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On the Meaning of K_m and V/K in Enzyme Kinetics

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Ever since Briggs and Haldane (*1*) introduced the steady-state hypothesis and demonstrated that the rapid-equilibrium assumption was not necessary for the Michaelis–Menten equation (*2*) to be applicable to an enzymatic reaction, there has existed some confusion and ambiguity of exactly what the Michaelis–Menten constant, K_m or K, really means. But as will be demonstrated below, the larger problem has to do with the meaning of V/K (or V_{max}/K_m or k_{cat}/K_m or $1/\phi_1$ in other nomenclatures). In the Michaelian world, the fundamental constants are K, the dissociation constant of the substrate S from the enzyme–substrate complex ES (which evolved into the steady-state Michaelis–Menten constant), and V, the maximal velocity at saturating concentrations of substrate. Binding and the rate of catalysis were split into two domains described, respectively and historically, by these two parameters.

But with the advent of the steady-state hypothesis, and later because of empirical data such as isotope effects, it became clear that the Michaelis–Menten constant itself had a kinetic dimension that was difficult to grasp or define. For example, large deuterium isotope effects have been observed as K_H/K_D (*3*) despite theoretical arguments and empirical evidence in opposition to any significant effect of deuterium on binding of ligands to enzymes (*4*). To deal with this kinetic dimension, the duality of Michaelian kinetics was split along a different plane,[1] and the two fundamental kinetic constants were deemed V/K and V, the apparent rate constants at very low and very high [S], respectively (*5*). The Michaelis–Menten constant became a derivative parameter, obtained from the ratio of the fundamental ones. This duality can be clearly diagramed as in eqs 1–3. V/K is determined by everything from free enzyme E up to and including the first irreversible step (6).

$$E + S \rightleftharpoons ES \rightleftharpoons EPQ \rightarrow EQ + P \quad (1)$$

V is determined by everything from the enzyme–substrate complex ES onwards to reformed free enzyme:

$$ES \rightleftharpoons EPQ \rightarrow EQ + P \rightarrow E + Q \quad (2)$$

Hence, if K is the ratio of these two fundamentals, it must be determined by the entire mechanism:

$$E + S \rightleftharpoons ES \rightleftharpoons EPQ \rightarrow EQ + P \rightarrow E + Q \quad (3)$$

Algebraically, the Michaelis–Menten constant is even more complex compared to V or V/K than eq 3 is to eqs 1 or 2. It is not surprising then, that students of enzymology might be just a little confused about what it means.

To resolve this confusion, it is necessary to first resolve confusion about V/K. Most textbooks begin with the Michaelis–Menten equation, where v is an initial velocity:

$$v = \frac{V[S]}{K + [S]} \quad (4)$$

and note that when $[S] << K$

$$v_{[S]<K} = \frac{V}{K}[S] \quad (5)$$

Hence, V/K has the dimensions of a first-order rate constant, and when multiplied by the concentration of substrate it yields a rate, just as a first-order rate constant does. The question is, V/K is a rate constant for what? Because product formation is generally being measured for initial velocities, the *rate* at low substrate is considered one measure of a catalytic turnover, governed by this apparent first-order *rate constant*. But this formalism cannot be true in eq 1 because V/K fails to measure a complete turnover. By definition, V/K only encompasses steps "up to and including the first irreversible step", which is often the release of the first product; and the release of the second product (Q in eq 2) is often rate-limiting for many real enzymes (*7*). Obviously, a rate-limiting release of Q in eq 1 would have no effect on V/K. The paradox is nicely illustrated graphically in the kinetic mechanism known as a ping-pong mechanism (*8*), in which two substrates, A and B, react with different forms of enzyme, E and F:

$$E + A \rightleftharpoons EA \rightleftharpoons FP \rightarrow F + P \quad (6)$$

$$F + B \rightleftharpoons FB \rightleftharpoons EQ \rightarrow E + Q \quad (7)$$

