more clearly by Briggs and Haldane in 1925 [34] (citing Van Slyke and Cullen [33]). This approach is the quasi-steady-state approximation originated by Bodenstein in 1913 [35], which implies that the concentration of the enzyme–substrate complex is nearly constant in time. As shown by Briggs and Haldane, this also works if the binding step is reversible. Because Briggs and Haldane did not cite Bodenstein, it is unclear whether they were aware of his method or rediscovered it.

Michaelis's enzyme kinetics 100 years later: the long-lasting effect of fruitful research

The terms 'Michaelis–Menten kinetics' and 'Michaelis constant' have found an enormous resonance in the biochemical community. All students in biochemistry, biology and medicine are taught the Michaelis–Menten kinetics. In French, the terms *cinétique michaélienne* and *enzyme michaélienne* are used. That is, Michaelis's name has given rise to a new adjective. Michaelis and Menten's seminal paper of 1913 was completely translated into English for the journal *Biochemistry* in 2011 [28], complementing Boyde's translation [36], which has recently been revised for the journal *FEBS Letters* [37]. In the 5 years from 2008 until the end of 2012 only, the original German paper received the impressive number of 398 citations in the Web of Knowledge using the Science Citation Index Expanded database.

Michaelis and Menten discussed enzyme kinetics in much more detail than their predecessors and gave a thorough and insightful interpretation to the binding constant, which now bears their names. Moreover, they defined an operating procedure for kinetic experiments so that widely useful information can be obtained from them. In particular, they were the first to make clear the relevance of controlling the pH and showed that initial rates are easier to analyse and interpret than time courses [17,27]. In this way, and by considering the mutarotation of glucose, they could test the kinetic equation experimentally,

[3]Hill (as well as many later authors) used n for the exponent. However, n fosters the misconception that it is the number of binding sites (also often symbolized as n), and the International Union of Biochemistry and Molecular Biology [30] recommends h or n_H.

whereas Henri could not do so satisfactorily [28]. The experiments by Michaelis and Menten were performed with high precision and with consideration of many effects (such as the influence of competing substrates), which is comparable to modern standards of experimentation.

The enormous scientific resonance outlined above contrasts with the unexpectedly weak reception of Michaelis's achievements in Germany and even in Berlin, the city where he developed the kinetics in question. Michaelis's name appeared in a few reviews and in a short section in a footnote of a study on the expulsion of Jewish scientists from Berlin in 1994 [38]. By renaming the *Abderhalden Haus* as the *Leonor Michaelis Haus*, the reception of Michaelis in Berlin has increased significantly. When one of the present authors (StS), studied at Humboldt University in Berlin from 1981–1986 and worked as a PhD student and postdoctoral fellow there afterwards for several years (teaching, among other places, in the building now carrying Michaelis's name), he never heard that Michaelis had worked on enzyme kinetics in Berlin. Several colleagues working in Berlin in the 1980s have recently confirmed this observation. By contrast, many other names such as Walther Nernst, Emil Fischer, Otto Warburg and, of course, Einstein and Planck, had been mentioned and praised as celebrities of Berlin science. For example, there is a lecture hall named after Emil Fischer in the chemistry building in Hessische Straße (not far from the current Leonor Michaelis Haus).

At the same building, a plaque recalls the theoretical physical and radiochemical contributions made to nuclear fission by the physicists Lise Meitner and Otto Robert Frisch, and the chemists Otto Hahn and Fritz Strassmann (the radiochemical work was carried out at the Kaiser Wilhelm Institute for Chemistry at Dahlem). Meitner was forced to leave Germany in 1938 because she was Jewish. Despite her crucial role in nuclear physics, she too was rarely remembered in Germany and her work was ignored for many decades after 1945. The fact that she was a woman also contributed to her lack of recognition. If she was remembered, it was as a 'co-worker' of Otto Hahn, not as his equal partner, who had for that matter initiated the research that led to nuclear fission. Only from the 1980s have historians, in particular Ruth Lewin Sime [39], examined her life and work more fully, with the result that Meitner is increasingly recognized in Germany and elsewhere for her pioneering work in nuclear physics and her essential role in the discovery of nuclear fission.

The low credit extended to Michaelis in Germany until today may be because:

1 Michaelis did not have a full professorship or an equivalent academic position and his work was not appreciated by his peers in Germany. Other people had better connections and networking (such as Abderhalden) in the German academic system.
2 Michaelis did not receive a Nobel Prize. Although not always justifiable in comparison to other excellent researchers who did not receive it, this Prize increases the reputation of a researcher enormously.
3 Michaelis was Jewish. Although academic anti-Semitism in Germany in the 1920s was not as virulent as in the 1930s, it already prevented several outstanding Jewish scientists from making academic careers (see the section on Leonor Michaelis in Berlin above). During the Nazi era, Michaelis's entry as *außerplanmässiger* (unpaid) professor in the calendar of Berlin University was deleted. Many outstanding Jewish émigré scientists, with a few exceptions such as Einstein and, much later, Lise Meitner, were, regrettably, largely forgotten in Germany after 1945. From the 1990s, several studies examined the impact of the forced emigration of Jewish scientists from Nazi Germany but Michaelis was not a refugee *sensu stricto*. Although his name and work were mentioned a couple of times [38], the 1998 article by U. Deichmann and B. Müller-Hill in *Nature* [40] was the first widely circulated reminder of his life and the outstanding work conducted in Berlin.

The controversy of Michaelis and Abderhalden in 1914 and the renaming of the Abderhalden Haus of Humboldt University as the Michaelis Haus in 2011

Michaelis's emphasis on exact experimentation brought him into opposition with the highly speculative, inexact research prevalent in some areas of medical biochemistry, as he noted in a letter to Loeb:

> People such as your M. H. Fischer[4] are in high esteem here, too. I consider Abderhalden one of them, even though he cannot be denied great organizational talent. But I detest his way of working (*Arbeitsweise*). My position in Germany has suffered because of my opinion against his pregnancy test. Even though there are already many who see through him, nobody dares to say anything against him. [7]

[4]An American colloid scientist of German origin who promised spectacular medical applications but whose work was highly questionable.

Emil Abderhalden was full professor of physiology and physiological chemistry at the University of Halle from 1911. He was President of the oldest German Academy of Science, the Leopoldina, from 1932 to 1950. Similar to Michaelis, he focussed his research on the biochemistry of proteins and enzymes. Unlike Michaelis's research, however, Abderhalden's research was highly questionable, and, in regard to what he considered his major discovery, untenable [26,40,41]. Michaelis was the first to question it. He did so in a paper in which he refuted Abderhalden's claim of the existence of 'defence enzymes' specific for foreign protein in the blood; in this case, specific for pregnancy (with Lagermarck in 1914) [42]. Abderhalden considered his alleged discovery of defence enzymes as his major scientific achievement. Michaelis showed that it was based on experiments that turned out not to be reproducible when exact methods were applied. His published refutation, which followed a request by the director of the hospital where he ran the laboratory to test the validity of Abderhalden's alleged pregnancy test, provides valuable insights into Michaelis's critical approach and methodological mastery. His conclusions were clear-cut:

> In spite of 'pedantically following Abderhalden's instructions' and spending some time at Abderhalden's laboratory in order to study the method in greater detail, we cannot confirm that a serum of pregnant women behaves differently in any recognisable, regular, practical usable way from sera of non-pregnant women or men. [42, p. 316; translation by UD]

Michaelis criticized the inadequacy and imprecision of Abderhalden's methods, such as his colour methods (biuret and ninhydrin) for the detection of specific peptides after the alleged protein degradation through defence enzymes:

> It is simply incomprehensible to us how [Abderhalden] could possibly have received apparently very unambiguous results with such an imprecise method. [42, p. 317; translation by UD]

As for Abderhalden's blaming the imperfection of some technical devices [the tubes for dialysis (*Dialysierhülsen*)] for incorrect results, Michaelis and his colleague observed wryly on 'the psychological influence of the investigator who regards the uselessness of the tubes to be a comfortable explanation of the wrong result'. After testing the tubes using a more appropriate method: 'The tubes are absolutely impermeable for protein, and if there may perhaps be occasionally bad and permeable ones, the Abderhalden method of testing is not appropriate to reveal this in the right way' [42, p. 318].

The sharpness with which Michaelis not only rejected Abderhalden's claims, but also pointed to the dubiousness of his approach in general, contrasted markedly with the deference usually displayed to German professors at the time. In Germany, the hierarchical university system and the great institutional power of a professor contributed to the fact that at least public criticism was very rare. Members of the elites, usually professors, too, would not critically examine the work of colleagues. Therefore, Michaelis's paper was a rare exception (and the editors of the *Deutsche Medizinische Wochenschrift* considered it necessary to add words of justification to its publication).

