Application of Topological Methods in Enzyme Kinetics

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A criticism [Cornish-Bowden (1976) *Biochem. J.* **159**, 167] of an algebraic method for deriving steady-state rate equations [Indge & Childs (1976) *Biochem. J.* **155**, 567–570] is theoretically unfounded.

The schematic method (King & Altman, 1956) of deriving rate laws for enzyme-catalysed reactions, like the analogous topological methods used in applied graph theory (Dodd, 1967; Mayeda, 1972), involves two fundamental operations. These are: (1) the generation of a set of specified patterns of reaction steps, or subgraphs, of a graph of the mechanism and (2) the evaluation of the product of the rate constants (and appropriate ligand concentrations) associated with each pattern of reaction steps. The requisite subgraphs, which are known as directed trees in applied graph theory (Chen, 1966; Dodd, 1967), can be readily identified by direct inspection of the graph of a simple mechanism. However, it is impractical to generate the complete set of directed trees of a complex mechanism in this way. For example, the general reaction scheme considered by King & Altman (1956) contains 3, 125 or 16807 directed trees based on each of the n enzyme-containing species when n is 3, 5 or 7 respectively (Mayeda, 1972). This is a general problem associated with this type of topological method of analysis, and efficient and reliable techniques for generating the required directed trees or other subgraphs of linear graphs have accordingly been developed (Chen, 1971). In the method of Indge & Childs (1976) the directed trees of the graph of an enzyme mechanism are identified by algebraic manipulation of sets of symbols denoting the reaction steps. It is essential, therefore, that each reaction step is labelled with a distinct symbol. The relevant mathematical techniques are described by Chen (1966, 1969).

In contrast with the problems of identifying the directed trees, the second stage of the topological analysis of an enzyme mechanism is straightforward. Where the directed trees are represented algebraically, it is advantageous to denote a reaction step and to specify the magnitude of its associated rate constant either by the same symbol or by related symbols, e.g. k_{+n} is the velocity constant of reaction step $+n$. The product of the rate constants associated with the reaction steps in a directed tree can then be written immediately without referring to the original graph.

The latter system of nomenclature is consistent with the scheme of numbering rate constants recommended by the CBN [see *Biochem. J.* (1976) **153**, 6].

Cornish-Bowden (1976) disregards this convention and fails to recognize that it is sometimes imperative to distinguish clearly the elementary processes of a reaction and the rate constants which characterize these processes. If two or more rate constants in a specific mechanism are of the same magnitude, he chooses to denote them by the same symbol. Cornish-Bowden (1976) then criticizes the method of Indge & Childs (1976) on the grounds that incorrect results are obtained if it is applied to mechanisms which contain 'repeated rate constants', whereas the schematic method (King & Altman, 1956) still provides meaningful rate equations. The criticism, in effect, involves the erroneous assumption that in the method of Indge & Childs (1976) the symbols represent simply the magnitudes of the rate constants. Furthermore, since the topology of a system is independent of the weights (e.g. rate constants) associated with its elements, the interpretation of the algorithm of Indge & Childs (1976) adopted by Cornish-Bowden (1976) makes no provision for the generation of subgraphs of the mechanism. In short, the criticism has no theoretical foundation and embodies, moreover, a misleading conception of topological methods of analysis.

Certain specific points made by Cornish-Bowden (1976) require comment. The number of directed trees in the graph of an enzyme mechanism is unaffected by the assumption of identical rate constants. Therefore the implication that the derivation of a rate law is simpler for these special mechanisms is not generally true. If attempts are made to analyse the isotope-exchange reaction formulated by Cornish-Bowden (1976) by misapplying the method of Indge & Childs (1976) either or both incidence sets $(1+1+1'+1')$ $(2+2+2'+2')$ are used. Since both are zero by Wang addition (Chen, 1969), there can be no result, despite claims to the contrary (Cornish-Bowden, 1976). However, the reaction must be at

chemical equilibrium, and the derivation of its rate equation by steady-state methods is unnecessarily complex. In fact, exchange reactions were not considered by Indge & Childs (1976), although in other work (unpublished) they applied another type of subgraph, the directed k -tree (Chen, 1966), to that problem.

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References

- Chen, W. K. (1966) *SIAM J. Appl. Math.* **14**, 550–560
Chen, W. K. (1969) *Int. J. Electron.* **27**, 101–117
Chen, W. K. (1971) *Int. J. Electron.* **31**, 301–319
Cornish-Bowden, A. (1976) *Biochem. J.* **159**, 167
Dodd, G. G. (1967) *IEEE Trans. Circuit Theory* **14**, 154–159
Indge, K. J. & Childs, R. E. (1976) *Biochem. J.* **155**, 567–570
King, E. L. & Altman, C. (1956) *J. Phys. Chem.* **60**, 1375–1378
Mayeda, W. (1972) *Graph Theory*, John Wiley and Sons, New York