V/K_a is determined by the rate constants in eq 6 and is independent of the concentration of B or of any rate constant in eq 7. This means that as [B] is varied—or even extrapolated to zero—K_a/V (the slope in a double-reciprocal plot of $1/v$ vs $1/[A]$) remains unchanged, giving rise to the diagnostic initial velocity pattern of a set of parallel lines. But it also means that V/K_a cannot be a measure of catalytic turnovers because no turnovers are completed at zero [B]. The preceding examples have two products, and product release is considered irreversible within initial velocity measurements, so a portion of a complete catalytic turnover cannot be associated with V/K. But even for a single product reaction, V/K need not encompass a complete turnover for many enzymes because some of them, such as carbonic anhydrase, have hidden but partially rate-limiting iso-mechanisms (*9*). Moreover, even in the most general case where V/K does encompass a complete turnover, at least formally in terms of including rate constants from all steps of a kinetic mechanism, it can be shown within a *congruent thermodynamic cycle* (*10*) that V/K can change

without a change in the catalytic power of an enzyme, *V/K* and catalytic power can change together, or *V/K* can remain unchanged as catalytic power is changed. Clearly, there is no linkage between *V/K* and catalytic power.

So, if *V/K* does not measure a rate of a catalytic turnover, what does it measure? How do we identify the measurement in a way that will apply equally and meaningfully to all the examples given above? One way to approach an answer to this question is to consider the Michaelis–Menten equation in the familiar reciprocal form of Lineweaver–Burk (*11*):

$$\frac{1}{v} = \frac{K}{V}\frac{1}{[S]} + \frac{1}{V} \qquad (8)$$

The reciprocal form became popular initially because plots of initial velocities yield straight lines, and was exploited later in the development of steady-state kinetics because it separated the fundamental parameters and variables (*12*). But it has a more subtle utility that made it attractive to Dalziel (*13*), who wrote eq 8 in the following way:

$$\frac{e}{v_0} = \frac{\phi_1}{[S_1]} + \phi_0 \qquad (9)$$

The difference between eqs 8 and 9 is that the former is written in apparent *kinetic constants* whereas the latter is written in apparent *time constants*. Much of the difficulty students have with enzyme kinetics lies in the awkwardness of multiple reciprocation, both algebraic and graphical, and the fact that none of us lives in reciprocal time. We live in real time, and in Dalziel's world, eq 9 states that the time necessary for an enzymatic reaction to occur is the simple sum of the time it takes for enzyme and substrate to come together in a productive complex, $\phi_1/[S_1]$, plus the time it takes for that complex to generate and release product, ϕ_0. (Time constants are additive whereas rate constants are not, which makes them easier to grasp and manipulate.)

Returning to the world of rate constants, *V/K* is the reciprocal of ϕ_1 and its true definition is now apparent: *V/K* is the rate constant for the coming together of substrate and product into a productive complex. "Productive complex" needs some qualification: it does not include those complexes of ES that form and then dissociate back to free E and S, but it does include multiple complexes in mechanisms with multiple steps (e.g., ES and EPQ in eq 1). When a substrate is particularly "sticky", virtually every collision between E and S yields a complex(es) that goes on to product, which drives *V/K* to the limit of diffusion control (k_1 in eq 10, below), somewhere in the neighborhood of 10^9 $M^{-1}s^{-1}$. Note, however, that the time required for the productive complex to actually be productive and go on to release a formed product is not specified by *V/K*. Therefore, a new label is needed to encompass this subtle distinction between total ES (which is real and can be observed) and productive complexes (which are so designated only because of their *potential*, which cannot be observed but only measured after the fact); the term *capture* is offered here for this purpose, symbolized by k_{cap}. *V/K* may be said to provide a measure of the rate of *capture* of substrate by an enzyme into a productive complex(es), in juxtaposition to *V*, which provides a measure of the rate of *release*[2] of enzyme and product from the same productive complex(es). It is further suggested that the symbol k_{cat} be replaced by k_{rel}. Capture and release are equally necessary to generate a complete catalytic turnover, but they are determined by different things.[3] This new construct allows us to return to the simpler Michaelian world in which the duality of enzyme kinetics was split between binding and catalysis. The differences are (i) Michaelian binding was a static concept, whereas capture is kinetic; and (ii) k_{cat} focused on a single chemical step, whereas release embodies multiple steps that may or may not include the chemical step and is often limited by the actual release of a product (*7*).