As shown elsewhere, Michaelis's results were confirmed within the next couple of years in the USA by, amongst others, the above-mentioned Donald Van Slyke at the Rockefeller Institute in New York. As a result, research on defence enzymes was essentially stopped outside Germany. Michaelis thus contributed decisively to the internationally widespread calling into question of defence-enzyme research. In Germany, too, this research decreased after 1914. However, because Michaelis lacked institutional authority, Abderhalden could go on with defence-enzyme research undisturbed for decades (it especially flourished during the Nazi era), extending its scope to a vast area of medical applications, such as the diagnosis of infectious diseases, cancer and race; details on defence-enzyme research are also provided elsewhere [10,40,43,44]. Only from the 1960s did defence enzymes disappear from German textbooks. However, there was no clarifying 'obituary' on defence enzymes before Benno Müller-Hill and one of the present authors (UD) analysed the background to this case of scientific misconduct in 'The fraud of Abderhalden's enzymes' [40]. In this paper, Abderhalden's self-betrayal and, finally, fraud was characterized as an example of a science that had developed into a pseudoscience. Clarification of the meaning of the term 'scientific fraud' is also provided elsewhere [44].

Müller-Hill and Deichmann received many letters in agreement with them, most of them from biochemists who were well acquainted with Abderhalden or his research. To quote from a letter by Peter Karlson, then Professor of Biochemistry at the University of Marburg, to UD:

> … It is honourable that you and B. Müller-Hill now carried them [the defence enzymes] to their grave. I still experienced the end of their flourishing period as well as the controversies. Many biochem-

> ists were still of the opinion that 'something about it must be true' (*irgendetwas dran sein müßte*) and yet everything was nothing but mass suggestion.
>
> (P. Karlson to U. Deichmann, June 1998; translation by UD)

The decision of Humboldt University to rededicate the *Abderhalden Haus* to Leonor Michaelis emphasizes the international orientation of its research in the life sciences.

Michaelis's research on biological redox reactions

The topic of biological redox reactions became a major focus of Michaelis's research after his emigration to the USA, where he examined in particular the role of heavy metals as catalysts and semiquinone radicals in reversible oxidations. Reversible oxidations are of crucial importance for the energy-providing reactions in the cell, in which cytochromes (i.e. iron-containing porphyrin proteins with the ability to transfer electrons) play a major role. Their rediscovery, in 1925, by David Keilin, and Otto Warburg's discovery and characterization three years later of what he called 'oxygen transferring ferment' (today known as cytochrome oxidase, or cytochrome a_3), a major enzyme of the respiratory chain in mitochondria, revealed the first oxidation–reduction systems through which foodstuff molecules might transfer electrons to molecular oxygen to provide energy in the form of ATP.

Michaelis made a decisive contribution to the elucidation of the mechanism of this electron transfer. His discovery [45] (simultaneous with that of the Dutch biochemist Elema [46]) of the two-step reduction of pyocyanine in acid solutions was the starting point for Michaelis to develop his principle of the intermediary formation of free radicals during oxidation of systems that require transfer of two electrons. Based on his research with model systems, such as the synthetic dye methylene blue, which he had studied with Paul Ehrlich in Berlin, he postulated that the oxidation–reduction process takes place in two successive steps, each characterized by a particular level of the oxidation–reduction potential, and that each step involved the detachment or acceptance of one electron. The intermediary compound thus had the constitution of a chemical radical [47].

Michaelis's theory of a free radical, stable in aqueous solutions (a semiquinone) as intermediate was initially rejected by nearly all prominent chemists. Michaelis recalled: 'The existence of such free radicals, existing in equilibrium with their "parent substances," even in aqueous solution, appeared to be unbelievable to most organic chemists' [3]. His first paper on this subject was rejected by American journals; one reviewer went so far as to declare, 'a principle of modern scientific philosophy [was] violated'. For Michaelis, it was a 'depressing experience'. He began to examine 'with his usual perseverance' a large number of oxidation–reduction systems [48]. To answer charges of lack of conclusive evidence, he began to study quantum mechanics and magneto-chemistry, an unusual step for an accomplished biochemist.

Through extended correspondence with Linus Pauling, Michaelis discussed his ideas about the stability of organic radicals and the experiments he devised to tackle the questions. From 1939, he also drew on Pauling's book *The Nature of the Chemical Bond* (1939) [49]. Although Pauling did not agree with every detail of Michaelis's interpretations, he appreciated his theories and experimental approach (Pauling Papers, Oregon State University, Corvallis).

Michaelis's demonstration of the paramagnetism of the compounds under study left no doubt about their nature as free radicals, finally convincing most organic chemists. Michaelis's conclusions were later confirmed by others [50] and became generally accepted. This research led to the formulation of the novel concept of a compulsory two-step monovalent oxidation; in other words, the loss of one electron at a time, with the intermediate formation of a semiquinone (now an established concept if biochemistry), rather than the simultaneous loss of two electrons. This finding of the two-step oxidation profoundly influenced the concept of the nature of intracellular respiration. It provided a clue to the mechanism whereby the oxidation of a metabolite system with two electrons is linked to one-electron haem–protein systems, such as cytochrome a_3 and molecular oxygen.

In 1939, Sam Granick joined Michaelis's laboratory as a postdoctoral fellow to study iron metabolism. Between 1942 and 1946, he and Michaelis published a series of papers on ferritin and ferric compounds in the *Journal of Biological Chemistry*. Contradicting previous assumptions about the structure and composition of this protein, the series to which it belonged forms the basis of the current knowledge of ferritin [51].

Michaelis also used his thermodynamic equations of semiquinone formation to conduct further research on dyes and other molecules, as well as a renewed examination of the metachromic effect (the ability of a dye to produce different colours with various histological or cytological structures), a topic that had already interested him as Ehrlich's student [52].

One example of Michaelis's research that does not readily fit in with the present review, but which has had considerable commercial consequences, was his discovery that keratin can be dissolved in thioglycolic acid: this reduces the disulphide bridges in the protein molecule to free sulphide groups, although it has no other chemical effects [53]. From the 1930s onwards, this formed the basis of methods of producing a 'permanent wave' in hairdressing without the use of machines or heating.

Conclusions and outlook

This special issue commemorates the centenary of Leonor Michaelis's ground-breaking work on enzyme kinetics, which culminated in his famous publication with Maud Menten in *Biochemische Zeitschrift* during 1913 [25]. It is one of only a few cases in the history of modern science in which the implications of a publication have remained fertile for such a long time; it has been cited ever since. Moreover, Michaelis published seminal work in a number of different fields, such as the physical chemistry of proteins, redox reactions, and the characterization of iron-transferring proteins. At the close of this essay we therefore raise the question: What caused this unusual scientific success?

The following traits of his personality and the influences of his working environments appear pertinent:

Personal characteristics. Here, Michaelis's deep and also broad knowledge stands out. Well-versed in fields as different as biology, biochemistry, physical and organic chemistry, as well as mathematics, he could apply exact scientific methods to research in areas of biology and biochemistry at a time when this was still exceptional. As one of the present authors (UD) explains elsewhere [7,8,10], Michaelis shared this characteristic with several of his contemporary German-Jewish biochemists, who similarly became internationally outstanding scientists, such as Otto Meyerhof, Fritz Lipmann and Rudolf Schoenheimer. After their forced emigration from Nazi Germany, these scientists contributed decisively to the development of biochemistry into a highly respected science in the USA, which, as a consequence, replaced Germany as the scientific centre of biochemistry and related fields [52,54].

In addition to his broad capabilities, Michaelis had at his disposal an unusual determination and clarity of thought, as indicated in the following assessments, which also emphasized his supportive attitude to students and young colleagues: according to Sam Granick, mentioned above, and Duncan A. Macinnes:

> Leonor Michaelis was certainly the most influential scientist, during the past half century, in the introduction of the methods of physical chemistry into biology and medicine… Michaelis could have made substantial contributions to nearly any branch of human endeavor that he chose to enter. And in doing so he would have brushed away obstacles that would have completely discouraged a man of ordinary capabilities. Not always tactful with his colleagues, he was invariably patient and kindly in his dealings with younger workers. [3, pp. 282, 291]

Guzman Barron emphasized Michaelis's ability to perceive immediately the important points in any discussion, as well as his wide knowledge in many fields, and concluded: 'There was more than genius in the mind of Professor Michaelis. Joint to his gift was another: the astounding clarity of his thoughts, so that the most abstruse and intricate subjects, once passed through his mind, were expressed with brilliant simplicity and precision'. [48, p. 1]

A critical attitude, which included self-criticism, has to be emphasized as another outstanding characteristic. This led him, for example, to realize that organic chemistry alone was not sufficient to explain many of the phenomena in biochemistry, such as the behaviour of proteins, enzymes and antibodies. It also prompted him to perceive quickly that some of the far-reaching promises of new approaches, in particular of colloidal chemistry, were completely untenable.

