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Algebraic Methods for Deriving Steady-State Rate Equations

PRACTICAL DIFFICULTIES WITH MECHANISMS THAT CONTAIN REPEATED RATE CONSTANTS

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Methods of deriving rate equations that rely on repetition of terms for identification of redundant or invalid terms give incorrect results if used with mechanisms in which some rate constants appear more than once.

Indge & Childs (1976) have recently described a new method using 'Wang algebra' for deriving steady-state rate equations. Unfortunately, it is not general and can lead to incorrect results if any rate constants appear more than once in the mechanism. This is because the method incorporates an assumption that any product of rate constants that occurs more than once in the derivation must correspond to a cyclic pattern in the method of King & Altman (1956), which is not necessarily true.

There are at least three circumstances in which the same rate constant may describe more than one step of a reaction. First, it is sometimes convenient to simplify the analysis of a complex mechanism by examining special cases in which two or more steps have identical rate constants, e.g. in the several types of pure non-competitive effect discussed by Cornish-Bowden (1976). Secondly, mechanisms for enzymes with two or more identical active sites always contain repeated rate constants. Although these can be removed by the use of 'statistical factors' or by addition of parallel branches (Volkenstein & Goldstein, 1966), the principles governing the use of statistical factors are not always well understood, and it is sometimes instructive to derive rate equations without them. Thirdly, repeated rate constants can arise in the analysis of some types of isotope exchange.

A classic method for showing that a group-transfer reaction proceeds through a substituted-enzyme intermediate is to show that the enzyme can catalyse isotope exchange in an incomplete reaction mixture (Doudoroff *et al.*, 1947). In this mechanism the transfer of radioactive label (represented by an asterisk *) between two species A and P involves the following four steps, in which E and E′ are two different forms of the enzyme:

$$E+A \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} (EA\text{–}E'P) \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} E'+P$$

$$E'+P^* \underset{k_{+2}}{\overset{k_{-2}}{\rightleftharpoons}} (E'P^*\text{–}EA^*) \underset{k_{+1}}{\overset{k_{-1}}{\rightleftharpoons}} E+A^*$$

If isotope effects are negligible the second half of the reaction is described by the same rate constants as the reverse of the first half, as shown. The correct rate constant for the exchange may be simply derived by the method of King & Altman (1956), and, if terms in [A*] and [P*] are assumed to be negligible in comparison with other terms, the denominator contains terms in [A], [P] and [A][P]. But the method of Indge & Childs (1976) leads to a rate equation in which the denominator contains a single incomplete term in [A][P] in the denominator, because the 'Wang algebra' causes several valid terms to be erroneously deleted. In this example the result is so obviously wrong that the error would probably be noticed immediately. However, similar techniques have been used to simplify the kinetics of complex mechanisms, and in such cases the incompleteness of the derived rate equation might not be obvious. An example of this is provided by the work of Cedar & Schwartz (1969), who studied ^{32}P exchange between ATP and pyrophosphate catalysed by asparagine synthetase in the absence of ammonia.

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