A more sophisticated definition of the Michaelis–Menten constant can now be formulated. The steady-state Michaelis–Menten constant is a kinetic term, albeit with a thermodynamic dimension, that describes the concentration of substrate at which the time for capture is equal to the time for release; hence, a turnover at [S] = *K* takes twice as long in real time as either process by itself. At very low [S], the time for capture becomes long and limiting because of the low rate of collision between substrate and enzyme, causing the time for release to become insignificant in comparison and a minor component of the apparent turnover time. At very high [S], capture becomes insignificant because collisions are infinitely fast at infinite [S] and virtually all of the enzyme is captured instantly, allowing the time for release to make up the whole of the apparent turnover time. Parallel to a dissociation constant, defined as $K_d = k_{off}/k_{on}$, a steady-state Michaelis–Menten constant in this new nomenclature is $K_m = V/(V/K) = k_{rel}/k_{cap}$; linguistically and kinetically, *release* is analogous *off* and *capture* is analogous *on*, and the transition from Michaelian to steady-state kinetics requires only those two simple substitutions.

This new definition has practical utility in other aspects of enzyme kinetics. For example, consider the popular textbook mechanism

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P \qquad (10)$$

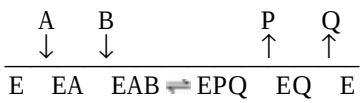
In case 1, the Michaelian model, $k_3 << k_2$ and $V = k_3$, $K_m = K_d = k_2/k_1$, and $V/K = k_1k_3/k_2$. In case 2, the steady-state model, $K_m = (k_2+k_3)/k_1$ and is numerically *larger* than the dissociation constant. What is often not recognized in textbooks is that Michaelis–Menten constants are frequently *smaller* than dissociation constants.[4] For example, in a slightly more complex case 3:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} EP \xrightarrow{k_5} E + P \qquad (11)$$

$K_m = k_5(k_2 + k_3)/k_1(k_3 + k_5)$ (*15*). If product release is slow relative to the chemical step so that $k_5 << k_3$, then $K_m \approx (k_5/k_3)(k_2 + k_3)/k_1$ and the factor k_5/k_3 can drive the Michaelis–Menten constant to values less than k_2/k_1. If in addition, in case 4, $k_3 >> k_2$ (making the substrate "sticky"), then $K_m \approx k_5/k_1$. In the first case, capture and release are controlled by the same thing and are in balance in the Michaelian region of substrate concentrations. In the second case, the time for release is shorter, so a relatively high concentration of substrate is required to hasten capture and bring capture and release into balance. In the third case, the time for release is long, so a relatively low concentration of substrate is required to slow capture and achieve balance. But the concepts of release and capture are most cleanly defined in the extremes of the fourth case of eq 11, where release is exactly that—the release of the last product ($k_{rel} = k_5$)—and capture is nothing more

than the molecular collisions between enzyme and substrate ($k_{cap} = k_1$). To understand enzyme kinetics in a general way, start here with this extreme example; the meaning of the common kinetic parameters will remain the same as mechanisms become more complex than case 4; they just become obscured by a lot of algebra.

Another aspect of kinetics where capture has utility is ordered binding:



(12)

Saturation with substrate *B* captures all the free enzyme in the EAB complex, making collisions between E and substrate A irreversible and V/K_a fully diffusion-controlled (*16*). Note that in both the extreme case 4 and the ordered example, *V/K* does not contain any rate constants for chemical steps. Whatever definition one applies to *V/K*, it must include these kinetic mechanisms as well as the more traditional varieties because they are not at all uncommon; for example, fumarase shows extreme capture and release (*17, 18*) and numerous dehydrogenases show compulsory ordered nucleotide binding.

The kinetics of inhibition is simplified by this approach. Competitive inhibition can be described as being caused by an inhibitor that interferes only with the time of capture (and it ceases to be expressed at high [S] where the time for capture is made short), uncompetitive inhibition is caused by an inhibitor that interferes only with release (which explains why an inhibitory analog of B in a ping-pong mechanism changes the apparent K_m of A even though binding of A and B have nothing to do with one another), and mixed or noncompetitive inhibition is caused by an inhibitor that interferes with both. That simplicity should ease teaching the kinetics of inhibition; so long as *V/K* was considered to have some measure of the rate of catalysis within it, the conceptual grasp of inhibitors could never be that clean.