Scientific influences and work conditions. Michaelis received his medical and scientific education during a time when Germany was emerging as the international leader in scientific fields, such as chemistry and physics. Scientific colloquia at the University of Berlin and various Kaiser Wilhelm Institutes set high standards, and included among Michaelis's teachers were internationally renowned German scientists, such as Emil Fischer and Paul Ehrlich.

These beneficial conditions contrasted markedly with Michaelis's poor work conditions in a city hospital in Berlin. Remarkably, he reached his greatest scientific success through research alone at his self-built small laboratory without students and assistants. At first sight, this appears to confirm the idea that scientific creativity can be enhanced by academic marginalization [55]. However, we would argue that, on the contrary, Michaelis's internationally acknowledged research would have lost its marginal status in Germany had he been given an academic position. We conclude therefore that he was successful despite and not because of the adverse working conditions to which he was confined. His success can be attributed

to his capabilities and connectedness to leading scientists in his own field. For example, he had close contacts to Emil Fischer's and Paul Ehrlich's institutes. He was well aware of the most modern scientific developments in his field and beyond. In the USA, he worked under excellent conditions, which enabled him to continue his pioneering and prolific research.

There is a widespread assumption that major discoveries usually take place without precursors but, in reality, this is only rarely the case. Very often, as for example in the cases of Newton and von Laue, it was the combining and further developing of already existing concepts and developments in such a way that they were cast in a 'concrete and binding form' that strongly changed a scientific field [56]. This scenario can often be seen in the history of the life sciences: Darwin, Koch, Morgan, and Watson and Crick all had prominent precursors [57].

There are many other examples of scientific fields that were initiated by early pioneers and were thoroughly and profoundly established only some years later. For example, metabolic control analysis (MCA) was founded in 1973 by Heinrich and Rapoport [58] in Berlin and, simultaneously, by Kacser and Burns [59] in Edinburgh. However, earlier ideas in that direction had been proposed approximately a decade earlier by Higgins [60]. Although he certainly was an early pioneer in that field, it is justified to consider Heinrich and Rapoport as well as Kacser and Burns as the founders of MCA because they provided a firm mathematical basis, a thorough biochemical interpretation and manifold examples of application. Thereafter, it took approximately another decade until MCA was widely applied in biochemistry [31].

A similar development can be observed in enzyme kinetics. Using the lock-and-key concept of enzyme–substrate interaction suggested by Emil Fischer, there were several pioneers in enzyme kinetics, with Victor Henri being outstanding among them. Combining biochemistry and mathematics, experimental skills and theoretical skills, their approach was interdisciplinary, thereby anticipating recent research in molecular modelling and systems biology. On the basis of earlier work, and in part following research he pioneered, it was Leonor Michaelis, approximately 15 years after the publication of the first ideas on enzyme–substrate molecular interaction, who became founder of enzyme kinetics as a coherent and well-established field. For this reason, one of the present authors (UD) strongly disagrees with the conclusion of Part 3, in which Henri is considered as founder of enzyme kinetics. Michaelis was able, more than the other pioneers, to combine the theories and practices in the physical chemistry of proteins and enzyme kinetics, and to place them into a 'concrete and binding form'.

To summarize, Michaelis's personal characteristics, an unusual determination, passion and a critical attitude, together with his broad capabilities and knowledge (including in biology, chemistry, biochemistry and mathematics) played a decisive role in shaping the direction of certain areas of biochemical research in the first half of the 20th century.

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Part 2: Maud Leonora Menten (1879–1960): her career as an experimental pathologist

by Athel Cornish-Bowden

Summary

As well as her well known work in the development of enzyme kinetics, Maud Menten carried out research in other subjects, both before and after her time in Berlin, and retired from a major university as a full professor. However, she was mainly an experimental pathologist and, in consequence, her full career is not very well known to biochemists. She made a number of distinguished contributions that are worthy of being remembered, in relation to the hyperglycaemic effects of *Salmonella* toxins, the electrophoretic mobility and sedimentation of different forms of haemoglobin, and a method for the histochemical detecton of alkaline phosphatase.

Introduction

Maud Menten's participation in the development of the Michaelis–Menten equation [25] is so well known that it hardly needs a detailed description here. Suffice it to say that when Johnson and Goody [28] applied modern methods of data analysis to her experimental results they found that they could reproduce her conclusions.[5] It is probably correct to write *her* results here because, although one cannot know exactly who did what in Michaelis's laboratory a century ago, he was involved in so many different projects, with 94 publications in the 5 years before the First World War [17], that it is difficult to believe that he carried out many of the experiments himself.

Biochemists tend to think of Menten's career as beginning (and perhaps ending) in 1913 but, as may be seen in Table 1, this is very far from being true. When she went for her research period with Michaelis in Berlin, sandwiching it between two fellowships at Western Reserve University, Cleveland,[6] she was about 33 years old and was already an established researcher, with a doctorate in medicine: she had published work on the distribution of chloride and potassium ions in nerve cells [61,62], and she had been co-author of a book on tumours in animals [63]. After her time in Germany, she had a long and distinguished career at the University of Pittsburgh, from which she retired as Full Professor in 1950. She also continued her research after her retirement, at the Medical Research Institute of British Columbia in Vancouver. Her work as an independent researcher is little known to biochemists because, although much of it touched on biochemical questions, her primary contributions concerned experimental pathology. This independent research is the focus of the present review, which takes some of its information from her obituary in *Nature* [64],[7] some from an account of her career by Gjedde [65] and some from a popular account of her life and career in the magazine of the University of Pittsburgh Medical School [66]. It deals in particular with her work on *Salmonella* toxins [67], on her ground-breaking work on sedimentation and electrophoretic mobility of different haemoglobins [68], and on her development of a method for detecting alkaline phosphatase in the kidney [69].

Menten's early career

Maud Menten, pictured as a young woman in Fig. 4, studied at the University of Toronto, where the Ontario Heritage Foundation erected the plaque in her honour shown in Fig. 5 in heavy rain during the International Congress of Biochemistry in Toronto in 1979.[8] She obtained her BA from Toronto in 1904, and worked as a demonstrator in physiology in the laboratory of Archibald Macallum; her first publication [61] arose from this time. Subsequently, she was a Research Fellow at the Rockefeller Institute for Medical Research, where she worked with Simon Flexner and J. W. Jobling, with sufficient success to become co-author with them on the first monograph from the Institute, on the subject of radiobromide and cancer [63]. She returned to Toronto, where she obtained her MD in 1911. Both before and after her work in Germany, she was a Research Fellow at Western Reserve

[5]In a recent article [17], I incorrectly stated that they repeated her experiments.

[6]When I read her career notes in *American Men of Science* (*sic*) many years ago, which were presumably prepared by her, I was puzzled that they listed the two fellowships in Cleveland consecutively, with no mention of what happened in between. The most plausible explanation is that she went to Germany at her own expense and had no formal status in Berlin. Michaelis himself was an *außerplanmässiger*, or unpaid, professor at the University of Berlin (see Part 1). He lived from his hospital work, and, having no paid academic position at that time, he would not have been in a situation to offer one to a visitor from the USA.

[7]I cited this obituary incorrectly in another recent article [17].

[8]I attended the Congress but, unfortunately, I forgot about the ceremony in Menten's honour, walking past it just as it was ending, and it was too wet to stop to see what was happening.

Table 1. Principal steps in Menten's life and career.

Dates	Stage	Location	Research
1879	Birth	Port Lambton, Ontario, Canada	
1904	BA	University of Toronto	
1905–1906	Assistant-demonstrator	University of Toronto	Ions in cells
1907–1909	Scholarship in pathology	Rockefeller Institute, New York	Animal tumours
1910–1912	Research fellow	Western Reserve University, Cleveland	Hydrogen ions in blood
1911	MD	University of Toronto	
1912–1913	Visiting scientist	Hospital Am Urban, Berlin	Kinetics of invertase
1913–1914	Research fellow	Western Reserve University, Cleveland	Hydrogen ions in blood
1916	PhD	University of Chicago	Effects of adrenalin on haemoglobin
1918–1950	Instructor…Professor	University of Pittsburgh	Various: see text
1950–1954	Retirement	Medical Research Institute of British Columbia, Vancouver	Cancer
1960	Death	Leamington, Ontario, Canada	

University, where she worked with George Crile. This collaboration led to a study of the hydrogen-ion concentration in blood [70] in which the influence of Michaelis's work on the hydrogen-ion concentration [71] is clearly visible. Subsequently she obtained a PhD in biochemistry from the University of Chicago, and so, during her time in Berlin, she was an MD but not a PhD. There is little doubt that her interest in working with Michaelis, and in obtaining a PhD in biochemistry, was motivated by a belief that a thorough knowledge of biochemistry would be essential for progress in the medical research that constituted her long-term objective. The fact that she interrupted her work at Cleveland to go to Berlin certainly suggests that she regarded it as important to understand the new concepts of pH and buffers, and where better to learn these than in the laboratory of the scientist who was rapidly becoming the leader of the field?

