reactions of a pathway and titrating the flux while varying the concentrations of enzymes involved Thus, it has been possible to calculate the sensitivity coefficients of three enzymes (hexokinase, glucose-6-phosphate isomerase and phosphofructokinase) among the set of enzymes involved in the pathway from glucose to glycerol 3-phosphate[10]

From these approaches it is possible to calculate sensitivity coefficients for enzymes but the control dependence of the flux on external effectors is difficult to quantify Thus, we agree with Crabtree and Newsholme[5]. 'In our opinion measured values of control coefficients are best used as an additional means of testing proposed control models' We believe that the approach of these authors, which gives an equation for the response of the flux to changes of an external effector, is valid

The essential question is how to calculate variations of flux with respect to changes in concentration of effectors (i e sensitivities) This can be either by following the criteria of Kacser and coworkers[1–3] or by the approach given and used by Crabtree and Newsholme[5] based upon power rate equations[11]

Are the coefficients (sensitivities or elasticities) calculated from experimental data the true values for these coefficients? In general we think that the answer is *no* They are normalized derivatives By simulation we have demonstrated that sometimes the coefficients calculated from experiments performed are not actual derivatives Hence, the sensitivity coefficients calculated from such values by applying the connectivity theorems will not always be the correct values (data in preparation)

Finally, we would like to repeat that the effect of external effectors on a given pathway is difficult to quantify in this form Thus, the approach of Crabtree and Newsholme might be more practically applicable

We have performed various metabolic simulations[12 13], calculating the variation of sensitivity coefficients for the enzymes of the citric acid and purine nucleotide cycles changing the concentrations of intermediate metabolites[13] Each cell of an organism having a particular set of concentrations for the metabolites of a given pathway will have different sensitivity coefficients for the corresponding enzymes We think that each cell having its own 'fingerprint' of metabolites will have a determined set of sensitivity coefficients, and hence, knowing the intermediate concentrations the control points can be derived

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Control of metabolism: where is the theory?

In spite of their admonishing others 'we should not start to set up an algebraic model incorporating *ad hoc* assumptions or *a priori* assertions', this is exactly what Kacser and Porteous have done in their recent *TIBS* article[1] Many of their assumptions are of questionable validity, others are at odds with current knowledge in the field Full documentation of the inadequacies embedded within the 'control analysis' approach and of the confusion that has followed its introduction is beyond the scope of a short note In what follows, I shall limit consideration to just a few examples taken from the Kacser and Porteous paper that illustrate faulty assumptions, logical inconsistencies and mistaken conclusions These shortcomings demonstrate that the approach they are promoting is inappropriate as a theoretical foundation for the field

They state that characterization of the 'elementary enzyme kinetics depends' upon the Michaelis–Menten formalism and that 'when fully stated' these mathematical functions 'describe the consequences of interactions between all the molecules which participate in a reaction' This statement is an assumption that is not supported by experimental evidence There has long been evidence for enzyme–enzyme interactions involved in metabolic channeling and regulation, and it is now clear that this 'structural organization' is widespread in intact cells[2–9] The Michaelis–Menten formalism does not apply in general to such enzymes as they function in the intact cell

The Michaelis–Menten formalism has a number of restrictive assumptions that lead to reaction rates that are linearly related to enzyme concentration, but this is typically not the case Nevertheless, Kacser and Porteous state, 'we expect [from our knowledge of enzymology] a change in local reaction rate proportional to any change in enzyme concentration', that is, a linear or first-order response This clearly ignores the documented differences in milieu between the cell and the enzymologist's test tube

The authors claim that they have presented 'the proper treatment of systems of enzymes' Examination of the referenced papers reveals the implicit assumption that systems are linear in the enzyme concentrations and molecular activities, and that one can independently alter each of the enzyme levels in the system They conclude that 'for any flux in a given steady state, there are as many flux control coefficients as there are enzymes and carriers in the system