An enzyme that illustrates the importance of both capture and release is chymotrypsin: the rapid and irreversible formation of a covalent acyl-enzyme intermediate signifies the mechanistic end of *V/K* and captures the substrate absolutely, committing it to a turnover, for there is no going back once the first product dissociates. Nevertheless, the slower hydrolysis of the acyl intermediate determines the rate of release from this captured complex and hence the rate of product formation. Capture and release have nothing to do with each other in chymotrypsin (when acting on a good substrate), yet both contribute to the enzyme's activity under normal conditions. Some authors have tried to grasp the kinetic contribution of *V/K* to enzymatic activity by calling it the "specificity constant" (*19*) to distinguish it from simple differences in binding; others compare values of *V/K* to the rate constant of the uncatalyzed reaction and call it the "catalytic proficiency" (*20*). Both terms are vague—and the latter, somewhat misleading—because *V/K* is clearly disconnected from real rates of net product formation and complete catalytic turnovers; capture has much clearer and more accurate imagery.

The concept of "committing to a turnover" is a good one, as it can be grasped without algebra. It correctly describes what capture is all about, but it does carry the risk of being confused with the "commitments to catalysis" used to describe isotope effects (*21*). For a reaction proceeding by the mechanism in eq 11, the isotopically sensitive step would be governed by k_3 and deuterium isotope effect expressed on *V/K* would be defined by the equation

$$\frac{(V/K)_H}{(V/K)_D} = \frac{k_{3H}/k_{3D} + C_f}{1 + C_f} \qquad (13)$$

in which C_f, the forward commitment to catalysis, is defined as k_3/k_2; it represents the tendency of the ES complex to proceed forward through catalysis as opposed to returning to free enzyme and substrate. Similarly (but not possible in the irreversible eq 11), a reverse commitment to catalysis, C_r, would be the tendency of an EP complex to return through catalysis as opposed to proceeding to free enzyme and product. Thus, C_f and C_r point in the forward and reverse directions, respectively, towards an isotopically sensitive chemical step. In contrast, capture points only in the forward direction all the way to the release of the last product. The concepts are related, however, in that *V/K* can be written in terms of C_f and C_r (*14*). The general expression without designating the number of steps or the identity of the isotopically sensitive step is

$$V/K = k_1 \frac{\prod_{i=3}^{i=ir} \left(k_i/k_{i-1}\right)}{C_r + C_f + 1} \qquad (14)$$

where *ir* is the rate constant number for the forward isotopically sensitive step used to define C_f (using the Cleland numbering system [*8*]). Equation 14 shows that *V/K* is simply a fraction of k_1. Moreover, that fraction only relates relative energy levels of transition states to each other; neither the order of energy barriers after binding nor the energy levels of intermediate reactant states are important. These points are illustrated in the activation diagrams *a–c* of Figure 1. In these three cases, $V/K = 0.07\ k_1$ and an intrinsic isotope effect of $k_H/k_D = 7$ on the step with the highest barrier would be expressed as $^D(V/K) = 5.4$. As [S] approaches zero, all enzyme becomes free and there is virtually no enzyme present in subsequent forms; hence, the curves are represented by dotted lines at the energy minima representing intermediate forms of enzyme because these minima do not matter to *V/K*; they could be as shown, or higher, or lower, or different from one another, without disturbing either the magnitude of *V/K* or the expression of an isotope effect.[5] The only thing that does matter is the relative vertical positioning of intermediate transition states, as illustrated by curve *d*, where the transition states after the binding barrier have been lowered relative to curve *a*. As a result, *V/K* is now more diffusion-controlled at $0.94\ k_1$, and the expression of a deuterium isotope effect would drop to $^D(V/K) = 1.3$. What *V/K* "sees" is some sort of singular collective barrier whose only significance is whether it is higher or lower than the binding barrier, illustrated in curves *e* and *f* (shown with only an energy minimum for free E), which were derived from curves *a* and *d*, respectively. When higher, as in curve *e*, most collisions between enzyme and substrate do not result in captured productive complexes; when lower, as in curve *f*, they do. The concept of capture is that simple, and all the kinetic information that can be conveyed by *V/K* is represented symbolically in curves *e* and *f*.