All of Menten's early collaborators were scientists of great distinction, and all of them are remembered in their different fields today. Archibald Macallum (1858–1934) founded the National Research Council of Canada, he played a major role in the development of Medical School of Toronto, and, at various times, held professorships of physiology and of biochemistry at Toronto, and later at McGill. Simon Flexner (1863–1946) was the first director of the Rockefeller Institute for Medical Research,[9] after previously being Professor of Experimental Pathology at the University of Pennsylvania. George Crile (1864–1943), a distinguished surgeon and founder of the Cleveland Clinic, is credited as being the first to carry out a successful blood transfusion; the Lunar Crater Crile was named in his honour, as was a 'Liberty ship' of the US Navy during the Second World War. Leonor Michaelis (1875–1949) is, of course, too well known to biochemists to need further description. The fact that four such distinguished scientists accepted Maud Menten into their laboratories surely indicates that she showed great promise from the outset, and, moreover, that she set out to work in the best laboratories of her time. According to Skloot [66], however, she was not impressed by previous achievements, and 'If a Nobel laureate was men-

Fig. 4. Maud Menten as a young woman (photograph kindly supplied by Mr John R. Barberie).

[9]It was Flexner who, as Director of the Rockefeller Institute, appointed Michaelis to the position that he occupied from 1929 until the end of his life (see Part 1).

Fig. 5. Plaque in front of the Medical Sciences Building of the University of Toronto. The text reads: 'MAUD LEONORA MENTEN 1879–1960. An outstanding medical scientist, Maud Menten was born in Port Lambton. She graduated in medicine from the University of Toronto in 1907, and 4 years later became one of the first Canadian women to receive a medical doctorate. In 1913, in Germany, collaboration with Leonor Michaelis on the behaviour of enzymes resulted in the Michaelis–Menten equation, a basic biochemical concept which brought them international recognition. Menten continued her brilliant career as a pathologist at the University of Pittsburgh from 1918, publishing extensively on medical and biochemical subjects. Her many achievements included important co-discoveries relating to blood sugar, haemoglobin, and kidney functions. Between 1951 and 1954 she conducted cancer research in British Columbia and returned to Ontario six years before she died. Erected by the Ontario Heritage Foundation, Ministry of Culture and Research'.

tioned, Menten was likely to ask, "What has he done since?"'

Research as an independent investigator

Salmonella toxins

Menten and Manning observed that guinea pigs and rabbits with *Salmonella* and other bacterial infections showed hyperglycaemia, and their study of paratyphoid in rabbits [67] was motivated by a desire to understand the relationship between the hyperglycaemia and the endotoxins produced by the bacteria. Initially, they worked with guinea pigs, although they moved to rabbits to have access to animals that could supply sufficient blood without incurring injury. The essential conclusion, amply confirmed by later work [72], was that bacterial endotoxins cause a depletion of glycogen reserves.

Sedimentation and electrophoretic mobility of haemoglobin

This study [68] on sedimentation and electrophoretic mobility was potentially the most important of Menten's career but had the misfortune not only of being published in wartime, but also of being eclipsed by work based on the same ideas, published in a very high-profile paper by one of the world's most prominent groups [73] some few years later. The result is that the latter work has now been cited more than 1250 times, and is regarded as a major advance in our understanding of protein structure, whereas that of Menten and her collaborators is almost forgotten, with fewer than 60 citations in total. Understanding sickle cell disease clearly caught the imagination more than understanding the difference between foetal and adult haemoglobin, although the oblivion is hardly justified.

Jope and O'Brien [74] reported in a symposium that unpublished experiments of H. Hoch did 'not confirm the results of M. A. Andersch, D. A. Wilson and M. L. Menten although their buffer conditions [were] reproduced exactly'. However, later publications by Hoch [75,76] referred to the earlier work with no mention of any difficulties in reproducing it, and so Jope and O'Brien's report must be regarded as hearsay. In any case, there can be no doubt that Menten and her collaborators conceived the possibility of using sedimentation and electrophoresis to distinguish between haemoglobin variants well before the work on sickle cell disease. They started from the observation, already well known at the time, that the haemoglobin of newborn babies is different from that of adults, and they argued that the different 'hemoglobins might be differentiated by electrophoretic mobilities, if the variation exists in the protein part of the molecule'. They first made measurements on oxyhaemoglobin but replaced this with carboxyhaemoglobin to take advantage of its greater stability. They concluded that at least two molecular species must be present, in proportions markedly different in adult and foetal haemoglobin.

Histochemical detection of alkaline phosphatase in the kidney

Alkaline phosphatase catalyses the hydrolysis of many different organic phosphates, such as glycerol phosphate, releasing inorganic phosphate. It is widely distributed in different animal tissues, and its importance as a histochemical marker led Menten and her collabo-

rators [69] to develop an azo dye method for the detection of alkaline phosphatase in the kidney, based on detection of the alcoholic product of hydrolysis of the phosphate ester used as the substrate. Pearse [77] subsequently wrote in a major textbook of histochemistry that 'it is not too much to say that the use of this principle was a stroke of genius' because it opened up the field of enzyme histochemistry. The technique used previously for detecting the inorganic phosphate produced by hydrolysis of glycerol phosphate required precipitation as calcium phosphate, followed by conversion to silver phosphate, which could be rendered visible by blackening in sunlight. This was effective but it was less than ideally specific, and there were doubts about whether the test medium was sufficiently alkaline to render residual silver chloride soluble, so that it could be washed away. The azo dye method introduced a different approach: instead of using silver to detect the phosphate form in the enzyme-catalysed reaction, it used a different substrate for which the organic product could be made visible as an azo dye. Tests of various possible substrates showed that the phosphate ester of β-naphthol gave the best results, both because of its insolubility and on account of the deep red colour of the diazotized α-naphthylamine.

Other research at the University of Pittsburgh

Menten's research covered such a wide field (albeit always of a medical nature, apart from her most famous paper [25]) that it is hardly possible to describe all of it in a short account of her career. The topics mentioned already can be regarded as the most important, although there were others of note, such as her work on the nature of vitamin C deficiency, and, in particular, the harmful effects that occur well before scurvy appears [78,79] as well as her investigation of the histochemical distribution of glycogen in kidneys [80–82].

Cancer research after retirement

Menten retired in 1950 as Full Professor of the University of Pittsburgh, and moved to British Columbia. However, her research did not end then because she returned to one of her earliest interests, cancer research [63], at the British Columbia Medical Research Institute in Vancouver. There, she studied the nucleic acid content of the bone marrow of leukaemia patients [83], using normal and leukaemic mice as an animal model [84].

Maud Leonora Menten's name

Maud Menten's middle name, Leonora, has sometimes led to speculation about whether its similarity to the given name of Leonor Michaelis was more than a coincidence. For example, after examining records at the University of Toronto in which she is listed simply as 'Maud Menten', the distinguished kineticist Keith Laidler suggested in a letter to me (now lost, unfortunately) that she might have added Leonora as a tribute to Michaelis. However, Mr John R. Barberie, her great-nephew, has found more than one Leonora in records of earlier generations of her mother's family (personal communication). This does not completely exclude Laidler's hypothesis but it renders it less plausible. A more definite result comes from examination of her first publication [61], which shows that the 'L.' was already present in 1905, long before there was any question of collaboration with Michaelis. Unfortunately, however, this conclusion is less clear than it appears because Mr Barberie has very recently obtained a copy of the birth certificate, in which there is no 'L' and no Leonora.

All three components of her name are frequently misspelt. It is given correctly on the plaque shown in Fig. 5, but there are articles that give her first name as Maude, her second as Lenora or Lenore, and her last, most often, as Menton. There are many web pages, and even some textbooks, that refer to something called the 'Michaelis–Menton equation'; a major textbook [85] has it correct in the text but incorrect in the index.

Acknowledgements

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Part 3: before Michaelis and Menten: Victor Henri's equation

by Jean-Pierre Mazat

Summary

The long road that led Victor Henri in 1902–1903 to the fundamental equation of enzyme kinetics is described, focussing on the rigour and the originality of his thought processes, despite the fact that his experimental work is open to criticism. Furthermore, he approached research in full awareness of how science has to be conducted, with concepts which anticipate those of Karl Popper later.

Introduction

This part of the review presents the beginning of the history of enzyme kinetics, taking the opportunity to emphasize the usefulness of describing such a history.