The response of the original system flux to a small change in any one enzyme in the system [with all other enzymes unchanged] is defined by the flux control coefficient' But when enzymes interact, as they generally do the conditions required for determination of a control coefficient cannot be met Moreover, in enzyme–proenzyme cascades, in several hormonal systems, and in the regulation of gene expression, enzyme levels are not fixed parameters or independent variables, as Kacser and co-workers assume Rather they are

dependent concentration variables over which the experimentalist has no direct influence, their values are determined by the values of the independent variables and parameters of the system[10,11], and no 'flux control coefficient' can be determined for such enzymes

Kacser and Porteous implicitly assume that their models possess steady states and that these states are stable with respect to small perturbations Real systems might possess these characteristics, but clearly these are critical properties that must be established for any *model* that is to represent such systems validly Explicit tests for the existence of a steady state and its stability are a part of more general theories of control (see, for example, Refs 12 and 13)

The formalism underlying our theory and that described by Kacser and Porteous is the same although these authors have failed to realize this (Savageau *et al*, submitted) They assume particular definitions for their parameters, because 'it is convenient in eliminating units of measurement', and make no reference to the underlying formalism that is implied In fact, the underlying formalism is totally obscured in their approach In contrast, our own theory makes the underlying formalism and the rationale for its selection explicit from the very beginning[12-14], the appropriate definitions of parameters then follow accordingly[10,12,15]

At one point the authors conclude that 'The magnitudes of all [the] . . elasticity coefficients at all of the steps in a particular intact system must then determine the behaviour of the whole system in the steady state' This conclusion is clearly wrong, as can be seen by examining the explicit steady-state solution[11,12] Indeed, the behaviour is determined in steady state by all the apparent kinetic orders (redefined as 'elasticity coefficients' by Kacser and co-workers), all the apparent rate constants (which are fundamental parameters of the underlying formalism but are not present in the approach of Kacser and co workers), and all the independent concentration variables

The cornerstones of their approach are the summation and connectivity relationships, from which they indirectly determine some (but not all) of the steady-state properties They claim that 'rigorous and logical analysis of the behaviour of metabolic systems demonstrated that [the] summation equals unity, whatever the complexity of the system' The truth is, these relationships are valid only under the restrictive assumption that each reaction is linearly related to the concentration of a single enzyme and the systems are simple, for example, they have no cascades

The authors demonstrate the summation and connectivity properties by stating that the flux control summation property follows directly from expressions for the individual flux control coefficients in terms of the elasticities, and from expressions for the individual flux control coefficients in terms of the elasticities it follows that one obtains the connectivity property This is a circular argument since derivation of the expressions required these properties in the first place

They also claim that 'addition of [more complex but] common aspects of metabolism in no way invalidates the fundamental conclusion of control analysis about the behavior of metabolic systems of any complexity' and that 'no new analytical techniques are required 'We have seen above that these assumptions are not valid for cascades and enzyme–enzyme interactions in general, and that this approach has notable deficiencies in analytical techniques, even the authors themselves acknowledge that 'additional important theorems' are necessary to treat branched pathways

The conclusion that 'allosteric inhibition of any one enzyme necessarily changes the values of all other control coefficients and transfers more control on flux to other enzymes in the system distal to the [metabolite that is exerting the feedback]' is based upon an unstated assumption that amounts to a very special way of realizing the feedback control In fact, in real cases that might be characterized by their model the distal control coefficients could increase, decrease, or remain unchanged. For a more complete discussion see Refs 11 and 16.

Kacser and Porteous go on to claim that 'the various summation and connectivity properties . . . are . . fundamental to any discussion of metabolic control. . Discussion and experiment in the absence of an understanding of these summation and connectivity properties can only proceed in a kind of intellectual vacuum' This is clearly not the case Not only are the author's properties not fundamental, as noted above, they are not even valid in general Furthermore, it has been demonstrated that systematic and predictive understanding of metabolic control is available through a more general theory without ever invoking such properties (for example, see Refs 16–18 and references therein)

Anyone seriously interested in acquiring the tools to understand rigorously intact biochemical systems is well advised to brush up on differential calculus and linear algebra, consult elementary texts on control theory and systems analysis, and critically study the original literature that deals specifically with intact biochemical systems. Attempts to obscure existing foundations, and to bypass the building of a solid theoretical foundation with over-simplified, intuitive and *ad hoc* approaches, can only lead in the end to confusion and error

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