One aspect of modern enzymology that requires reexamination in this new light is the concept of *catalytic perfection*

and its associated *perfect enzyme*, which has become a centerpiece in chapters on enzymes in most biochemistry textbooks. Albery and Knowles (*23*) invented the concept by addressing ways that the evolution of enzymes might take to improve catalytic efficiency and they proposed three mechanisms to achieve this end. The first was termed *uniform binding* and consists of changes in which "the positions of all the internal states are shifted energetically up or down [on activation diagrams] by the same amount." The second was termed *differential binding* and consists of "changes in the relative stabilities of the internal intermediates, and the consequential effects of the internal transition states." The third was termed *catalysis of elementary steps* and consists of mechanisms to "reduce the free energy of activation of those elementary steps whose intermediates and transition states are kinetically significant." According to Albery and Knowles, all three contribute to catalytic improvement and the ultimate in catalytic perfection was identified as reaching diffusion-controlled kinetics.

The problem is, only the third way actually represents an increase in the rate of release concomitant with the increase in capture. For a contrary example, moving from curve *a* to *d* in Figure 1 illustrates Albery and Knowles's first way to evolve towards a more diffusion-controlled *V/K*. But *V* has the same value, 0.38, in both curves. Obviously, this increase in *V/K* comes from a drop in *K*, from 5 to 0.4, which may not be desirable physiologically. Moreover, making a substrate sticky in this fashion will ultimately lead to a sticky product, causing a decrease in *V*. It may be true that real catalytic perfection is always accompanied by a diffusion-controlled *V/K*, but the converse need not be; a mutation leading to diffusion-controlled kinetics may bring catalytic ruination as well as perfection. The reason for this lies in the distinction between *capture* and *release*, both of which are important in vivo because most enzymes operate with Michaelis–Menten

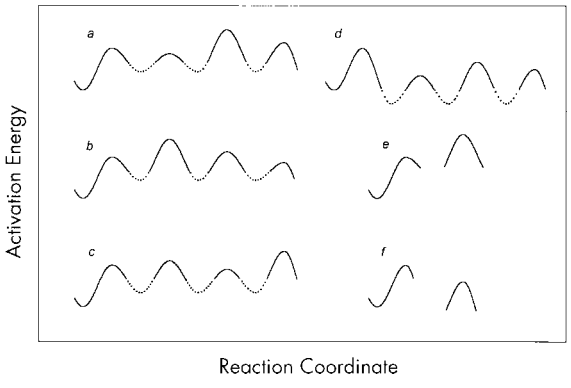


Figure 1. Activation energy diagram relating the energetics of *V*/*K*. Each curve begins with E + S at the left and proceeds with multiple forms of ES and EP towards the right. Relative rate constants used in each construction are (using the Cleland [*8*] numbering system): curve *a*: $k_1 = 1$, $k_2 = 10$, $k_3 = 20$, $k_4 = 20$, $k_5 = 1$, $k_6 = 1$, $k_7 = 5$; curve *b*: $k_1 = 1$, $k_2 = 10$, $k_3 = 1$, $k_4 = 1$, $k_5 = 5$, $k_6 = 5$, $k_7 = 20$; curve *c*: $k_1 = 1$, $k_2 = 10$, $k_3 = 5$, $k_4 = 5$, $k_5 = 20$, $k_6 = 20$, $k_7 = 1$; curve *d*: $k_1 = 1$, $k_2 = 0.05$, $k_3 = 20$, $k_4 = 20$, $k_5 = 1$, $k_6 = 1$, $k_7 = 5$; curve *e*: $k_1 = 1$, $k_2 = 10$, $k_3 = 0.8$; and curve *f*: $k_1 = 1$, $k_2 = 0.05$, $k_3 = 0.8$. Values for *V*/*K* are identical in curves *a*–*c* and *e*; larger but identical values are found in curves *d* and *f*. Values for *V* are 0.38, 0.65, 0.30, and 0.38 for curves *a*–*d*, respectively.