First, it illuminates the whole history of the sciences and, in particular, the idea of a scientific model, to the extent that it contrasts two attitudes to the experimental facts: one that only seeks a descriptive (phenomenological) model, and one that sets out from a theory (or, more modestly, from a series of hypotheses) to produce a model that can explain the facts and, finally, to test the model. Moreover, this history illustrates the whole history of biology, as we find in it, concentrated in a short period of approximately 30 years, all the philosophical ideas that have traversed biology, in particular vitalism. This history also illustrates well the relationship of biochemistry with chemistry and, in particular, with kinetics and chemical catalysis.

I would also like to show that the fundamental equation of enzyme kinetics, most often called the Michaelis–Menten equation, was actually published 10 years earlier by Victor Henri: this appears to be a great injustice, not for having forgotten Henri's name but for remaining ignorant of the rigour of his work and the spirit with which he treated enzyme kinetics for the first time.

Finally, from a pedagogical point of view, enzyme kinetics is often poorly understood, and evoking the difficulties that were encountered historically at the same time as establishing the fundamental equation of kinetics can help our understanding of it today. After all, establishing this simple equation was not so easy and took able scientists more than 10 years. Should we not also allow our students some years to understand it? Here, the first part of the history of the establishment of the Michaelis–Menten equation, from about 1890 to 1913, is examined in detail, followed by a more brief account of the last part of this history, ending in 1925 with the paper of Briggs and Haldane [34].

The first attempts to establish the experimental facts

The material

The enzyme[10] (then called a 'ferment', in reference to catalysis of the reactions of alcoholic fermentation) that was used most often as the starting point for all the studies of enzyme kinetics was invertase, or invertin, which is extracted from brewer's yeast, and which catalyses the reaction:

$$\text{sucrose} \rightleftharpoons \text{fructose (laevulose)} + \text{glucose (dextrose)} \quad (12)$$

'Invert sugar' is a name given to an equimolar mixture of glucose and fructose. The name comes from the inversion of the polarization plane of polarized light: sucrose solution displaces the plane to the right (sucrose is said to be dextrorotatory) but an equimolar mixture of glucose and fructose resulting from the hydrolysis of the sucrose displaces it to the left (the fructose is laevorotatory). Thus, there is an inversion of the rotation plane, hence the name of 'invert sugar'.

This reaction has the property of being able to occur in the absence of enzymes but in the presence of acid, and is then a first-order reaction. Was it the same in the presence of the ferment? Approximately 10 years were needed to arrive at broad agreement about the experimental facts, although the disagreements had less to do with the experimental facts than with their interpretation. All individuals analysing enzyme kinetics before Henri did so in relation to first-order chemical kinetics.

O'Sullivan and Tompson's research (1890)

C. S. O'Sullivan and Tompson [18] reported an exponential law for the production of invert sugar by invertase (i.e. one fully comparable with the rate of reaction

[10]The term 'enzyme' was introduced by Kühne [86] in 1877. It is usually said to derive from ἐν ζύμη, meaning 'in yeast' but, as Boyde [36, pp. 71–72] has discussed, the reality is less clear, and the word, derived from modern Greek ἔνζυμος, 'leavened', was already used in English as early as 1850 to refer to the leavened bread used as sacramental bread in the Orthodox Church. In Henri's time, the word 'diastase' (from διαστάσις, 'separation') was used more often. In the text, both of these terms are used equivalently, together with the word 'ferment' in a somewhat more general sense. In the same way, the words 'invertin', 'invertase' and 'sucrase' refer to the same enzyme.

of acid hydrolysis, with minor deviations). They showed that the rate was proportional to the enzyme concentration. However, in 1903, Henri [23] commented:

> But they do not calculate the constant
>
> $$k = \frac{1}{t}\ln\frac{a}{a-x} \quad (13)$$
>
> where a = initial substrate concentration and x = concentration of product released at time t. When these calculations are made it is evident that the value of k does not remain constant, but it increases smoothly from the beginning to the end of an experiment.

Another criticism of C. S. O'Sullivan and Tompson's work is that 'they have not studied the dependence on the sucrose concentration'.

However, J. O'Sullivan [87], working with whole yeast, showed, in 1892, that the constant varied with the initial substrate concentration and that it is inversely proportional to it. This study was also performed by Duclaux [19], who stressed C. S. O'Sullivan and Tompson's error.

Duclaux's research (1898)

Duclaux [19] criticized C. S. O'Sullivan and Tompson's results, and showed that the quantities of sucrose inverted after a given amount of time did not by any means vary in proportion to the sucrose concentration, as occurred for acid hydrolysis; in other words, in contrast to this latter case, the kinetics were not of first order. Duclaux made the following observations: at the beginning, the rate of reaction is constant (zero-order); the reaction products have a retarding effect. From these observations, he suggested that at the beginning, and in the absence of appreciable amounts of product, the rate is:

$$\frac{dx}{dt} = k \quad (14)$$

where x is the product concentration at time t. He then proposed that the retarding action of the products could be defined by the following term:

$$k_1\frac{x}{a} \quad (15)$$

where a is the initial substrate concentration. These assumptions led to the following rate equation:

$$\frac{dx}{dt} = k - k_1\frac{x}{a} \quad (16)$$

which can be integrated to give

$$t = \frac{a}{k_1}\ln\frac{ka}{ka - k_1 x} \text{ or } x = \frac{k}{k_1}a\left[1 - \exp\left(-\frac{k_1}{a}t\right)\right] \quad (17)$$

If the catalysed reaction goes to completion (as is the case for invertin), one must have $k = k_1$, and so

$$k = \frac{a}{t}\ln\frac{a}{a-x} \text{ or } x = a\left[1 - \exp\left(-\frac{k}{a}t\right)\right] \quad (18)$$

This is almost the expression of a first-order law with a factor a.

Henri's empirical equation of 1901

Henri [88] showed experimentally that the value of the 'constant' k defined by Eqn (18) for the pseudo-first-order law increased as the proportion of inverted sugar increased. He therefore proposed to follow a procedure recommended by Ostwald (in whose laboratory in Leipzig he had worked) and to write empirically

$$k = k_1\left(1 + \varepsilon\frac{x}{a}\right) \quad (19)$$

As k_1 and ε were constant he then deduced that

$$\frac{dx}{dt} = k_1\left(1 + \varepsilon\frac{x}{a}\right)(a - x) \quad (20)$$

which can be integrated to give

$$k_1(1 + \varepsilon) = \frac{1}{t}\left[\ln\frac{a}{a-x} + \ln\left(1 + \varepsilon\frac{x}{a}\right)\right] \quad (21)$$

For his experimental results with invertase, he showed that one could put $\varepsilon = 1$, and so the formula could be simplified to give

$$2k_1 = \frac{1}{t}\ln\frac{a+x}{a-x} \quad (22)$$

As we shall see later, it was on the basis of this empirical (and incorrect) equation that he could show that the enzyme did not lose its activity over the course of the reaction. A more qualitative indication of this had been given as early as 1890 by O'Sullivan and Tompson [18], who reported that a sample remained active after catalysing the hydrolysis of more than 100 000 times its own weight of sucrose.

Bodenstein's formula

Bodenstein was an assistant in Ostwald's laboratory in Leipzig. After studying Henri's results, he proposed the following mechanism, which he published later [89]. Henri cited Bodenstein's equation in his paper [22] and detailed it in his thesis [23, p. 77]:

> When a certain quantity ϕ of ferment is added to a mixture containing a_1 sucrose and i invert sugar, the activity of the ferment is decreased, first with respect to sucrose and second with respect to invert sugar. In this way one can argue that instead of the quantity ϕ of ferment one had a proportion $\phi/(ma_1 + ni)$, where m and n are constants.

In these conditions, the rate equation becomes as follows:

$$v = \frac{dx}{dt} = \frac{K_2\Phi}{m(a_1 - x) + n(i + x)} \cdot (a_1 - x) \qquad (23)$$

By trial and error Bodenstein found the values $m = 2$ and $n = 1$, which allowed him to simplify the integrated equation as follows:

$$K_2\Phi = \frac{a}{t}\left[\frac{x}{a} + \ln\left(\frac{a}{a - x}\right)\right] \qquad (24)$$

Following the usual practice, Henri, probably with Bodenstein's help, verified that his experimental results agreed with the constancy of K_2. As a matter of fact, with the values of Henri [23], an optimization procedure to identify the best values of m, n and $K_2\Phi$ gives values of m and n that are different and are not integers.

For sucrose concentrations greater than 0.1 N (which for sucrose is the same as 0.1 M), K_2 is constant:

> On the contrary, we see in the preceding series that for lower concentrations (0.05N) the values of K_2 deviate from the mean.