constants near physiological concentrations of substrates. A single mutation may change one or the other, or both; but *V/K* sees only the former, whereas catalytic power depends only on the latter (*10*). The posited criterion for catalytic perfection, a diffusion-controlled *V/K*, is founded on the mistaken notion that *V/K* is somehow related to the intrinsic rate of enzymatic catalysis. It is not, as this discussion clearly shows. Rather, *V/K* determines how much of the total enzyme is engaged in what will *become* a catalytic event: how much has been *captured* and is thereby committed to producing product. Maximal catalytic power is available at $[S] = K$ and $[S]\ V/K = V$ (*10*); therefore, evolution favors holding *K* constant while increasing both capture and release in constant ratio. Diffusion then dictates the upper limit of capture, which indirectly sets the maximal rate for release.

An example of the importance of capture, and an exception to the normal evolutionary pressure, can be seen in the kinetics of bacterial enzymes responsible for antibiotic resistance. The common measure of resistance in vivo, the *minimum inhibitory concentration*, correlates with *V/K* measured in vitro, but does not correlate with either *V* or *K* (*24*). Rates of release are not very impressive for these enzymes, because they do not matter. It is in the interest of the bacterium to keep the concentration of active antibiotic as low as possible, and it achieves this goal not by evolving higher catalytic power, but rather by making lots of enzyme (*25*) with high rates of capture (*26*). The concentration of enzyme probably exceeds that of the antibiotic in the periplasmic space, so that individual enzyme molecules engaged in catalysis can afford to take their time about carrying out the actual chemistry of inactivation—after a speedy capture.

A clear understanding of enzymatic catalysis requires coming to grips with the kinetic nature of capture, regardless of what name is given to it.[6] Once one has arrived at that, then *catalysis* or *catalytic power* or *catalytic efficiency* or the more profane "how enzymes work" kind of question can be easily understood within the simpler concepts of the older Michaelian world: binding is arbitrary and can be adjusted up or down to suit physiological needs, and a good enzymatic catalyst must simply lower the energy minima[6] and energy barriers that come *after* substrate binding.

Acknowledgments

I thank W. W. Cleland for firmly conveying the importance of *V/K* (three decades ago), for a critical review of the manuscript which included identifying errors in previous renditions of eq 14, and for suggesting that conditions in which $K_m < K_d$ be included in the discussion. This work was supported by NIH research grant GM46695.

Notes

1. This duality led to other problems, the most significant being the faulty concepts that binding energy can be used to drive catalysis and that reactant-state stabilizations waste catalytic power, which are discussed elsewhere (*10*).

2. An earlier draft attempted to pair *catalysis* with *capture*, consistent with the original duality of Michaelis and Menten and the popular nomenclature of k_{cat}. But this led to confusion by readers who associate "catalysis" either with a single, rate-limiting chemical step or with a complete catalytic cycle. The standard textbook mechanism of eq 10 stresses $k_{cat} = k_3$, which is unfortunate as it presents a somewhat misleading symbol for catalysis. Given the formal definition of a catalyst, i.e., something that accelerates a chemical reaction but is recovered un-

changed by the process, the cyclical nature ought to be more closely associated with the word. In contrast, k_{rel} may or may not include the chemical step, it may include the chemical step but not express it, and it never includes all the rate constants for a complete turnover. Cyclical catalysis requires both capture and release, and saturating concentrations of substrate simply make capture infinitely fast and kinetically insignificant to the cycle.

3. An analogy to "different things" would be to consider traveling by air, say from New York to Boston. Getting from home to an assigned seat on a plane is like substrate binding and capture, and because of contending with rush hour traffic, finding a parking place, checking baggage, and waiting to board the plane, this phase of the trip is likely to take longer than the actual flying time, which is analogous to *V* and release. Nobody considers the former to have anything to do with how an airplane flies, yet it must be taken into account when planning a trip. The proposal here is, in a direct analogy, that capture has nothing to do with how an enzyme carries out the chemistry of catalysis.

4. Klinman and Matthews (*14*) make the important observation that $K_m = K_d$ when isotope effects on *V* and *V/K* are the same, and provide an equation for calculating K_m/K_d when they are not the same.