Segal [90] suggested that K_2 was not constant at low sucrose concentrations on account of competitive inhibition by the products formed. However, the real reason may be simpler. The experiments for which Henri tried to apply Bodenstein's equation were carried out in the initial absence of products. If we compare the expression for the initial rate ($x = 0$), Bodenstein's formula becomes in the absence of products ($i = 0$):

$$v = \frac{K_2\Phi a_1}{ma_1} \qquad (25)$$

which differs from the true expression (see below):

$$v = \frac{K_3\Phi a_1}{1 + ma_1} \text{(or in modern symbols) } v = \frac{V_{max}a_1}{K_m + a_1} \qquad (26)$$

by the term 1 in the denominator. If ma_1 is large compared to 1 (i.e. if a_1 is large compared to K_m), Bodenstein's expression is applicable until the 1 is no longer negligible. Starting from Henri's values we can calculate that K_m for sucrose, which is 0.1 N, corresponds well with the threshold of validity found by him and Bodenstein.

The situation at the very beginning of the 20th Century

Various other researchers, including Tammann [91], Herzog [92] and Medwedew [93–95], also proposed rate laws for diastases, although no one arrived at the true equation. However, in 1901–1903, the following points were well established:

1 The action of a 'diastase' is proportional to its concentration.
2 The rate as a function of time is constant at the beginning of the reaction.
3 The kinetics of appearance of the products do not truly obey the laws of first-order chemical kinetics without excessive deviations.

All this led most authors to 'massage' the first-order law to apply it to enzyme kinetics. Indeed, these different approximations did not bring any knowledge about the real mechanism of catalysis, nor did they provide any answer to the question of whether enzymes obeyed the laws of chemistry. It was a reflection on the different possible models of chemical catalysis that would lead Victor Henri to the solution.

Henri's approach

Henri made his study of the 'General laws of diastase action' the subject of his thesis [23]. He treated it with extreme rigour, constantly guided by the idea that diastases must obey the laws of chemistry, and in particular the law of mass action.

> In the study of the general phenomena of life of organisms two groups of theories have been proposed: some consider that living behaviour depends on physical chemistry; others reject this reductionism and recognize the existence of *sui generis* new forces [specific new forces] or types of energy, a 'vital force', as it is called. As experimentation is based on the data from chemistry and physics, the vitalist theories exclude the possibility of experimenting on this vital force; they constitute a sort of brake that prevents experimental research, i.e. scientific research, and convert discussion of experiments into speculation. The effect of these theories is thus harmful, as the utility of any theory must be judged by the number and importance of the new facts that it leads one to discover…
>
> …The present work has for its subject the study of the general laws of diastatic[11] action. The aim is to

[11]This would be 'enzymatic' in modern terminology.

> study actions of the diastases in following the methods and results of physical chemistry.
>
> [23, Preface]

He began by making an extensive critical bibliography, starting with chemical catalysis. In effect, if diastases are catalysts, their mechanism of action should be similar to that of chemical catalysts. In consequence, argues Henri, if we can know the laws and mechanisms of chemical catalysis, we shall have, perhaps, by the same token, those of enzyme catalysis. Henri was, as far as I know, the first to proceed in that way (i.e. to consider enzymes as they really are, as catalysts). In making different models systematically, he showed that the laws of the complete catalysed chemical reactions might appear different from those of simple reactions without, however, violating the laws of chemistry. He became fully conscious that one of these models of catalysis would be applicable to the diastases. This model is described on p. 15 of his thesis under the title 'Formation of intermediate complexes produced very fast', and, on p. 17, the equation appeared that he would demonstrate later on and apply to enzymes. Henri finished that paragraph by saying:

> We shall see later on that for the diastases there is an analogous reasoning that will allow us to arrive at the general law for their action.
>
> [23, p. 18]

After having examined the different models of catalysis and having derived the kinetic laws, he made a very critical review of the research on the laws of action of the diastases, including his own, and always with the same theme: no empirical formula brings any knowledge of the underlying mechanism of action of the diastases. Thus, on p. 59, he wrote about his own empirical formula of 1901:

> But this expression $2K_1$ has the fault of being established in a purely empirical way, and of changing when one moves from one sucrose concentration to another, as we shall see later on.
>
> [23, p. 59]

His judgement on Duclaux's theory was more severe:

> Finally Duclaux's theory has the fault that it assumes two different laws for the action of diastase on the substance to be transformed and for the action of the products of the reaction on the diastase, and neither of these different laws satisfies the law of mass action. Diastases are thus envisaged as obeying these laws *sui generis* [specific laws], constituting in this way a class outside the phenomena of general chemistry.
>
> [23, p. 42]

Finally, his criticism was also experimental, and he imagined and put into practice definitive experiments to test the set of hypotheses that had been proposed to explain the deviations from a first-order chemical process.

1 One of these hypotheses (put forward by Tammann in particular) [91] to explain the observed kinetics was that the enzyme was 'worn out' and became less active. Henri, using his empirical equation of 1901, confirmed that the enzyme remained unchanged throughout the period of inversion.
2 Another hypothesis (of Duclaux in particular) [19] was that the products slowed down the reaction. Henri showed that 'Addition of invert sugar to a mixture of sucrose and invertin slows the reaction down. For addition of the same amount of invert sugar the slowing down is the weaker as the sucrose concentration is bigger'. He showed, in addition, that the slowing down is 'caused almost solely by the laevulose'. He would therefore take account of this information for setting up his general equation.
3 He then studied the effect of the initial sucrose concentration on the rate of inversion and the effect of the invertin concentration, showing that the rates are proportional to the concentration of ferment but not to that of sucrose.

Henri's equation: the concept of the enzyme–substrate intermediate

Starting from the model of catalysed chemical reactions, Henri arrived at the idea of the existence of complexes between enzyme, substrate and product. In fact, this idea of a complex was 'in the air'. C. O'Sullivan and Tompson had also used the formation of an enzyme–substrate complex to explain the fact that invertase could tolerate a higher temperature in the presence of its substrate than in its absence [18]. In 1902, Adrian J. Brown [21] proposed that the enzyme formed a complex with its substrate that required a certain amount of time before decomposing into enzyme and product, and, with increases in substrate concentration being unable to increase the concentration of complexed enzyme, the rate of reaction would remain constant. Thus, Adrian J. Brown well understood the concept of maximum rate and he checked it experimentally. Following publication of this article, another by Horace T. Brown and T. A. Glendinning

[96] gave the results of a study of starch hydrolysis catalysed by amylase. These authors wrote explicitly:

> But ($a + b'$) must be strictly speaking be considered the starting point[12] from which the hydrolysis commences, the true hydrolyte, in fact, and the velocity of the inversion will depend on the concentration of ($a + b'$).

However, these were only qualitative explanations: none of these authors proposed a rate equation. Henri was the first to analyse the formation of enzyme–substrate and enzyme–product complexes quantitatively. Below, the end of his 1902 paper [22] is reproduced. The beginning of this article in English is adapted from the translation of the the whole article in Boyde's book [36]:

> Suppose we have a mixture of a quantity $a-x$ of the compound to be transformed with a quantity x of the products of hydrolysis; to this mixture we add the quantity Φ of diastase. I suppose that a portion z of the ferment combines with a portion of the compound to divide in two; that another portion y of the ferment combines with a portion of the products of hydrolysis; and finally that there remains a portion X of the ferment which remains free. I further suppose that these combinations are produced following the law of mass action. One obtains these three following equations:[13]

$$(a-x)X = \frac{1}{m}z,\ xX = \frac{1}{n}y,\ \Phi = X + y + z \qquad (27)$$

> From these equations, one may deduce values for X and for z.
>
> Two different hypotheses may be advanced:
>
> 1 One may suppose that it is the uncombined portion of the ferment, X, which acts upon the compounds to be divided in two; in this case the velocity of the reaction is proportional to X and to $a-x$; whence one obtains

$$\frac{dx}{dt} = \frac{K\Phi(a-x)}{1 + m(a-x) + nx} \qquad (28)$$

> 2 On the contrary, one may suppose that it is the complex z between the compound to be divided in two and the ferment is an unstable intermediate compound, which decomposes restoring some of the ferment. In this case the velocity of the reaction will be proportional to the amount of this complex z; whence one will deduce

$$\frac{dx}{dt} = \frac{Km\Phi(a-x)}{1 + m(a-x) + nx} \qquad (29)$$

> It is remarkable that these two different hypotheses lead to the same law.

Finally, he gives the expression for the initial rate (by setting $x = 0$) in his thesis.