5. The energy minima do matter to *V*, equally so as energy maxima, but not equally to each other. For example, in a five-step reversible mechanism with all rate constants equal to the same value, the distribution of enzyme forms would be [E1] = 0, [E2] = 0.4[E_t], [E3] = 0.3[E_t], [E4] = 0.2[E_t], and [E5] = 0.1[E_t]. A fully optimized enzyme has each subsequent energy minimum lower than the previous one so that the enzyme runs "downhill" (*22*). The practical significance of the absence of energy minima in *V/K* is that the rate of capture cannot be enhanced by binding the substrate more tightly, as represented by the vertical positioning of these minima. This mode of "tighter binding" would simply drop ES down a hole out of which the two escape routes, forwards and backwards, would remain in constant ratio no matter how deep the hole. In contrast, getting out of a deeper hole would matter a great deal to *V*.

6. Retaining the symbols of *V/K* and k_{cat} in our rate equations, and "V-over-K" and "k-cat" in our kinetic vocabulary, may impede this understanding. V-over-K diverts our attention to a ratio of other things which neither contain nor imply capture; k-cat diverts our attention to chemical steps which often are completely missing from k_{rel} and maximal velocities.

Literature Cited

1. Briggs, G. E.; Haldane, J. B. S. *Biochem. J.* 1925, *19*, 338.
2. Michaelis, L.; Menten, M. L. *Biochem. Z.* 1913, *49*, 333.
3. Simon, H.; Palm, D. *Angew. Chem. Int. Ed. Engl.* 1966, *5*, 920.
4. LeReau, R. D.; Wan, W.; Anderson, V. E. *Biochemistry* 1989, *28*, 3619.
5. Cleland, W. W. In *The Enzymes*, 3rd ed.; Boyer, P. D., Ed.; Academic: New York, 1970; Vol. 2, p 1.
6. Cleland, W. W. *Biochemistry* 1975, *14*, 3220.
7. Cleland, W. W. *Acc. Chem. Res.* 1975, *8*, 145.
8. Cleland, W. W. *Biochim. Biophys. Acta* 1963, *67*, 104.
9. Rebholz, K. L.; Northrop, D. B. *Methods Enzymol.* 1995, *249*, 211.
10. Northrop, D. B. *Adv. Enzymol.* 1998, manuscript submitted.
11. Lineweaver, H.; Burk, D. *J. Am. Chem. Soc.* 1934, *56*, 658.
12. Cleland, W. W. *Adv. Enzymol.* 1977, *45*, 273.
13. Dalziel, K. *Acta Chem. Scand.* 1957, *10*, 1706.
14. Klinman, J. P; Matthews, R. G. *J. Am. Chem. Soc.* 1985, *107*, 1058.
15. Northrop, D. B. *Biochemistry* 1975, *14*, 2644.
16. Frieden, C. *J. Am. Chem. Soc.* 1957, *79*, 1894.
17. Blanchard, J. S.; Cleland, W. W. *Biochemistry* 1980, *19*, 4506.
18. Sweet, W. L.; Blanchard, J. S. *Arch. Biochem. Biophys.* 1990, *277*, 196.
19. Fersht, A. *Enzyme Structure and Mechanism*, 2nd ed.; Freeman: New York, 1985; p 105.
20. Radzicka, A.; Wolfenden, R. *Science* 1995, *267*, 90.
21. Northrop, D. B. In *Isotope Effects on Enzyme-Catalyzed Reactions*; Cleland, W. W.; O'Leary, M. H.; Northrop, D. B., Eds.; University Park Press: Baltimore, MD, 1977; p 122.
22. Hackney, D. D. In *The Enzymes*, 3rd ed.; Sigman, D. S.; Boyer, P. D., Eds.; Academic: New York, 1990; Vol. 19, p 4.
23. Albery, W. J.; Knowles, J. R. *Biochemistry* 1976, *15*, 5631.
24. Radika, K.; Northrop, D. B. *Antimicrob. Agents Chemother.* 1984, *25*, 479.
25. Williams, J. W.; Northrop, D. B. *Biochemistry* 1976, *15*, 125.
26. Williams, J. W.; Northrop, D. B. *J. Biol. Chem.* 1978, *253*, 5908.