1 In the absence of invert sugar:

$$\text{Initial rate } = \frac{K_3}{1 + ma} \text{ with } K_3 = K\Phi \qquad (30)$$

He concludes:

> The relationship between the sucrose concentration and the initial rate is represented graphically by a hyperbola through the origin and with an asymptote parallel to the x-axis at a distance equal to K_3. This is indeed the general shape one gets experimentally. [23, p. 91]

2 In the presence of a concentration i of invert sugar:

$$\text{Initial rate } = \frac{K_3 a_1}{1 + ma_1 + ni} \qquad (31)$$

3 Finally, the expression of the integrated equation in the general case:

$$\frac{dx}{dt} = \frac{K_3(a-x)}{1 + m(a-x) + nx} \text{ or } dx = \left[\frac{1 + na}{a-x} + m - n\right] = K_3 dt \qquad (32)$$

$$K_3 t = (1 + na)\ln\left(\frac{a}{a-x}\right) + (m-n)x \qquad (33)$$

This indicates a reaction rate of order 1 (the logarithmic term) and a reaction rate of order 0 [$(m-n)x$].

After V. Henri: Michaelis and Menten (1913)

The article of Michaelis and Menten that is always cited [25] is above all an experimental criticism of Henri's work on invertase, an experimental study that they repeated; their conclusion was that the equation proposed by Henri was valid. At the very beginning of their paper they wrote:

[12]Here ($a + b'$) refers to the enzyme–substrate complex.

[13]These equations correspond, in modern notation, to the two following reactions and the conservation equation: $E + S \rightleftharpoons ES$ (equilibrium constant m) and $E + P \rightleftharpoons EP$ (equilibrium constant n) with $[E] = X$, $[S] = a-x$, $[ES] = z$ and $[EP] = y$.

> Henri's investigations are of particular importance since he succeeded, starting from rational assumptions, in arriving at a mathematical description of the progress of enzymatic action that came quite near to experimental observations in many points. We start from Henri's considerations in the present work. That we have gone to the lengths of reexamination of this work arises from the fact that Henri did not take into account two aspects, which must now be taken so seriously that a new investigation is warranted. The first point to be taken into account is the hydrogen ion concentration, the second the mutarotation of the sugar(s). [28]

They criticized Henri's work in two ways: he had taken account neither of the concentration $[H^+]$ of hydrogen ions (recall that the idea of pH had been developed in 1909 by Sørensen[14]), nor of the mutarotation of glucose (a criticism that had already been made by Hudson [98] in 1908). The form of glucose produced by hydrolysis of sucrose is, in fact, α-D-glucose (specific rotation +110°) and is converted spontaneously into an equilibrium mixture with β-D-glucose (specific rotation +19°) until the specific rotation of the mixture reaches +52.5°. However, Henri used the decrease in the polarimeter reading at different times to calculate the quantity of invert sugar directly, without waiting for the mutarotation to be complete. Nonetheless, Michaelis and Menten emphasized the importance and validity of the fundamental work that Henri carried out:

> If Henri's research is susceptible to be improved in relation to these points its shortcomings are not as serious as Hudson thinks… On the contrary, we think that the fundamental ideas that Henri has developed are completely rational, and we will now attempt to use improved techniques to demonstrate this. [28]

After V. Henri: the equation of Van Slyke with Cullen and Zacharias

Around 1914, Van Slyke and his collaborators Cullen [33] and Zacharias [99] described an equation to explain their results with urease. They imagined a reaction with two irreversible steps, $E + S \rightarrow ES \rightarrow E + P$ with rate constants k_1 and k_2, respectively. They reasoned as follows: the time θ for one molecule of substrate to be converted into product is the sum of the times for the two steps:

$$\frac{\Delta(ES)}{\Delta t} = k_1(E)(S) \text{ and } \frac{\Delta(P)}{\Delta t} = k_2(ES) \qquad (34)$$

Thus, to make a molecule of ES from a molecule of enzyme it takes a time $\Delta t_1 = 1/k_1(S)$. In the same way, to make a molecule of P (and to restore the initial molecule of enzyme from a molecule of ES) takes a time $\Delta t_2 = 1/k_2$. The total time needed is therefore $\theta = \Delta t_1 + \Delta t_2 = 1/k_1(S) + 1/k_2$. The total rate of product formation is thus $1/\theta$ times the concentration of enzyme:

$$v = \frac{k_2(E)(S)}{\frac{k_2}{k_1} + (S)} \qquad (35)$$

This is therefore an equation fully comparable with Henri's, with, however, a different underlying hypothesis. Note that this analysis of processes in terms of times has not been much used afterwards, although it remains useful, even (or especially) for multi-enzyme systems [100].

In 1925, Briggs and Haldane [34] showed that it is not necessary to assume that the complex is in equilibrium with E and S, and even that there is a real possibility that it is not (thus making the connection with Van Slyke and co-workers). They showed, however, that, after a very short time, the rate of formation of ES is essentially the same as that of its conversion to products,[15] so that (ES) remains constant.

With the scheme: $E + S \rightleftharpoons ES \rightarrow E + P$ (rate constants k_1 and k_{-1} for the first reaction and k_2 for the second one), we can write: $d(ES)/dt = k_1\ (E)(S) - (k_{-1} + k_2)\ (ES) = 0$ with the quasi-steady state hypothesis. From there we obtain classically:

$$v = k_2(ES) = \frac{k_1(E)(S)}{\frac{k_{-1} + k_2}{k_1} + (S)} = \frac{V_{max}(S)}{K_m + (S)} \qquad (36)$$

Henri's equation is then seen to be a special case in which $k_{-1} \gg k_2$, such that K_m is the dissociation constant of the complex, whereas the equation of Van Slyke and co-workers is the special case in which $k_2 \gg k_{-1}$. In that case, there is no equilibrium of formation of complex. The parameter K_m is then a characteristic of the enzyme kinetics, and no longer a measure of the affinity of the substrate for the enzyme $K_1 = k_{-1}/k_1$. It

[14]The use of indicators to estimate the hydrogen ion concentration had been described a little earlier [97], although still after Henri's thesis.

[15]It can be shown that $d(ES)/dt$ is strictly equal to 0 at one point only. It is for that reason that we use the term quasi-steady state. Experimentally, the linearity of the formation of product as a function of time justifies this approximation.

is remarkable that the two different hypotheses, equilibrium and steady state, lead to the same equation. It will not be the same, however, for some scarcely more complicated systems.

This last contribution of Briggs and Haldane is particularly important. It is one of the first examples in biochemistry of the analysis of a dynamic system in the steady state.

Who was Victor Henri (1872–1940)? His other research

Victor Henri's career was astonishingly wide-ranging, and, with hundreds of papers to his name, it is impossible to do justice to it in a few words. He was born in Marseilles to a Russian unmarried mother, Alexandra Lyapunova, but was adopted by his natural father, Alexei Krylov, and his wife (the sister of his natural mother), who took him to St Petersburg, where he lived with his three parents and half-brother, and was educated in the German school. His family was aristocratic and well connected: his mothers' cousin was the mathematician Alexander Lyapunov, and his niece married Peter Kapitsa, the physicist who developed low-temperature research. Information about Henri's origins has been given by A. P. Kapitsa [101], correcting the sanitized account of his birth that was circulated for 130 years.

Afterwards he studied in Paris, and became the first collaborator of Alfred Binet, the pioneer in intelligence testing, with whom he studied hysterical patients from Charcot's department at the Salpétrière hospital. After a long period of time in Germany, particularly in Leipzig and Göttingen, he obtained his first doctorate in psychology in 1897 at Göttingen; his thesis concerned tactile sensations. Afterwards, he came back to Paris and performed new experiments with Binet on intellectual fatigue [102] that led him to the physiology laboratory in the Sorbonne to perform some chemical analysis to study nutritional exchanges during intellectual work.[16] He obtained a position of *préparateur* (a sort of lecturer) in this laboratory directed by Albert Dastre, a pupil of the great physiologist Claude Bernard, where he began the research on diastases, which forms the main subject of this part of the review. He received his second doctorate from the Sorbonne in 1903 [23]. In 1907, he was nominated Professor of Physiology in Paris. He then moved to Russia where he was responsible for the organization of the chemical industry for defence. On his return to Paris, he presented, in April 1920, Einstein's principle of relativity to philosophers and psychologists, with its philosophical consequences. In 1920, he was nominated at Zürich University where he remained 10 years. After a brief period at Berre-L'Étang (near Marseilles), where he was to be in charge of a planned great institute of petrochemistry, he was nominated at the Science Faculty in Liège (Belgium).

His later work was mainly in physical chemistry, where he studied absorption spectra, from which he obtained a wealth of information on molecular structures, such as those of naphthalene [104] and phosgene [105]. His last publication recorded in the Web of Science concerned the use of ultraviolet spectroscopy for detection of aromatic compounds in mineral oil [106], and was a collaboration with Chaim Weizmann, later the first President of Israel. He died in La Rochelle after a pulmonary congestion contracted during the 1940 retreat that followed the German invasion of France. Edgar Morin relates the arrival in Toulouse (where he was living) of Henri's wife and her four sons in abject poverty after his death [107]. Fuller accounts of his career are given by Nicolas [108], emphasizing his work in experimental psychology, and by Boyde [36].

Conclusions

This brief history of the establishment of the laws of enzyme kinetics well illustrates what Thomas Kuhn calls a 'scientific revolution' [109]. Of course, it is here a microrevolution, not comparable to the Copernican revolution or the emergence of the relativity theory in the field of Newtonian mechanics. We pass here from an explanation using chemical kinetics in terms of the order of a reaction to a new explanation based on the mechanism of enzyme action when the first explanation consistently fails.

In the first 10 years of research, between 1890 and 1902, all the early biochemists analysed their results in the framework of the paradigm valid in chemistry, trying to 'bend' the theory of chemical kinetics to fit the new experimental facts of enzyme kinetics (mainly an order of reaction that is not always unity) by means of approximations. This attitude, well described by Thomas Kuhn, is also adopted by Henri in the beginning.

[16]The expression 'nutritional exchanges' is the literal translation of the words *échanges nutritifs* that Henri used many times in his paper [103]. It refers to the differences produced by different degrees of intellectual effort in the composition of the excreta of subjects following a controlled diet and controlled muscular activity.

> To find this formula empirically I have followed a procedure recommended by Ostwald in his *Grundriss der allgemeinen Chemie*. As the value of K increases as the proportion of invert sugar increases, we can replace K in the expression for the rate of reaction by $K_1\ (1 + \varepsilon \cdot x/a)$.

He criticized this attitude himself: 'But this expression $2K_1$ has the fault of being established in a purely empirical way' [23, p. 59] and defends the idea 'to study the actions of the diastases in following the methods and results of physical chemistry' [23, Preface]. The change of paradigm here merely consists of considering the enzymatic reaction not simply as a chemical reaction (which takes place in the case of hydrolysis of sucrose in acidic medium in absence of enzyme), but as a catalyst with the formation of complexes, enzyme–substrate and enzyme–product. Henri's achievement was to have derived all the quantitative consequences of this mechanism on the basis of the laws of physical chemistry. His paper [22] is very interesting in the sense that it shows the two attitudes and presents the change of paradigm between them with the list of all the experimental results that have to be taken into account. Henri saw the advantages of this change of paradigm very well:

> The second conclusion that follows immediately is that is useful to study the rates of catalytic reactions. If we study the law that a catalytic reaction follows, discussion of it will allow the reaction studied to be placed in one of the classes above, and in consequence such a study will give important evidence of the detailed mechanism of the catalytic reaction. Sometimes the answer will not be absolutely clear-cut, but one will be able, in contrast, to state with certainty that a whole series of hypotheses about the catalytic action can be eliminated. Thus for example one can affirm from studies of diastase kinetics that Arthus's physical theory is untenable.
>
> In consequence, kinetic study of catalytic reactions will always teach us something new in relations to these reactions. That is why it is necessary to try to repeat the studies of the rates of all the diastatic reactions: it is only in that way that we shall be able to understand their mechanisms. [23, p. 24]

The change of paradigm is also visible in the way of representing the results: in chemistry, the reaction is studied as a function of time; in biochemistry, it will be mainly studied as a function of substrates and products concentrations. In Henri's work, this idea is simply noted in his thesis [23, p. 90] when he writes: '*Vitesse initiale* (initial rate) = $K_3a/(1 + ma)$', and later, taking into account the concentration i of invert sugar, '*Vitesse initiale* = $K_3a_1/(1 + ma_1 + ni)$', and remarks:

> Graphically, the relationship between the sucrose concentration and the initial rate is represented by a hyperbola through the origin and with an asymptote parallel to the x-axis at a distance equal to K_3. It is well the general shape one gets experimentally. [23, p. 91]

But he does not present such a hyperbolic graph (Fig. 6) in his thesis.[17] There are few graphical representations in Henri's work, but rather tables in which the results are fitted by trial and error, establishing the constancy of parameters that have to remain constant. The influence of chemical kinetics is also apparent in the way he presents his results by looking at the time course of the reaction (hence the integrated equation with the discussion on the mixed order 0 and 1 of the reaction) rather than at the initial rates. In a sense, Victor Henri remained a prisoner of chemical reasoning. It can be noted here that Henri's idea of how to conduct scientific research is essentially what Karl Popper proposed much later (i.e. the idea of 'falsifiability' of theories more than their 'verifiability'). Henri also appeared to approach another important concept of Popper, that of a 'demarcation criterion' between science and religion or science and beliefs when he criticized the vitalist attitude:

> The vitalist theories renounce the possibility of experimenting on this vital force; they constitute a sort of brake that prevents experimental research, i.e. scientific research, and convert discuss of experiments onto the field of speculation. [23, preface]

Michaelis and Menten criticized the experimental part of Henri's work but they recognized his pioneer work in establishing the rate equation of enzyme action:

> It will become apparent that the basic tenets of Henri are, at least in principle, quite correct (*völlig richtig*), and that the observations are now in better accord with them than are Henri's own experiments.

They cite Henri's work extensively, including his thesis (ref. 4 in the first page of the original paper in

[17]Thus, a plot often misleadingly called the 'Michaelis–Menten plot', but never in fact used by Michaelis and Menten, was first described (but not illustrated) by Henri.

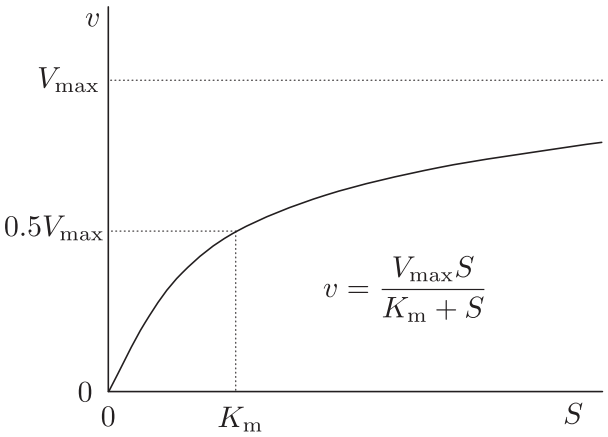

Fig. 6. Plot of *v* against *S* as defined by Eqn (6). Henri described this hyperbola in his thesis (see text) but did not illustrate it. This type of plot is sometimes called a 'Michaelis–Menten plot', a name that is acceptable if only intended to mean that it is one of the many ways in which the relationship defined by the Michaelis–Menten equation can be plotted, although this is misleading if taken to mean that it is the way that Michaelis and Menten plotted the equation, which is shown in Fig. 3.

German [25]; ref. 7 in the translation of Johnson and Goody [28]).

Their main strong improvements are in the experimental set-up, and also in using the plot of initial rate as a function of the concentration of substrate (in fact, the logarithm of the substrate concentration) to derive the parameters of the enzyme kinetics rather than the time course of the reaction with the integrated equation of Henri. They show the advantage of such an approach [17]. It should be noted that, although they probably read the comment of Henri on the hyperbolic representation of the initial rate, they used the logarithmic representation, influenced by their previous work on acid dissociation [17].

That Henri's equation is traditionally called the Michaelis–Menten equation is not in itself shocking, and it is far from being the first time in the history of science that such a thing has happened. The fact of referring generally to Michaelis and Menten's paper shows that one has not read it, as they themselves explicitly referred to Henri's work. One can regret, however, that Henri's work, carried out ten years earlier with such rigour, should have faded into obscurity. And even if his experiments are subject to criticism, this is not a reason to doubt Henri's paternity of the equation. It is unarguable that Henri must be considered the founder of enzyme kinetics not only because he discovered the 'equation of Michaelis and Menten', but above all because, with full consciousness of the laws of scientific procedure, he supplied the methodology for the general study of enzymic processes. The fundamental equation of enzyme kinetics should be cited as the Henri–Michaelis–Menten equation, as is done, for example, in the documentation of the metabolic simulation software COPASI [110]: and in a recent paper by Kell in this journal [111], although this is to forget also the fundamental contribution of Briggs and Haldane [34]. Furthermore, the custom of calling it the Michaelis–Menten equation is now well established after 100 years. May I simply propose that reference is made to the three seminal papers [22,25,34] when the Michaelis–Menten equation is cited, and, following a suggestion by Stefan Schuster (see Part 1), that 'Henri's equation' is used for the equation involving substrate and product binding:

$$V_0 = \frac{V_{max}\frac{S}{K_S}}{1 + \frac{S}{K_S} + \frac{P}{K_P}} \quad (37)$$

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